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Validation of models commonly used to predict feed intake of lactating dairy cattle

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Introduction Accurate prediction of daily food intake is a fundamental prerequisite of any nutritional model designed to provide feeding recommendations for lactating dairy cattle. Consequently, much research effort has been expended over the last twenty years in developing food intake prediction models. These range from relatively simple multiple regression models to much more complex theoretical models embracing animal and food characteristics and environmental influences. However, given the major changes in the types of diet now offered to dairy cows, coupled with changes in genetic merit/milk production potential, it is important to examine if the food intake prediction models currently used within the British Isles are appropriate for to-day's dairy cow. This paper examines the accuracy of prediction of a range of intake models for diets based on grass silage, concentrates and brewers grains.

Materials and Methods Twenty seven recent dairy cow studies, 6 from ADAS Bridgets, 11 from the Agricultural Research Institute of N. Ireland and 10 from Scottish Agricultural College, were selected containing data on dietary and animal characteristics and *ad libitum* food intake. Data for 2424 cows were used to validate the Vadiveloo and Holmes (1979), Lewis (1981), Rumint (Oldham *et al.*, 1998), Cornell Net Carbohydrate and Protein System (CNCPS) 3.0 (Milligan *et al.*, 1981) and the French Fill Unit (FFU) (Dulphy *et al.*, 1989). Total dry matter intake (DMI) ranged from 8.5 to 29.4 kg/d, silage DMI 3.8 to 18.8 kg/d, concentrate DMI 1.5 to 21.4 kg/d, milk yield 7.7 to 49.7 kg/d and liveweight from 351 to 802 kg. Predicted intakes for each model were determined. Mean-square prediction errors (MSPE) were calculated and used to compare the prediction accuracy of the models between actual and predicted total DMI.

Results The results of the present validation are presented in Table 1. The models of Oldham *et al*. (1998) CNCPS and FFU over predicted intake by proportionately 0.12, 0.13 and 0.17 respectively while Lewis (1981) under predicted intake by 0.15. The Vadiveloo and Homes (1979) model, which used concentrate intake as the only dietary variable, was the most accurate equation for predicting intake as indicated by the lowest MSPE and mean prediction error (MPE). The equation of Lewis (1981) which is derived predominantly from feed variables was the second most accurate equation for predicting total feed intake. The CNCPS, Oldham *et al*. (1998) and FFU models which are derived from animal and environmental, feed and cow liveweight and feed and animal variables respectively, had similar MSPE's, although the MSPE for the CNCPS model being slightly lower. The prediction error was largely derived from the over prediction of DM intake by the CNCPS, FFU and Oldham *et al*. (1998) models, which resulted in large mean bias to MSPE ratios relative to the models of Vadiveloo and Holmes (1979) and Lewis (1981). Consequently the CNCPS, FFU and Oldham *et al*. (1998) models were the least accurate in predicting total feed intake.

Table 1 Prediction precision of different models for predicting intake of dairy cattle (n=2424)

Model	TDMI (kg/d)			R^2	MSPE	MPE	Proportion of MSPE		
	Actual	Predicted	Bias				Bias	Line	Random
Vadiveloo & Holmes (1979)	16.96	16.99	-0.03	0.69	2.9	0.101	0.00	0.00	1.00
Lewis (1981)	16.96	16.08	0.88	0.50	5.5	0.138	0.14	0.00	0.86
CNCPS	16.96	19.14	-2.18	0.58	8.9	0.176	0.54	0.02	0.44
French Fill Unit	16.96	19.84	-2.88	0.68	11.4	0.199	0.73	0.01	0.26
Oldham *et al.*(1998)	16.96	19.02	-2.06	0.49	10.8	0.194	0.39	0.17	0.44

Conclusions It is concluded that with diets based on grass silage, actual dry matter intakes are likely to be much lower than those predicted by the models of Oldham *et al*. (1998) CNCPS and FFU. New models are urgently required to accurately predict dry matter intake for dairy cattle offered grass silage based diets. The Vadiveloo and Holmes (1979) and Lewis (1981) models with respect to overall means provided the most accurate prediction of food intake.

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Meal patterns of cows offered complete diets with different ratios of concentrate to silage

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Introduction It is frequently assumed that energy intakes from mixed foods with a high proportion of silage (HS) are lower than those from mixed foods with a high proportion of concentrate (HC), because of short-term constraints, i.e. gut fill, that physically limit the amount of food a cow can consume. It was the aim of the present study to analyse how different proportions of concentrate in mixed foods affect short-term feeding behaviour. We hypothesised that cows offered HS are likely to have more meals that are more spread out during the day and vary less in size than cows offered HC. Alternatively, we expected higher correlations between meal size and the length of intervals before (pre-prandial) or after (post-prandial) meals for cows offered HS than for cows offered HC. We tested the hypotheses with a data set of 21195 meals.

Materials and methods We analysed 127213 visits by 22 lactating cows to 12 computerised feeders supplying two foods consisting of grass silage and concentrate to test for effects of food quality on meal patterns. Data were collected during an experiment described by Friggens *et al*. (1998). Foods consisted of the same ingredients but the proportion of concentrate DM in food DM differed between HC (0.59) and HS (0.27). HC and HS contained 457 and 331 g DM/kg of food and 343 and 429 g NDF/kg of DM, respectively. Each food was offered in six feeders to 11 cows from the start of lactation until 156±9 days after calving. After estimating meal criteria (Yeates *et al*., 2001), visits were grouped into meals. Sine waves were fitted to intake per hour of a feeding cycle (i.e., meal size divided by the sum of meal duration and length of the pre-meal interval). ANOVA based on 22 individual values was used to test for treatment effects.

Results Mean daily food intake (49.2 kg as fed) was not affected by treatment but DM intake was: 22.7 (HC) vs. 16.9 kg (HS). Means of individual meal criteria (25.9 min), daily number of meals (6.6) and the intake of fresh food per meal (7.6 kg) were not affected by treatment (Fig. 1[A]). Meal size did not vary less for cows consuming HS (Fig. 1[B]). Cows offered HS had longer meals (41.3 vs. 31.3 min) but lower feeding rates (78 vs. 156 g DM/min), than cows offered HC. Pre- and post-prandial correlation coefficients were sometimes significant but always low (r^2's ranged from 0 to 0.05) and were not affected by treatment. For both treatments, the largest meals were recorded after fresh food was supplied in the morning; meal size decreased systematically thereafter. Food intake per hour of the feeding cycle showed diurnal patterns in the shape of sine waves. Fitted sine wave models were significant for all cows and showed low and high mean values of about 1.5 and 3.5 kg/h for cycles starting just after midnight and just after noon, respectively. The means (2.37 kg), the relative amplitudes (0.41) and the shifts (7.3 h) of the sine-wave were not affected by treatment (Fig. 2). In short, food type did not affect the diurnal distribution of daily intake.

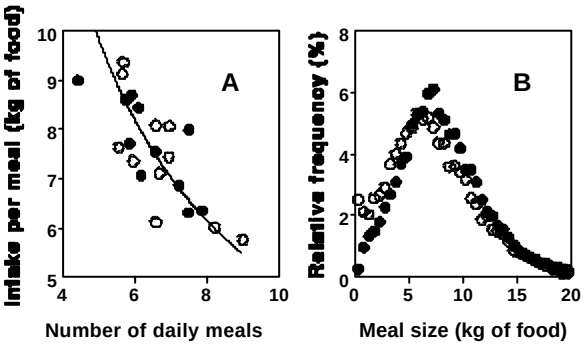

Figure 1 Individual mean intake per meal vs. number of daily meals and a line indicating combinations that result in average daily intake (that is: 49.2/x; A); the frequency distribution of meal size (B) for cows fed HC (●) or HS (○).

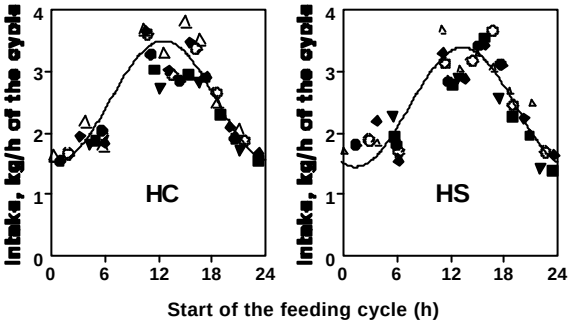

Figure 2 Food intake per hour of the feeding cycle for days during which cows consumed 4 (▼), 5 (■), 6 (●), 7 (○), 8 (◆) or 9 (Δ) meals of a high concentrate (HC) or a high silage (HS) food.

Conclusions HS cows consumed less DM per meal but their meal patterns were virtually identical to those of HC cows. No evidence was found that the diurnal meal pattern of cows offered HS deviated from that of cows offered HC, implying no differences between these foods in short-term constraints related to the physical properties of the diets.

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The effect of replacing grass silage with pea/wheat bi-crops in dairy cow diets on feed intake, concentrate utilization and milk production.

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Introduction. One of the ways by which UK farmers can maintain profitability in the current low milk price climate is to increase home-grown forage utilization in livestock diets. Previous work has shown that pea/wheat bi-crops are energy and protein rich forages that give higher intakes and nitrogen (N) retention than grass silage but only marginal improvements in milk production (Adesogan *et al.*, 2000). This study determined whether the milk response from such bi-crops could be improved by using a short-straw pea variety instead of the conventional tall straw variety.

Materials and methods The varieties of peas compared were Magnus, a tall straw, low tannin, pink flowered spring variety and Setchey, a short straw, relatively high tannin, purple flowered spring variety. The wheat (spring *var. Axona*) was drilled at a seed rate of 62.4 kg/ha with Magnus at 188 kg/ha or Setchey at 117 kg/ha. The bi-crops were harvested at week 14 when the peas and wheat were at the yellow wrinkle and soft dough stages respectively. Harvesting entailed cutting with a disc mower fitted with a conditioner, wilting overnight, precision chopping with a forage harvester and clamping in 40 tonne concrete, clamp silos. Chemical composition was measured and the bi-crops were fed to dairy cows along with a control, first-cut perennial ryegrass silage that was supplemented with either 4 (GS4) or 8 (GS8) kg/day of a dairy concentrate (CP 240 g/kg DM). The Magnus-wheat (MW) and Setchey wheat (SW) bi-crops were also supplemented with 4 kg/day of the concentrate. 10 multiparous cows in weeks 10 to 12 of lactation were used in each treatment. A continuous, 7 week, completely randomized design was used and feed intake, milk yield and milk composition were measured. The data were analyzed by analysis of variance using the one-way design model (Genstat 5, Lawes Agricultural Trust, 1995).

Results The chemical composition of the bi-crops and grass silages are presented in Table 1. The MW and SW bi-crops respectively contained 800 and 500 g/kg DM of peas. The bi-crops and the GS had similar total nitrogen (N), soluble N and ammonia (NH_3) N contents. The DM and starch contents of the GS were however lower ($P<0.001$) than those of the bi-crops. The DM intake (DMI), milk yield and composition of cows on bi-crops or GS are presented in Table 2. Irrespective of the level of concentrate intake, forage DMI was higher ($P<0.001$) for the bi-crops than for GS. Forage DMI was highest ($P<0.001$) on SW4 and this treatment also gave the highest total DMI. Milk yields and the fat corrected milk yield from cows fed GS8 and SW4 were similar, and higher ($P<0.001$) than those from cows fed GS4 or MW4. Milk constituent compositions were not significantly different between treatments. However, milk fat, protein and lactose yields were significantly increased by GS8 and SW4 compared to levels in GS4 or MW4.

Table 1: Chemical composition (g/kg DM) of MW, SW and GS

	MW	SW	GS	SED
pH	4.38	4.07	3.88	0.02
DM (g/kg)	301	292	211	8.09
Total N	31.9	28.7	29.7	5.55
SN (g/kg TN)	559	525	505	26.3
NH_3N (g/kg TN)	101	93.0	106	4.93
WSC	13.5	9.25	21.4	0.62
Starch	180	188	5.60	2.04
NDF	507	488	531	3.43
Lactic acid	50.9	38.0	83.3	3.60
Acetic acid	12.5	24.9	18.1	1.72

SN: Soluble N
WSC: water soluble carbohydrate
NDF: Neutral detergent fibre

Table 2: DM intake (kg/d), milk yield (kg/d) and milk composition (g/kg) in dairy cows fed GS, MW and SW

	GS8	GS4	MW4	SW4	SED
Forage DMI	10.7^{a}	11.8^{a}	14.0^{b}	15.8^{c}	0.50
Total DMI	17.5^{b}	15.4^{a}	17.2^{b}	19.3^{c}	0.71
Milk yield	24.5^{b}	20.1^{a}	20.8^{a}	24.0^{b}	0.81
4% Fat CMY	24.1^{c}	20.4^{a}	22.3^{b}	26.5^{d}	1.44
Composition (g/kg)					
Milk fat	40.2	41.5	42.3	43.5	1.64
Milk protein	32.4	31.1	31.5	31.7	0.43
Milk lactose	47.1	46.1	46.7	46.9	0.38
Yield (g/d)					
Milk fat	985^{b}	829^{a}	873^{a}	1037^{b}	49.7
Milk protein	792^{b}	620^{a}	651^{a}	758^{b}	26.3
Milk lactose	1151^{b}	933^{a}	977^{a}	1133^{b}	41.0

CMY: corrected milk yield

Conclusions This study shows that using a Setchey pea/wheat bi-crop as the basal part of the ration instead of grass silage, halved the concentrate requirement for dairy cows without adversely affecting milk yield or quality. Therefore pea/wheat bi-crops may be a viable cost-saving option for dairy farmers. The study also demonstrates and confirms the high intake characteristics of pea/wheat bi-crops and demonstrates that such forages can have similar nutritive values to good quality grass silage.

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Adesogan, T.A., Salawu, M.B. and Deaville, E.R. (2000) The effect of proportion of peas to wheat and harvest date on the voluntary feed intake, *in vivo* digestibility and nitrogen retention of pea-wheat bi-crop silages by sheep. *Proceedings of the Winter Meeting of the British Society of Animal Science, Scarborough*

The effect of feeding rations containing heat treated rapeseed meal, lupins and beans to lactating dairy cows on milk yield and quality

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Introduction The UK ruminant industry is currently reliant on soyabean meal and fishmeal as sources of high quality, digestible undegraded protein (DUP). However, there is increasing concern over the sustainability of fish stocks, and the world market price fluctuation and traceability of soya bean meal. There are a number of protein crops which will grow under UK conditions including sweet white lupins, peas, field beans and oilseeds (rapeseed meal and linseed meal). However, these proteins tend to be more rumen degradable than fish meal and soyabean meal (Moss and Givens, 1994). Heat moisture treatment has been shown to be particularly effective in reducing the rumen degradability of protein without reducing intestinal digestibility (Herland, 1996). This experiment was undertaken to determine the effect of feeding moist heat treated rapeseed meal, lupins and beans to dairy cows.

Material and Methods Sweet white lupins (*Lupinus albus*), field beans (*Vicia faba*) and solvent extracted rapeseed meal were obtained within the UK and heat treated at 120°C for 35 minutes in a batch process using steam. Five iso-energetic and iso nitrogenous (metabolisable energy = 12.3 MJ/kg dry matter (DM); crude protein = 190 g/kg DM) diets, based on grass silage wheat, sugar beet feed and rapeseed meal, were formulated. Each diet differed in the combination of protein sources to meet DUP requirements as follows: 21 g/kg DM fish meal and 41 g/kg DM soyabean meal (Control), 113 g/kg DM heat treated rapeseed meal (HR), 124 g/kg DM heat treated lupins (HL), 167 g/kg DM heat treated beans (HB) and a combination of these heat treated proteins (HC).

Sixty multiparous Holstein dairy cows in early lactation (mean 9 weeks *post partum*) were all fed a standard ration for the covariate week. The cows were blocked according to parity and days in milk, and fed one of the five experimental diets for eight weeks with milk yield (daily) and composition (fortnightly) monitored. Data were analysed using analysis of variance with treatment and week as factors, and data collected in the covariate week as the covariate.

Results Compared with the control diet, feeding rapeseed meal (HR), beans (HB) or a combination of proteins (HC) had no had no significant effect upon milk yield, milk protein content and yield or milk casein-nitrogen or non-protein nitrogen (NPN) content (Table 1). Inclusion of the heat treated lupins (HL) however led to a reduction in dry matter intake ($P < 0.05$), milk protein content ($P < 0.01$) and milk casein-N content ($P < 0.001$) compared with Control.

Table 1 Mean milk yield, composition and dry matter intake for the five dietary groups of cows.

Variable	Control	HR	HB	HL	HC	s.e.	P
Dry matter intake (kg/day)	19.3[a]	18.9	19.9	18.5[b]	19.9	0.37	*
Milk yield (kg/day)	33.0	32.9	33.4	33.9	33.2	0.71	ns
Protein yield (kg/day)	1.06	1.04	1.05	1.00	1.05	0.024	ns
Protein content (g/kg)	32.3[a]	32.1	31.6	30.6[b]	31.7	0.35	**
Milk casein-nitrogen content	4.0[a]	4.1	3.9	3.8[b]	4.0	0.05	***
Milk NPN content (mg/kg)	293	286	293	298	289	3.0	ns

[a,b] Means within a row with different superscripts to Control are significantly different to the Control.
Means adjusted for performance during the covariance period.

Conclusion Results indicate that fish meal and soyabean meal can be replaced with either heat treated rapeseed meal, heat treated beans or a combination of heat treated rapeseed meal, lupins and beans without any reduction in intake, milk yield or composition. There were also no adverse effects on milk non-protein nitrogen content. However, feeding heat treated lupins led to a reduction in dry matter intake, milk protein and casein-nitrogen concentration.

Acknowledgement This work was sponsored by HGCA, MAFF, MDC and the PGRO.

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The effect of fodder beet inclusion on milk production and nitrogen and energy utilization of grass silage based diets by lactating dairy cattle

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Introduction In a previous experiment with dairy cows at this Institute, in which fodder beet was included as a third component of the diet along with grass silage and concentrate, fodder beet considerably increased metabolisable energy intake (MEI), but there was little effect on milk yield *(unpublished data)*. The main objectives of the present study were to examine the effects of including fodder beet versus concentrate in a grass silage diet, on milk production, digestibility of nutrients and the utilization of nitrogen (N) and energy at equal ME intake in lactating dairy cattle.

Materials and methods A total of twelve Holstein-Friesian dairy cows were used (mean lactation stage 108.4d; s.d. 18.2) in a partially balanced changeover design with four periods (4 weeks per period). T1, T2, T3 and T4 each consisted of fodder beet, concentrate and grass silage at 0.5 beet plus soya mix, 0.2 concentrate and 0.3 grass silage ratio (DM basis), and T5 (offered to double the number of animals of each of T1-T4) comprised concentrate plus grass silage at a 70:30 concentrate:forage (C:F) ratio (DM basis). The concentrations of N in the DM were similar in all diets. The diets were presented to individual animals as follows: T1, offered to appetite; T2, 1.5kg DM/d below T1; T3, 3.0kg DM/d below T1; T4, 4.5kg DM/d below T1 and T5, offered at 2.25kg DM/d below T1. Three blocks were formed based on *ad libitum* intake. Ration digestibilities and energy exchange data using indirect open-circuit respiration calorimetry, were determined during the last 9 and 3d of both periods 2 and 4 respectively. The data were subjected to analysis of variance. Predicted output values for the fodder beet diet (T6) at the same MEI as the concentrate diet (T5), were obtained by interpolation. The estimated means were compared with the concentrate treatment by using a t-test to test for significance.

Results Predicted digestibility of dry matter (DM) and energy (E) were significantly higher ($P<0.01$ for both) for the beet diet at similar MEI to that recorded with the concentrate control diet (Table 1). However, predicted nitrogen (N) digestibility was significantly lower ($P<0.05$). Milk yield was significantly higher, but milk fat concentration was lower for T5 ($P<0.001$) resulting in a non-significant difference in total milk energy output between T5 and T6. Methane/GE intake was higher with the beet diet than the concentrate diet ($P<0.001$). While heat production was similar between T5 and T6, suggesting that source of ME had a minimal effect on the utilization of ME, the efficiency with which energy was used for milk production (k_l) was slightly higher for T5 than T6 ($P<0.05$). Differences in retained energy were not significant between T5 and T6.

Table 1 The effects of fodder beet inclusion on milk yield, milk composition and nutrient utilization

	Fodder beet treatments				Control		Predicted		Sig
	T1	T2	T3	T4	T5	s.e.d.	beet treat T6	s.e.d.	T6 vs Control T5
Digestibility									
Dry Matter	0.849	0.822	0.821	0.826	0.791	0.0132	0.823	0.0130	**
Nitrogen	0.773	0.732	0.724	0.728	0.755	0.0159	0.724	0.0165	*
Energy	0.838	0.812	0.805	0.814	0.776	0.0141	0.811	0.0140	**
ME intake (MJ/d)	225.6	198.5	175.5	159.9	184.6	5.31	184.6	-	-
Milk yield (kg/d)	23.47	21.91	21.59	21.04	24.60	0.800	21.74	0.626	***
Milk protein (g/kg)	33.5	33.61	31.57	31.53	32.53	0.859	32.71	0.834	NS
Milk fat (g/kg)	41.14	37.43	39.35	39.81	27.28	2.702	39.94	2.742	***
Milk lactose (g/kg)	48.38	49.24	48.96	49.39	49.37	0.454	48.97	0.302	NS
Milk energy (MJ/d)	75.59	66.66	65.06	65.06	65.17	3.388	68.03	3.470	NS
Methane energy/GEI	0.075	0.075	0.073	0.079	0.045	0.0060	0.075	0.0066	***
Urine energy/GEI	0.035	0.039	0.045	0.047	0.037	0.0060	0.042	0.0060	NS
Heat Production (MJ/d)	134.9	131.2	111.4	110.2	126.5	5.38	121.9	9.34	NS
k_l	0.534	0.538	0.567	0.607	0.529	0.0244	0.573	0.0264	*
Energy retention (MJ/d)	4.54	7.78	-6.89	-3.60	0.10	6.302	2.15	5.935	NS

Conclusions The results show an increase in the digestibility of DM and energy in the total diet with the inclusion of fodder beet along with concentrate, versus the inclusion of only concentrate in a diet with a basal forage of grass silage. Diet type had no effect on milk energy output. While diet type did not have a significant effect on heat production, k_l was significant higher with the fodder beet diet.

Milk production and N partitioning in early lactation dairy cows offered perennial ryegrass containing a high concentration of water soluble carbohydrates

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Introduction The efficiency of utilisation of grass nitrogen for milk protein production tends to be low, because rumen fermentable energy sources limit the amount of diet amino acids that are incorporated into microbial protein. As a consequence, absorption of ammonia from the rumen and excretion of high-N waste products is considerable. Previous studies (Miller *et al*., 1999) have shown that the efficiency of use of feed N can be increased in late-lactation dairy cows by feeding ryegrass bred to contain a high concentration of water soluble carbohydrates (WSC). The objective of this study was to investigate milk production and N partitioning in early lactation dairy cows using the same high WSC ryegrass variety, AberDove.

Material and methods Twelve multiparous Holstein-Friesian dairy cows in early lactation (mean of 42, s.e. 2.4, days of lactation) were used in a continuous design, zero-grazing experiment. Following covariate measurements taken from all animals on a standard grazing diet, six animals were each offered one of two varieties of perennial ryegrass at *ad libitum* rates: High Sugar (HS), a variety bred to express high WSC concentrations (cv. AberDove); and Control, a commercially available variety (cv. AberElan). The grasses were cut daily from five-week regrowth swards fertilised with about 62.5 kg N/ha prior to regrowth. The Control grass was cut at approximately 10:00 h and HS grass was cut at approximately 14:00 h to maximise WSC differences. Immediately following cutting, the grasses were chilled for 2 hours in a blast freezer and were then moved to a cold store at 4°C until being offered that evening and the following morning. In addition to the experimental forages, all animals received a standard dairy concentrate (225 g crude protein (CP)/kg dry matter (DM); 13.5 MJ metabolisable energy/kg DM), at a flat rate of 4 kg/d fed in two equal portions at each milking (08:00 h and 16:00 h). The first two weeks of the 20-day experiment were used for diet adaptation and the last six days were used for measurements of forage DM intake, milk yield and composition in all animals, and N partitioning and whole-tract diet DM digestibility in four animals of each treatment. Results were analysed using analysis of variance using a treatment structure of grass variety, with covariate adjustment for milk data.

Results The nutritional composition of the HS and Control grasses, as fed, was 202 and 167 g DM/kg, 236 and 152 g WSC/kg DM, 98.2 and 99.3 g CP/kg DM, 494 and 575 g neutral detergent fibre/kg DM. The low CP content of both grasses was probably due to the low rates of N fertiliser application. Animals offered the HS grass diet consumed significantly more grass DM than Control animals (Table 1). This, together with a small but statistically significant increase in the whole tract diet DM digestibility of the HS diet led to an increase in the digestible DM intake. Animals consuming the HS diet produced almost 25 kg milk/d more than Control animals. This difference was not significant, but milk protein yield (although low for both treatments) was significantly higher from HS cows. There were no differences in the yields of milk fat or lactose. Diet N intake was significantly increased on the HS diet, but the proportion of dietary N used for milk production was similar for both grasses. The gross efficiency of utilisation of feed N for milk production was very high for both diets, probably because of the low CP content of both grasses, but excretion of urinary N as a proportion of N intake was significantly lower from cows given the HS diet.

Table 1. *Mean treatment effects on feed intake, diet DM digestibility, milk production (covariate adjusted), and N partitioning.*

Grass Variety:	HS	Control	s.e.d.	Sig[†]
Forage DM intake, kg/d	15.3	13.1	0.78	*
Diet DM digestibility, g/g	0.75	0.72	0.01	*
Milk yield, kg/d	32.7	30.4	1.48	
Milk constituent concentrations, g/kg				
Fat	39.0	40.1	2.60	
Crude protein	26.0	26.8	0.77	
Lactose	48.1	48.0	0.87	
Milk constituent yields, g/d				
Fat	1272	1212	46.5	
Crude protein	866	757	39.2	*
Lactose	1573	1456	73.7	
N intake, g/d	362	320	11.9	*
N output, g/100 g N				
Faeces	37.8	38.4	0.01	
Urine	20.7	27.2	0.02	*
Milk	37.6	37.1	0.02	
Body (balance)	3.9	-2.7	0.03	+

[†] Significance of effect: + $P = 0.071$; * $P < 0.05$

Conclusion Animals offered the higher WSC grass consumed more, and produced more milk protein than those offered the conventional grass. While the efficiencies of utilisation of diet CP for milk production by cows on both diets were higher than normally achieved in dairy systems, the extra dietary WSC in the HS grass reduced urine N excretion and this would help decrease the environmental burden of the system.

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The effect of equi-molar dietary betaine and choline addition on performance and carcass quality of pigs

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Introduction Betaine has three chemically reactive methyl groups attached to the nitrogen atom of the glycine molecule. Therefore, it can be used as a methyl group donor partially to replace methionine in poultry and pig diets. Recent work also suggests that betaine has an energy sparing role by reducing maintenance requirement of the pig (Schrama and Gerrits, 2000). Betaine has improved performance and carcass leanness in some studies but the results are variable and seem to depend on age and sex of the animal, feeding level and diet composition.

Choline can also be used as methyl donor in animal feeds. In poultry, methyl groups are available after the conversion to betaine in the liver. However, dietary betaine is twice as efficient as the equi-molar dietary choline for increasing liver betaine levels in broiler chick (Saarinen *et al*., 2000). The aim of this study was to compare the response of pigs fed equi-molar betaine and choline in terms of pig performance and carcass characteristics.

Materials and methods A total of 70 individually penned Finnish Landrace and Yorkshire pigs (initial live weight 30 kg) were used. Five females and five castrated males were randomly allocated to seven groups. The experimental treatments were: control (basal diet) – no added betaine or choline, basal diet supplemented with 250, 500 or 1000 mg/kg of betaine (Betafin® S1) or with a similar molar amount of choline (578, 1155 or 2310 mg/kg of choline chloride, respectively). The basal, meal-form diet consisted of ingredients with low betaine content: maize (660 g/kg) and soyabean meal (213 g/kg), and its net energy content was diluted by oat hull meal (100 g/kg). The diet contained 8.55 MJ/kg NE, 155 g/kg crude protein and 7.4, 4.4 and 4.3 g/kg digestible lysine, threonine and methionine+cystine, respectively. The pigs were restrictedly fed, 1.5-3.0 kg feed/d. The experiment lasted 11 weeks (75 days). After slaughter, the lean percentage of the carcass, back and side fat thickness and the proportions of fat and lean+bones in the ham were measured. The data were submitted to a least square analysis of variance (SAS, GLM). The effects of treatment, sex and their interaction were included in the model. The linear, quadratic and cubic effects of dietary betaine or choline addition were tested by orthogonal polynomials.

Results Daily weight gain and feed conversion ratio of pigs improved linearly ($p<0.01$) with increasing dietary betaine (Table 1). Dietary choline additions had no effect on pig performance. The carcass weight of the pigs increased linearly ($p<0.01$) but the slaughter loss percentage, back and side fat thickness and lean percentages in ham and carcass were unaffected by dietary betaine level. Dietary choline additions had no effect on carcass characteristics. No interactions between treatment and sex were found. Growth and feed utilisation was similar in female and castrated male pigs but females had a higher carcass lean percentage (59.3 vs. 58.4, $p<0.01$).

Table 1 Effect of dietary betaine or choline addition on performance and carcass characteristics of pigs

Betaine, mg/kg	0	250	500	1000	0	0	0		
Choline choride (50%), mg/kg	0	0	0	0	578	1155	2310	s.e.‖	P (diet) ≤
Daily weight gain, g†	883	879	943	969	897	909	906	22.2	0.01
Feed conversion ratio, kg DM/kg†	2.44	2.45	2.31	2.27	2.42	2.38	2.41	0.05	NS
Feed consumption, kg DM/pig‡	161.1	160.9	163.2	164.0	161.9	161.4	163.1	1.42	NS
Lean in carcass, %	58.7	58.9	59.0	58.6	59.2	59.3	58.2	0.49	NS
Back fat, mm§	19.9	19.3	20.1	19.3	20.8	18.8	20.6	0.92	NS

†Linear effect of betaine ($p<0.01$) ‡Linear trend of betaine ($p=0.08$) §Carcass weight as a covariate ‖s.e. is presented for 9 observations

Conclusions The results indicated that dietary betaine addition improved the performance of pigs restrictedly fed diets with diluted energy concentration as found by Cromwell *et al.* (1999). A maximal response to betaine addition was not observed in this trial. However, betaine did not affect carcass composition, unlike the results of Cromwell *et al.* (1999) which indicated a reduction of back fat with betaine addition. Choline addition had no effect on performance and carcass characteristics. On an equi-molar basis it appears that pigs respond to betaine rather than choline supplementation, although more work is needed to clarify the mode of action.

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Methionine supplementation of whey globulin concentrate diets negatively affects weaner performance

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Introduction In a previous study (Slade and Miller, unpublished), weaners fed a protein source high in porcine immunoglobulin (Ig) outperformed contemporaries fed an isonutritious and amino acid balanced diet containing an Ig rich bovine protein source (WGC). Diet analysis indicated differences between the protein source contributions to total dietary methionine (Met); 21.5% and 12.5% of 5.2 g/kg total Met respectively for bovine and porcine materials. Hydrolysis of bovine IgG in the proximal and medial thirds of the weaner small intestine is limited (Morel et al. 1995) and Met absorption from the piglet large intestine is insignificant (Darragh *et al*., 1991). Small intestine failure to degrade bovine Ig would therefore limit dietary Met availability. This limitation may be further confounded by retention of dietary Cys within the disulphide bridges of non-degraded Ig thereby increasing metabolic demand for Met. The objective of this study was therefore to determine the effect of Met supplementation on the performance of weaners fed an isonutritious diets containing the same level WGC.

Materials and methods One hundred and twenty eight crossbred piglets (JSR Healthbred) were weaned from 16 mixed parity sows at a mean age of 26.0 ± 0.16 days (± SEM) and liveweight of 7.89 ± 0.14 kg. At weaning piglets were identified with an ear tag and housed in conventional, fully-slatted, flat-deck pens (1.99m^2) in groups of 8 each balanced for litter origin, weaning weight and gender profiles. Each group was allocated one of four dietary treatments differing only in Met content. DL-Met was added to a 17.5% WGC based weaner ration (16.5 MJ DE, 16.5 g lysine, 4.0 g Met per kg) to produce diets containing 4.0, 5.2, 6.5 and 7.8 g total Met per kg. Each treatment was replicated four times. Feed and water were provided *ad libitum* and one diet was fed throughout the trial. Daily feed disappearance (FI) and piglet liveweight on days 7, 14 and 20 following weaning were recorded. Data were analysed using the GLM and regression analysis procedures of Minitab 12.2.

Results The average performance of piglets by treatment is shown in Table 1. FI was numerically improved at the lowest and highest levels of Met inclusion in the diet however this was significant only during d 1-7 post-weaning. Although FCR differences were not significant, FCR was generally optimal at 5.2 g Met. Met supplementation significantly depressed LWG d 15-20 and overall although this response was not linear.

Table 1 *Feed intakes (FI, g/pig/day), daily liveweight gains (LWG, g/pig/day) and feed conversion ratio (FCR) of piglets by week and overall during the 20 day trial period.*

Dietary Met (%)	Days 1 -7			Days 8 - 14			Days 15 - 20			Days 1 - 20		
	FI	LWG	FCR	FI	LWG	FCR	FI	LWG	FCR	FI	LWG	FCR
0.40	202^{a}	200	0.99	401	326	1.25	567	437^{a}	1.30	381	315^{a}	1.14
0.52	170^{b}	155	1.03	337	326	1.06	506	384^{b}	1.30	329	283^{b}	1.10
0.65	168^{b}	167	1.03	349	324	1.09	515	356^{bc}	1.47	335	279^{b}	1.16
0.78	191^{a}	173	1.14	362	315	1.16	496	329^{c}	1.56	342	269^{b}	1.20
s.d.	8.0	13.5	0.07	24.1	16.0	0.08	22.4	15.9	0.08	15.0	9.5	0.04
Sig.	*	ns	ns	ns	ns	ns	ns	***	ns	ns	**	ns

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

Simple regression analysis indicated Met intake more accurately predicted d 15-20 and d 1-20 LWG than did FI per se (R^2= 31.4% vs 11.9% and 21.0% vs 8.1%). Inclusion of the quadratic term (x^2) in d 1-20 regression analysis indicated improvement in LWG beyond ≈ 2.6 g Met intake per day (R^2= 38.8%, *P<0.05*). Litter origin was observed to have a constant and highly significant influence on piglet LWG throughout the trial (*P<0.001*), however this effect was mediated by treatment d 8-14 (*P<0.05*).

Conclusions Growth performance of weaners fed diets containing 17.5% WGC was not compromised by sulphur amino acid availability, indeed our results indicate that dietary Met inclusion above 4.0 g/kg had a deleterious affect on LWG. This response appeared mediated by depression of FI and/or reduced feed utilisation efficiency that was not consistent with or proportional to Met intake. The apparently erratic response to Met supplementation may indicate temporal changes in weaner Met requirement. Similarly, the distinct litter:treatment differences in LWG might indicate Met sensitivity is mediated by piglet genotype and/or suckling period experiences.

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The effects of particle size and liquid feeding on the performance of young pigs offered mash and steam pelleted diets

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Introduction There is a well-established relationship between particle size of processed grains in diets and pig performance. It has been reported that as the particle size of feed is reduced there is an increase in the performance of pigs (Wondra *et al.*, 1995). Increasing the surface area of feed ingredients by reducing particle size facilitates greater enzyme activity. Liquid feed provides an excellent medium for endogenous enzyme activation and the opportunity to improve the availability of nutrients (Brooks *et al.*, 1996). The current study investigated the effect of particle size, processing and feed form on pig performance.

Materials and methods One hundred and twenty male, Large White x Landrace pigs, (weaned at 27d) were allocated by weight according to a 2 x 2 x 2 factorial design. The factors were feeding form (dry *vs*. liquid), processing method (meal *vs.* steam pelleted feed) and particle size (1200µm *vs.* 500µm). Pigs were adapted to their respective diets for 5 days before commencing the 21-day growth trial. Liquid diets were mixed in a ratio of 2.5 l water / kg DM at 25°C for 15hrs before feeding. Pelleted diets dispersed completely when mixed with water. A wheat-based (65%) diet was formulated containing 14.5 MJ DE and 0.85g available lysine per kg. A long chain alkane ($C_{36}H_{74}$) was added (200g/tonne) to the feed as a digestibility marker. Faecal samples were collected between day 13 to 15 of the trial for analysis of GE and hydrocarbon. Pig weight and feed intake was recorded at 0, 7,14 and 21 days. Statistical analyses were carried out using a multi-factor analysis of variance and least squares means. Covariance was used to remove the effects of any differences in start weight.

Results. In this study feeding diets differing substantially in particle size did not significantly effect the physical performance of the pig, nor were there any significant interactions between factors. Feeding the diet in liquid form significantly ($P<0.001$) improved feed intake, growth rate and 21 day weight and increased feed conversion ratio (FCR). Steam pelleting increased ($P<0.001$) growth rate and improved FCR, an additive effect on intake and growth rate was observed when steam pelleted diets were liquid fed. The best daily gains (516 g/d) were achieved by pigs on the treatments fed diets that had been steam pelleted prior to being presented to the pig in liquid form.

Table 1 Performance of male pigs between 8 and 20 kg liveweight (0-21 days)

	Particle size µm (PS)		Feed form (F)		Process (P)		SE_D	Significance[2]
	500	1200	Dry	Liquid	Meal	Pellet		
Start weight (kg)	7.9	7.9	7.9	7.9	7.8	8.0	0.18	NS
21-d weight (kg)	17.3	17.5	16.7	18.0	16.9	17.9	0.45	P*F**
Daily feed intake (g)	548	544	470	621	541	551	16.5	F***
Daily gain (g)	447	457	418	486	434	470	14.4	P**F***
FCR[1]	1.23	1.20	1.13	1.30	1.26	1.17	0.03	PF***

[1]100% DM basis; [2]***P≤0.001;**P≤0.01;*P≤0.05;NS P≥0.05

Conclusion. Improvement in gain as a result of steam pelleting appeared to have been a result of increased nutrient availability. In the case of feed form the improvement in gain when the diet was fed in liquid form rather than dry appeared to have resulted from a significant ($P<0.001$) increase in (apparent) feed intake; which was accompanied by a significantly ($P<0.001$) worse FCR. This suggests that there was considerable feed wastage by the pigs fed liquid diets. Consequently it is not possible from these data to determine whether nutrients were used any more or less efficiently when the feed was provided in liquid form. This would indicate that it is possible to take advantage of both, the improved digestibility of nutrients generally associated with the steam pelleting process, and the increased dry matter intake generally associated with liquid feeding. The study also highlights the difficulty of making meaningful comparisons of feed utilization when different treatments may affect both feed utilization and the extent of feed wastage.

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Performance of pigs post-weaning fed cereal-based diets with an enzyme complex added either before or after pelleting

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Introduction The responses of pigs to diets supplemented with enzymes have been variable (review by Partridge, 2000). The situation has not been eased due to an uncertainty over the usefulness of laboratory analyses of enzyme activity as a measure of performance. One issue has been the temperature of feed pelleting in which laboratory assays for apparent enzyme activity have shown very low levels compared with the known level included in the feed, whereas experimental data have indicated typical poultry responses from the enzyme-supplemented feed (Bedford and Pack, 1998). The experiment reported here studied the performance of pigs fed diets to which enzymes were added either pre- or post- pelleting (which was conducted at 85^0 C).

Materials and Methods Thirty, individually penned, commercial hybrid male and female pigs were used, with 10 pen replicates (5 of each sex) per treatment. The pigs were weaned at around 26 days of age; the trial started after a 4 day adjustment period (9 kg live weight) and was completed after each pig reached 27.5 kg live weight (or 42 days on trial). Pigs were allocated to 1 of 3 diets: either 1) control treatment – cereal based diets; 2) control treatment with 1 kg of a dry enzyme complex containing xylanase, β-glucanase and α-amylase having minimum guaranteed activities of 4000, 150 and 1000 U/g respectively (Porzyme® tp100G) added pre-pelleting; 3) control treatment with 1 kg Porzyme® tp100G added post-pelleting. All three treatments were offered *ad libitum* as a two phase feeding regime. Phase 1 was offered for the first 21 days and phase 2 from day 21 to completion. The diets were multi-ingredient (mainly wheat, barley, cooked wheat and soyabean meal) and contained 14.3 and 14.0 MJ/kg DE and 11.4 and 10.5 g/kg digestible lysine (phases 1 and 2 respectively). The ingredients were coarsely ground (4 mm sieve), mixed, pelleted at 85°C and crumbled. Enzyme was added during mixing for treatment 2 (pre-pelleting) and during crumbling for treatment 3 (post-pelleting) by Roslin Nutrition Ltd. Live weight gain was calculated as the linear slope of the response of live weight (recorded weekly in kgs) to time (in days) using GENSTAT 5.3 for Windows. This analysis was initially conducted over the entire duration of the trial. Solving the linear equations for each piglet for a live weight of 7.5 and 27.5kg allowed a precise estimate of the initial and final day on trial. This then allowed, if necessary, an adjustment to recorded food intake to give the actual amount of food required to grow from 7.5 to 27.5kg.

Results Apparent enzyme activity was reduced by 89 and 69% (xylanase), 82 and 60% (β-glucanase) ('Megazyme ') and 41 and 10% (? -amylase) ('Pharmacia') for phases 1 and 2 respectively when comparing laboratory analyses of treatment 2 before and after pelleting. Treatment 3 enzymes, added post-pelleting, were either above or within 10% of minimum guaranteed activity except one α-amylase measurement which was low. Treatments 2 and 3 were better than treatment 1 (control) for daily live-weight gain and food conversion ratio (at least $P < 0.01$, table 1). Pigs on treatment 1 consumed significantly ($P < 0.001$) more food than those on treatments 2 and 3 overall, but total food intake per pig on treatment 1 was lower for the first 21 days (12.2, 14.3 14.6, sed 0.92, kg; $P < 0.05$) compared with the enzyme-supplemented treatments 2 and 3 respectively. Daily live-weight gain and food conversion ratio were also worse ($P < 0.001$) over the first 21 days for the control treatment 1, after which no differences in the treatment responses were observed.

Table 1 Effect of treatment on performance of post-weaning pigs over the complete experimental period

Treatment	Control (1)	Enzyme added pre-pelleting (2)	Enzyme added post-pelleting (3)	s.e.d.	P value
Daily live-weight gain (kg)	0.510^a	0.582^b	0.585^b	0.022	0.003
Food conversion ratio	1.78^a	1.56^b	1.48^b	0.142	<0.001
Total food intake/pig (kg)	35.6^a	31.1^b	29.6^b	1.38	<0.001

Means in rows with different superscripts are statistically significantly different ($P< 0.05$).

Conclusions The main responses of pigs to supplementation with the enzyme complex Porzyme® tp100G occurred during the first 21 days after weaning for both pre- and post- pelleting treatments and the differences were maintained throughout the entire experimental period. The results indicated that laboratory measurements of enzyme recovery were not related to assessments of enzyme activity as measured by pig performance, and that piglets responded similarly to supplementary dietary enzymes whether added to feeds pre- or post- pelleting (85°C pelleting). It has been postulated that this might be due to enzyme activity during the conditioning process (Silversides and Bedford, 1999).

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Effect of protein nutrition on bone strength and incidence of osteochondrosis in gilts

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Introduction Leg-weakness in female breeding pigs has serious implications both in terms of economics and welfare, accounting for up to 0.2 of first litter gilt cullings at an estimated cost to the UK pig industry of up to £3.0 million (Douglas, 1992), with the principal predisposing cause in the growing pig being osteochondrosis. It has been reported that selection for increased lean tissue growth rate and increased growth rate accompanied by *ad libitum* feeding of diets with high energy and nutrient concentration may increase the incidence of osteochondrosis and leg- weakness (Jorgensen and Sorensen, 1998), although evidence is largely equivocal. The effect of protein level in the diet is uncertain. Although both high and low levels of dietary protein lead to bone growth failure and fracture in human studies (Reid and New, 1997), protein level has not been found to affect osteochondrosis in pigs (Jorgensen, 1995), thus the aim of the current study was to assess further the effect of protein nutrition on leg weakness in gilts.

Materials and Methods Two diet series based on Wheat, Barley, Soya, Fishmeal and Premix were formulated; Series 1 to meet the nutrient requirements for maximal protein deposition rate and series 2 for a protein deposition rate 0.8 of maximal. 8 x 50 kg commercial white hybrid gilts were allocated to one of four dietary treatments (see table 1b), with dietary cross-overs of group 3 and 4 taking place on week 6. Feed allowances were adjusted weekly as determined by dietary treatment (diet 1 being fed to previously obtained feed intake data for the genotype when achieving maximal protein deposition and diet 2 being fed according to assumed live weight using the function $FI = 0.01W^{0.75}$). Blood samples were taken from 4 gilts per treatment at 3rd oestrus in order to measure serum calcium and Alkaline phosphatase (ALP) activity. Gilts were slaughtered 12 days post 3rd oestrus. The 3rd metatarsal bones were removed from the left leg, and scored on a 5 point system for severity of osteochondritic lesions at the proximal and distal (ventral medial and lateral, and caudal medial and lateral) cartilage surfaces. The bones were then measured and mechanically evaluated (3 point bending test) using a Hounsfield tester.

Table 1a *Nutrient specification of diet series*

Diet	1A	1B	1C	2A	2B	2C
Week of Trial	1 to 4	5 to 8	9 to 12	1 to 4	5 to 8	9 to 12
CP (g/kg)	200	167	147	169	118	100
DE (g/kg)	14.6	14.4	14.2	13.3	13.3	13.1
LYS (g/kg)	12.7	10.8	9.5	10.6	7.5	6.4
Ca (g/kg)	5.5	5.7	5.5	7.2	7.6	9.2
P (g/kg)	4.5	4.5	4.5	5.2	6.2	4.5

Table 1b*Experimental design*

Group 1	Group 2	Group 3	Group 4
Diet 1	Diet 2	Diet 1/2	Diet 2/1

Results and Discussion The pattern of protein nutrition had no significant effects on bone mineralisation of the 3rd metatarsals as assessed by bone strength and mineral characteristics (Table 2). This is further supported by there being no significant differences in serum calcium or ALP activity, showing that the higher protein diet is not interfering with mineral absorption and that there is no apparent effect on bone re-modelling rate.

Table 2 *Effect of dietary treatment on 3rd metatarsal characteristics*

Variate	Group 1	Group 2	Group 3	Group 4	SED	Fpr
Bending moment (kg-cm)	139.3	142.8	143.9	142.1	11.6	0.98
Ash content of bone (g/kg)	530	575	585	588	4.0	0.46
Phosphorus content of bone (g/kg)	10	94	11.0	9.31	4.1	0.36
Lesion score (average of all sites)	1.5	1.2	1.7	1.7	0.2	0.03
Serum Calcium (mg/ dL)	9.2	9.7	9.55	9.08	0.5	0.59
Serum ALP activity (u/L)	118.8	101.9	107.1	91.6	15.0	0.37

There were significant effects of dietary treatment (Fpr = 0.033) and site at which lesions occurred (Fpr < 0.001) on osteochondritic lesion scores, suggesting that lower protein diets may be beneficial and periods of restriction and flushing may be detrimental to cartilage soundness, whilst maximal load bearing occurs on the distal caudal lateral surface of the bone . However, as there was no effect of treatment on growth rate and given that all gilts examined had mild to moderate osteochondritic lesions, there may be underlying genetic and conformational causes, thus further investigations are required before any firm conclusions may be drawn

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Do rabbits pose a risk of Johne's disease to grazing cattle?

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Introduction Paratuberculosis (Johne's disease) is a chronic invariably fatal enteritis of cattle caused by the bacterium *Mycobacterium avium* subspecies *paratuberculosis* and has recently been isolated from wild rabbits. One potential route of transmission of *M.a.paratuberculosis* from rabbits to cattle is the ingestion of rabbit excreta contaminating pasture. Here we (1) determine the prevalence and level of infection in rabbits and their excreta (2) quantify the level of rabbit faeces contaminating cattle pastures and (3) determine the impact of rabbit faeces on cattle grazing behaviour.

Materials and methods 83 rabbits were sampled from four study farms in east Scotland with a history of *M.a.paratuberculosis* in cattle and rabbits. Smears of lymph node, intestine and faeces were examined for acid fast bacilli (AFB). AFB positive samples were cultured and isolates of *M.a.paratuberculosis* identified using a polymerase chain reaction (PCR). Where possible urine was collected from rabbits and tested for *M.a.paratuberculosis* as above. Serial dilutions of faeces from infected rabbits were cultured to determine the level of infection present. The number of faeces present on grazing pasture at any given time (standing crop of faeces) and the rate of rabbit faeces deposition (over 6 weeks monitoring) on the 4 farms were estimated in winter (Nov-Feb) 1998 by random stratified quadrat sampling. The grazing behaviour of a herd of 57, one year old Friesian/Simmental cross heifers was monitored for eight field rotations in 1999 on one of the 4 study farms. Active transponder remote monitoring systems in conjunction with stratified surveys of sward height were used to quantify the grazing behaviour of the herd in relation to areas of sward contaminated with rabbit faeces. Prior to grazing each field, 4 sward treatments were created by contaminating 40 sward plots (0.5m x 0.5m) with 0 (control), 10, 50 or 250 rabbit faeces. 10 sward surface heights were taken every other day from the day of cattle release into the field throughout the grazing period. Active transponders monitored the behaviour of all cattle at a control and a 250 faeces plot in each field rotation.

Results *M.a.paratuberculosis* was isolated from rabbits on all four study farms with an overall prevalence of 14/83. Out of a sub-sample of 17 rabbits for which urine was available, *M.a.paratuberculosis* was isolated from 2 rabbits from different study farms. Infected rabbit faeces contained $1.6x10^4 \pm 1.4x10^4$ colony forming units (cfu) of *M.a.paratuberculosis* per gram of faeces (n=7). The mean number of faeces deposited by adult rabbits on pasture was 7357±2571 faeces/ha/day and the log-backtransformed (95% confidence interval) winter standing crop of rabbit faeces on pasture was 81000 (75600-86800) faeces/ha. Given the mass of a rabbit faeces pellet as 0.18g, a single infected faeces pellet carried a mean of $2.9x10^3$ cfu and rabbits contributed 3.6 million cfu of *M.a.paratuberculosis* /ha/day. These estimates of contamination by rabbits represent yearly minima by over-wintering pre-breeding adult populations. During the monitored grazing year, grazing pressure was low (maximizing the probability of cattle avoiding faeces contaminated swards) with a net mean sward off-take (based on sward height) of 18% per rotation. There were no significant differences between rabbit faeces treatments (0, 10, 50 & 250 faeces) with respect to the height (Figure 1) or proportion of sward removed, or between the numbers of contacts made by cattle on contaminated (250 faeces/plot) and non-contaminated (control) plots. 54/57 cattle were recorded on contaminated transponder plots with a total of 738 contacts over the 7 monitored rotations.

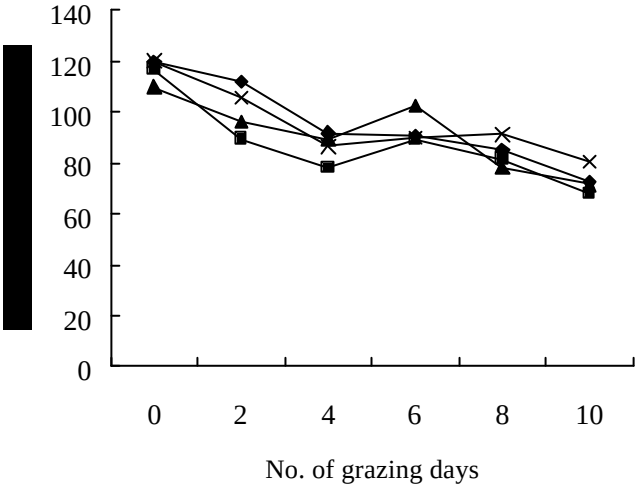

Figure 1. Sward depletion of grazing rotation 2 (4.92 ha of permanent pasture). Diamonds=0, squares=10, triangles=50 & crosses=250 faeces per plot respectively. SED= 20.5.

Conclusions The experimental doses of *M.a.paratuberculosis* needed to produce disease in domestic ruminants range from 10^3 to 10^9 organisms and so the ingestion of a few rabbit faeces may constitute an infective dose for cattle. This is the first reported incidence of lack of avoidance by grazing cattle towards swards contaminated with faeces of a wildlife species. The combination of high prevalence and levels of infection in rabbits and their faeces (Obj. 1), high levels of faeces contamination by rabbits of pastures grazed by cattle (Obj. 2) and the lack of avoidance by cattle to grazing rabbit faeces (Obj. 3), suggests that rabbits represent a significant risk of *M.a.paratuberculosis* to grazing cattle.

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Consequences of adding condensed tannins to low and high protein foods for parasitised sheep

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Introduction The consumption of condensed tannins has been associated with reduced faecal egg counts (FEC) and total worm burdens (TWB) in parasitised sheep. This can result from either a direct anthelmintic effect (Athanasiadou *et al*., 2000) and/or an indirect nutritional effect. Condensed tannins can protect dietary protein from rumen degradation and increase protein availability in the small intestine of the host; this could improve the expression of immunity towards parasites (Coop and Kyriazakis, 1999). The objective of the present study was to investigate the consequences of including a condensed tannin extract in foods of different protein content on the performance and development of immunity in parasitised sheep, during the phases of acquisition and expression of immunity.

Materials and Methods Forty-eight, parasite naive, three-month-old Texel × Scottish Greyface sheep (mean liveweight 32.2 kg, sd: 5.23) were housed individually and dosed with 2,000 infective larvae of the intestinal nematode *T. colubriformis*/day. Sheep were allocated to four groups (n=12) and were offered *ad libitum* one of four isoenergetic (10 MJ/kg fresh matter) experimental foods: a low protein food (L, 70 g crude protein/kg fresh matter), a high protein food (H, 145 g crude protein/kg fresh matter) and the L and H foods supplemented with 60 g/kg fresh matter condensed tannin (Quebracho) extract (LQ and HQ respectively). The experiment lasted 10 weeks and was divided into two periods: weeks 1-6, period of high worm establishment and acquisition of immunity (P_1) and weeks 7-10, period of established infection and beginning of expression of immunity (P_2). Six sheep from each group were slaughtered at the end of each period, i.e. weeks 6 and 10. Daily food intake and weekly faecal egg counts (FEC) were analysed by repeated measurements. Total worm burdens (TWB) were analysed by two-way ANOVA, with protein and Quebracho inclusion as factors. FEC and worm burdens were transformed (log (x+1)) prior to statistical analysis.

Results Food intake of sheep offered the HQ food was higher than in sheep offered the H food (mean food intake: 2387 vs 1835 g/day, respectively; sed: 153, $P<0.05$), whereas food intake of sheep offered the LQ food was not different from intake of sheep offered the L food (mean food intake: 1268 vs 1290 g/day, respectively; sed: 153, Fig 1). Sheep on the HQ food had higher liveweight gain compared to sheep on the H food (242 vs 191 g/day, sed: 24.6, $P<0.05$), whereas sheep on the LQ food had lower liveweight gain compared to sheep on the L food (52 vs 90 g/day, sed: 24.6). FEC of sheep offered the Quebracho-containing foods were reduced compared to sheep offered the Quebracho-free foods but only up to week 6 ($P<0.05$). Worm burdens were similar between groups at week 6, but at week 10 sheep offered the L foods ($P<0.05$) and the Quebracho-free foods ($P<0.01$) had lower worm burdens compared to sheep offered the H foods and Quebracho-containing foods (Fig 2).

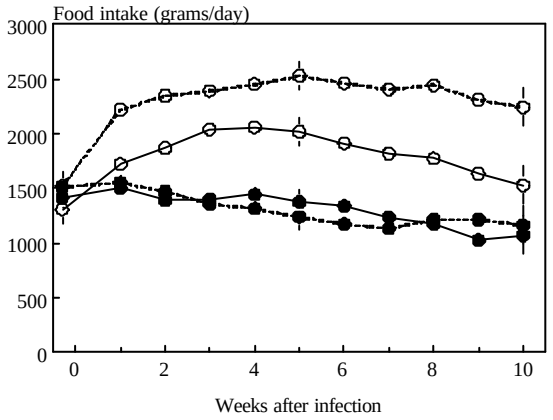

Fig 1. Food intake for sheep offered L (●) or H (○) protein foods supplemented (– –) or not ( ) with Quebracho extract over the 10-week experimental period. The bars indicate standard errors.

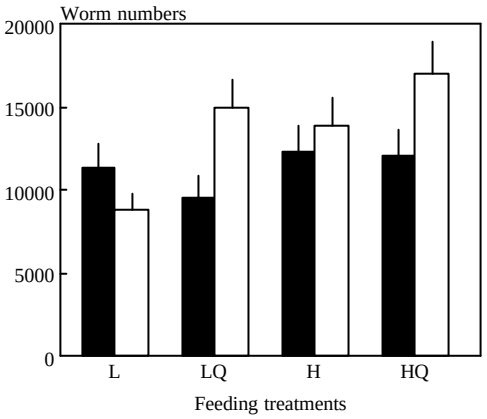

Fig 2. Backtransformed means of total worm burdens for week 6 (■) and 10 (□) of the experiment, with the upper limits of 95% confidence intervals.

Conclusions The results do not support the hypothesis that supplementation of a low protein food with condensed tannins may improve the performance and the development of immunity of parasitised sheep. Low protein food may impair the performance of the host, but might cause negative effects on parasites too. During the acquisition and the expression of immunity, condensed tannins supplementation enhanced the performance of sheep offered the high protein food only. The lack of a clear direct anthelmintic effect of condensed tannins could be due to the *ad libitum* feeding, which might have resulted in reduced concentration of condensed tannins over time in the digestive tract.

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Body protein reserves affect periparturient breakdown of immunity to nematodes in ewes

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Introduction Body reserves can be regarded as sources of nutrients at times of scarce nutrient intake. It has been suggested that the latter is responsible for the periparturient breakdown in immunity to parasites (BIP), since most scarce nutrients are expected to be allocated to the prioritised reproductive effort (Coop and Kyriazakis, 1999). Nutrient supply from body reserves may partly overcome scarce nutrient intake and, as a consequence, can be expected to affect BIP. It has been shown that an increased supply of dietary metabolizable protein (MP), but not of metabolizable energy (ME), reduces periparturient BIP (Donaldson *et al*., 1998). Therefore, we tested the hypotheses that body protein reserves, but not body fat reserves, affect BIP and its relationship with dietary MP supply in periparturient ewes.

Materials and Methods Sixty twin-bearing ewes were trickle infected with *Teladorsagia circumcincta* (10,000 infective larvae per day, three days per week) from day$_{-65}$, relative to expected parturition, onwards. Body protein- and fat reserves (assessed via ultrasound muscle- and back fat depth measurements) were manipulated during mid-pregnancy (day$_{-65}$ to day$_{-21}$) through feeding of different allowances of MP and ME. One of three feeds, calculated to either maintain body reserves (HH), or maintain body protein and lose body fat (HL), or lose both body protein and body fat (LL), were fed to 20 ewes each. During the periparturient period (day$_{-21}$ to day$_{42}$), one of two iso-energetic feeds, calculated to provide either scarce (LP) or adequate (HP) amounts of MP, was offered to 10 ewes each of groups HH, HL, and LL. Lambs were weighed at birth and weekly thereafter. Faecal egg counts (FEC, in eggs per gram faeces, epg) were assessed from day$_{-49}$ onwards as an indicator for immunity to gastrointestinal parasites, and were transformed according to log (FEC+1) prior to statistical analysis (ANOVA using a 3×2 factorial design with repeated measurements).

Results The muscle- and back fat depth of the HH-, HL- and LL-ewes was 26.9, 26.5 and 23.2 mm (S.E.D. 0.7, $P < 0.01$) and 7.8, 6.2 and 5.6 mm (S.E.D 0.3, $P < 0.001$), respectively, by the end of mid-pregnancy. The mid-pregnancy feeding treatments did not affect lamb birth weight, whilst lambs of HP- and LP-ewes grew at 317 and 258 g/day, respectively (S.E.D. 29, $P < 0.001$). Figure 1 shows the backtransformed means of the ewe FEC with 95% confidence intervals. The LL-ewes had higher FEC than HH- and HL-ewes by the end of mid-pregnancy ($P < 0.01$). Across feeding treatments and during lactation, LL-ewes had higher FEC than HH- and HL-ewes ($P < 0.01$) and LP-ewes had higher FEC than HP-ewes ($P < 0.05$). However, time, mid-pregnancy feeding treatments and periparturient feeding treatments significantly interacted; LL-ewes offered the LP feed had higher FEC than ewes for any of the combinations of feeding treatments, but only until 28 days into lactation (Figure 1). FEC of all ewes converged by the end of the experiment.

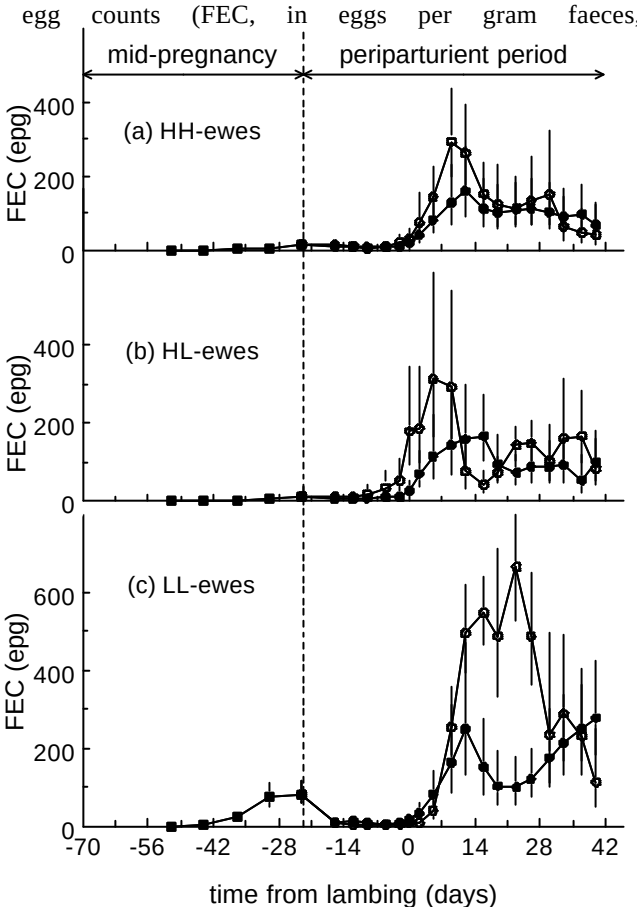

Figure 1. FEC of ewes offered a scarce (LP, ○) or adequate (HP, ●) allowance of MP during the periparturient period, following mid-pregnancy feeding strategies to manipulate body reserves

Conclusion This study supports the view that body protein- but not body fat reserves affect expression of immunity to gastrointestinal nematodes, and that body protein reserves play a role in overcoming effects of dietary MP scarcity on periparturient breakdown of immunity. The results suggest that a large proportion of the nematode eggs excreted onto the pasture could come from periparturient ewes with compromised body protein reserves. An improved protein nutrition targeting these ewes in particular would not only enhance pre-weaning lamb growth, but also lower pasture infectivity and dependency on anthelmintics for parasite control in young grazing lambs.

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Estimates of genetic parameters for test day somatic cell count fitting orthogonal polynomials

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Introduction Genetic evaluations in the United Kingdom (UK) for Somatic Cell Count (SCC) are currently based on a single trait repeatability model using the first five lactations. Only cows with completed lactations are included. However, to utilise information from cows with part lactation records and to achieve a better correction for environmental effects, a test day model (TDM) evaluation would be preferred. The objective of this study is to estimate genetic parameters needed for such a TDM evaluation by utilising a random regression (RR) approach.

Materials and methods Two data sets (Data I and Data II) of $\log_e$ transformed first lactation SCC for Holstein /Friesian heifers calving since 1992 were analysed. Data I consisted of 16205 heifers from 122 herds with a minimum of 190 cows per herd, which were daughters of 501 sires. Data II consisted of 24000 heifers from 228 herds, smaller in size (110-135 cows) and sired by 571 bulls. All heifers were required to have 10 test days and sires a minimum of 10 daughters. Both data sets were analysed with a sire RR model fitting herd-test-day, age at calving, orthogonal polynomials of order 5 as fixed lactation curves within season and orthogonal polynomials of order 3 for both random sire and permanent environmental effects. ASREML (Gilmour *et al*, 1999) was used for the two analyses but could not analyse both data sets together. The combined data sets (Data III) were analysed using Gibbs sampling with the sire RR model but in addition, fitting an animal RR model. Genetic parameters were generated from the RR coefficients for all analyses.

Results The parameters from the analyses of Data I and Data II were so similar that only estimates from Data II are presented. Figure 1 shows the daily heritability (h^2) estimates for SCC from Data II and Data III. The daily h^2 values from all the analyses were similar and generally about 0.06 at the beginning of lactation, increasing to 0.10 towards the end of lactation. The h^2 value for completed lactation SCC (1-305 days) was 0.16 for the sire models fitted and 0.18 for the animal model. These estimates are higher than the value of 0.12 for first lactation from an animal model using lactation average (Mrode *et al*, 1998) on UK data.

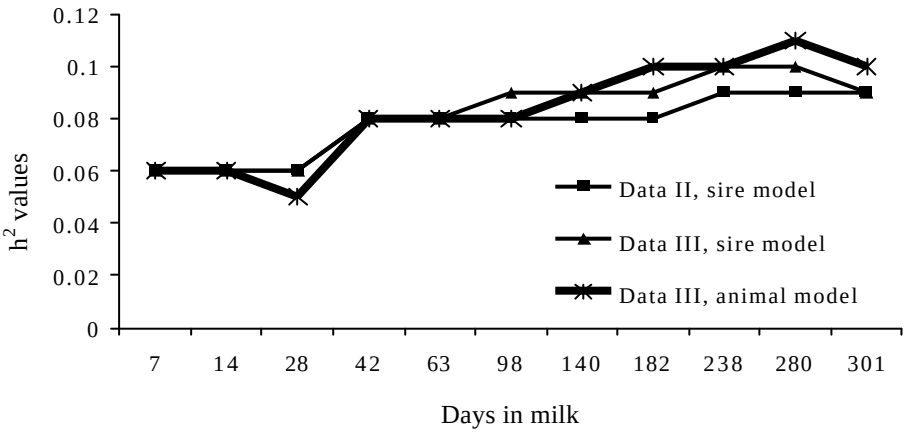

Figure 1 Estimates of heritabilities for SCC at different days in milk

Daily estimates of the variance of permanent environment (from Data III) expressed as a ratio of daily total phenotypic variance increased from 0.37 at the beginning of lactation (day 7) to 0.54 at end of lactation (day 305) from the sire RR model. Similar estimates for the animal RR model from Data III followed the same pattern but were slightly lower. Genetic correlations (r_g) between SCC at different days in milk (DIM) were again similar for all the analyses. The r_g were high between SCC in adjacent DIM, varying from 0.99 (between days 7 and 14) to 0.97 (between days 43 and 63). These r_g declined steadily as the interval between SCC test days increases. The lowest r_g was 0.33 between SCC at days 7 and 301 from the animal model.

Conclusion The results indicate that daily h^2 for SCC were low (0.06 to 0.10) but h^2 from the completed lactations from the random regression model (0.16 to 0.18) were higher than those estimated from lactation average. This should lead to more accurate evaluations. There were no major differences between parameters estimated from a sire or animal random regression model, although h^2 was slightly higher from the animal random regression model.

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The effect of genetic selection for lack of aggression towards humans on male reproductive physiology in the silver fox

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Introduction Most of silver foxes bred in captivity show predominantly defensive responses to human contact. Since early captive breeding humans have unconsciously carried out selection for tameness. Thus, artificial selection for the absence of aggressive and fearful responses to humans most likely played a key role in domestication process. To establish the genetic and physiological mechanisms of the early evolution of domestic animals, a model of silver fox domestication was developed at the Institute of Cytology and Genetics (Novosibirsk, Russia). A population of silver foxes has been produced in long-term selection for lack of aggression and fear towards humans (domestic behaviour). The method of selection and the behavioural changes in the course of selection have been described (Belyaev, 1979; Trut, 1995). Selected animals show no aggressiveness to humans and they are better adapted to captive conditions than their wild counterparts. The purpose of this study was to determine how behaviour selection influences testicular function in silver fox males. Plasma concentrations and testicular production of testosterone were determined in selected and control males in different parts of the reproductive cycle and prenatal life. In addition, reproductive behaviour and hormonal responses to opposite sex were estimated in the males of both behaviour groups. Sperm production and sexual activity were also compared between selected and control males.

Materials and methods
Testicular function in the silver fox (*Vulpes vulpes*) undergoes an annual cycle of characteristic changes in both steroidogenic and spermatogenic activity. To compare testicular steroidogenic activity in selected (S) and control (C) males, animals were killed before (in early December) and at the end (in the middle of March) of the breeding season. Each group consists of 28-41 animals. Body and testes weights were recorded and blood was collected. The portions of minced testicular tissue were incubated in Eagle's medium and incubates were used for testosterone assay. Sperm was collected from 29 control and 29 selected males during the expected peak of male sexual activity (February) by digital manipulation and examined for total number of spermatozoa and sperm defects. Reproductive performance (number of vixens bred, litter size and lasting of mating season) was recorded for 423 control and 305 selected males of different ages. During socio-sexual interactions, silver fox males (6 control and 19 selected) were placed with individual females in different parts of the reproductive cycle and the sexual and aggressive behaviours were recorded. The blood was taken from the males before and after the exposure to females and plasma samples were assayed for testosterone and oestradiol. To compare fetal testicular steroidogenesis in selected and control animals, serum concentration, testicular content and *in vitro* production of testosterone were studied in silver fox fetuses (from 59 control and 50 selected pregnant females) on days 35, 40, 45 and 50 of prenatal life. RIA was used for steroid hormone determinations.

Results At the end of the breeding season the plasma testosterone concentration (C: 1.07±0.20 ng/ml, S: 0.71±0.11, $P<0.05$) as well as the testicular testosterone production (C: 7.4±0.6 ng/100 mg/hour, S: 5.9±0.3, $P<0.05$) were lower in selected animals than in control although there were no differences in both parameters before the breeding season. The total number of spermatozoa in ejaculate was lower (C: 178.0±26.5 million; S: 79.8±11.8, $P<0.05$) and a proportion of abnormal spermatozoa was higher (C: 0.16±0.02, S: 0.23±0.03, $P<0.05$) in selected group as compared with control. Selected males mated with a smaller number of females (C: 8.3±0.5, S: 5.5±0.3, $P<0.05$) and their breeding period was significantly shorter compared with that of control (C: 35.0±1.4 days, S: 25.3±1.4, $P<0.05$) but fertility did not differ between the behaviour groups. During socio-sexual interactions selected males showed higher aggressiveness towards an anestrus female (C: 6.8±1.7 acts/hour, S: 15.0±2.0, $P<0.05$) and less sexual activity towards a receptive female (C: 12.5±5.4 mounts/hour, S: 2.3±0.5, $P<0.05$) in comparison to control. Both groups of males responded to the introduction of anestrus female with a significant increase in the plasma testosterone and oestradiol concentrations being lower in selected compared to control animals while the increased testosterone concentration after the exposure to a receptive female occurred only in selected males. During fetal life the serum concentrations, testicular contents and *in vitro* baseline production of testosterone did not differ between selected and control animals. When the fetal testes were stimulated by human chorionic gonadotrophin (hCG) *in vitro*, the hCG response appeared earlier in selected group than in control suggesting a timing shift in prenatal maturation of the pituitary-testicular axis.

Conclusions The data obtained support the idea that selection for domestic behaviour can bring about for a short time period a considerable destabilisation in the pituitary-testicular axis. At present time various wild species bred in captivity can be unconsciously selected for nonagression to humans, and this selection ultimately will provoke related alterations in male reproductive physiology.

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The influence of positive human-animal interaction during rearing on the welfare and subsequent production of the dairy heifer

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Introduction Anthrozoology (the study of human-animal interactions) is proving that human interactions with animals can have a severe impact on their welfare, production and ease of management; and can therefore have a substantial impact on productivity of a livestock unit. Recent research on dairy cattle shows how the quality of human-animal interactions has a potentially greater effect than with other intensively farmed species, due to the extended duration, intensity and frequency of contact. Early work by Bouissou and Boissy (1988) imposed various positive handling treatments on young heifers to determine the most sensitive time to socialise cattle to humans and investigate subsequent ease of handling; it revealed that the benefits of handling decreased with time, it was harder to establish a positive relationship with older animals but the work did monitor the heifers upon entry to the milking herd. Our research is designed to bring together techniques and hypotheses of previous pioneering work and carrying it to its commercially important conclusion when the dairy heifer calves, enters the herd and commences her productive milking life. The aim is to investigate whether the extent and nature of human contact during the rearing period has an effect on heifer welfare and subsequent production.

Materials and Methods 66 Holstein-Freisain heifers were allocated to one of two rearing treatments on one of the two University Farms for 3 months prior to calving (21-24months of age), i.e. 4 groups of 16/17. The control groups were exposed to minimal human interaction, only that associated with routine husbandry. The treatment groups received positive human-animal interaction consisting of additional handling where the experimenter brushed the heifer on her head, neck and shoulders and talked to her, activities which have been successful in building positive fear reducing relationships in other work with cattle (Bouissou and Boissy 1988). The allocation to treatment group ensured an even distribution for fear of humans (based on approach tests), weighted for dam and sire's temperament, balanced for £PIN 95 (predicted profit index) and liveweight. The heifers were managed in their treatment group with the positive group visited weekly during the 3 treatment months, targeting each heifer for 5 minutes. After calving a range of behavioural measurements were taken in direct association with the milking routine (flinch step [FS] & flinch, step, kick [FSK] in human presence at various stages of the milking process etc.) and production data (milk yield per day, milk let down, milk flow rate etc.) recorded during 40 observations over the first 20 weeks of lactation.

Results The analysis shows significant differences between rearing treatments, in favour of the positive group, in some of the areas studied.

Table 1: Paired t-tests for various parameters over the first 20 weeks

Milk Let down (secs)	Mean	Std. Error	Significance
Positive	11.6	0.37	P < 0.001 ***
Control	13.4	0.60	
FS & FSK at Udder Handling/milking			
Positive	0.7	0.12	P < 0.001 ***
Control	1.5	0.22	

Milk Flow (ml/sec)	Mean	Std. Error	Significance
Positive	30.4	0.82	P = 0.012*
Control	28.8	0.64	
Milk yield litres per day			
Positive	23.59	0.75	NS
Control	23.47	0.65	

The positive group kicked less overall in the parlour, both in the presence of a human and during milking. This has many beneficial commercial and welfare (both heifer and milker) implications. The positive treatment heifers were less fearful in the presence of humans, less disruptive to the milking routine, had reduced chance of injury etc. Milk let down implied the positive group were less stressed (an improvement in welfare) and as a result milked out quicker which is important for cow throughput, also longevity, as slow milking and belligerence can be a major factor in the premature culling decision.

Conclusions 3 months of positive treatment prior to calving has been shown to improve some of the behaviours that contribute to preferred milking temperament, which are also associated with low fear response to human presence, and indicate improved welfare. A second study is underway to investigate whether the extent of positive treatment can further influence these and other parameters. Minimal treatment reflects the majority of commercial rearing systems. Most interactions between human and animal in normal husbandry are actually aversive, reinforcing their innate fear of humans. It is the proportion and quality of interactions in relation to the total physical interactions that determine the animals'overall experience of the treatment. Supporting our work is a commercial study to highlight trends in heifer management and look for feasible ways of implementing positive experiences on our nation's dairy farms.

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The effect of positive and negative handling on the behaviour and stress response of Holstein Friesian heifers

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Introduction Extensive research into human-animal interactions, particularly in the pig industry, has led to the proposal that high fear of humans, through a stress response, can limit an animal's growth, reproduction and welfare (Hemsworth *et al*., 1993). In a recent study in the Australian dairy industry, a significant negative between-farm correlation was found between the avoidance of an experimenter by cows in a standard test and milk yield of the farm, suggesting that fear of humans may also have implications for the productivity of dairy cows (Breuer *et al*., 2000). The behaviour of stockpeople is a likely factor affecting fear of humans in dairy cows (Breuer *et al*., 2000). It was the aim of this experiment to investigate the effects of handling on the stress physiology and behaviour of dairy heifers.

Materials and Methods Forty-eight 5 - 14 month old nonlactating Holstein-Friesian heifers were studied in two handling treatments, positive and negative handling, over four time replicates. Positive handling involved patting, stroking or resting the hand on the back of the cow. Negative handling consisted of slapping or hitting an animal, using a piece of plastic pipe. In each replicate, 12 animals were studied over a 5-week period with 6 animals per treatment. Handling was imposed twice daily. Fear of humans was assessed by observing the approach behaviour of individual animals to a stationary experimenter over a 3-minute period in a standard test and by observing the avoidance behaviour of individual animals from an approaching human in the flight distance test. The stress physiology of the animals was studied by measuring plasma cortisol concentrations in response to the presence of humans (acute stress response) and basal cortisol concentrations in the absence of humans (chronic stress response), using indwelling catheters with extensions to remotely sample blood. In the fourth week of treatment, remote blood sampling before and after a 30s exposure to a familiar human in the home pen was used to assess the acute stress response of the animals to the presence of humans, and remote blood sampling at hourly intervals over 8 hours was used to assess the chronic stress response. The effects of treatment and replicate on the acute stress response, the chronic stress response and on the approach and avoidance behaviour were investigated using a two-way analysis of variance, blocking on pre-treatment liveweight.

Results Selected results are presented. In this handling study, the negative-handled heifers took longer to approach within 1m and 2m of an experimenter in a standard test, than their positive-handled counterparts (165 vs 121s and 139 vs 94s, respectively, $P<0.001$) and had a greater flight distance to an approaching experimenter (4.6m vs 2.2m, $P<0.001$). The negative-handled heifers had greater ($P<0.05$) increases in total cortisol concentrations 5, 10 and 15 minutes after exposure to a human and had higher ($P<0.05$) free cortisol concentrations in the afternoon than the positive-handled heifers (Table 1).

Table 1. *The effect of positive and negative handling on the total cortisol concentration following human exposure and on the concentration of free cortisol in the absence of a human*

Variable	Positive handling	Negative handling	s.e.d.	P value
Cortisol concentration after human exposure (nMol/L):				
0	19.4	17.6	2.54	NS
+ 5 minutes	19.4	28.5	2.93	< 0.01
+ 10 minutes	25.1	39.6	5.52	< 0.05
+ 15 minutes	23.2	32.5	3.98	< 0.05
+ 60 minutes	2.7	2.7	0.14	NS
	(17.9)	(17.3)		
Free cortisol concentration in the afternoon with no human exposure (nMol/L)	0.11 (1.90)	0.28 (2.78)	0.09	<0.05

(For data log transformed, the nontransformed means appear in brackets).

Conclusion There is evidence that the nature of the human contact directed to cows affects the subsequent behavioural response of heifers. In addition, fear of humans results in an acute stress response in the presence of humans and there is some evidence of a chronic stress response. These stress responses may have detrimental effects on milk production in fearful cows.

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Effect of breed on behaviour of lactating dairy cows in an open-field test

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Introduction The open-field test can be used to measure temperament in dairy cows (Kilgour, 1975). Temperament or emotional reactivity reflects an animal's ability to cope with an environmental change or challenge (Lawerence *et al*, 1991; Manteca & Deag, 1993), and consequently assessment of this can be effective in the development of novel production systems. The aim of this experiment was to identify temperament differences between two breeds of dairy cattle, Dutch Holsteins (DH) and Norwegian Dairy Cattle (NC), in two production systems.

Materials and Methods Fifty-two DH and fifty-two NC first lactation heifers, with an average age of thirty-two months, were used in the study. Thirty-two animals of each breed were housed indoors (I), while twenty animals of each breed were at grass (G). The animals were brought from a home pen individually according to a pre-determined order, balanced for system and time of day. Each animal was moved along a passage-way towards the test arena, and scored by an observer on its ease of progress. The open-field test arena measured 9m x 7m, and was divided into 12 equal sized rectangles, numbered in an anti-clockwise direction. A camera was positioned directly above the arena to record the animal's behaviour. Each animal was left in the arena for 10 minutes in total. After 2 minutes, a novel object was gently lowered down, to within 1 metre of the ground, in the centre of the arena. The number of vocalisations and eliminations were recorded by direct observation, by an observer positioned outside the arena. The video recordings were analysed to determine the duration and frequency of behaviours by the animal and its location in the test arena. Due to the unequal numbers of animals in each system, a REML analysis was applied to determine if there were any significant breed or system differences.

Results Significant breed (B) effects on behaviour were observed during the open-field test. DH cows interacted with the novel object more frequently than NC cows (DH 0.09/min; NC 0.04/min; sed=0.025; $P<0.05$), and they also vocalised more often (DH 3.74; NC 2.35; sed=0.375; $P<0.001$). Breed also influenced the location of animals within the open-field test arena. NC cows visited the squares furthest from the entrance more frequently (Square 6: DH 0.32/min; NC 0.41/min; sed=0.042; $P<0.05$; Square 7: DH 0.46/min; NC 0.58/min; sed=0.055; $P<0.05$), while DH cows spent almost twice as much time as NC cows near the entrance (DH 9.0%; NC 5.4%; sed=1.48; $P<0.05$).
The different systems (S) affected the locomotory behaviour of the animals. Animals that were housed indoors were inactive for less time than those at grass (I 47.8%; G 56.7%; sed=2.70; $P<0.001$). The indoor animals spent more time walking (I 16.9%; G 12.5%; sed=1.63; $P<0.01$) and, as a result, visited more squares than the grazing animals (I 6.26; G 4.36; sed=0.516; $P<0.001$).
Significant differences in behavioural patterns between the two breeds were observed within each system (BxS), as outlined in Table 1. Management system had a greater effect on the behaviour of DH cows than on NC cows.

Table 1 *Time spent in (expressed as a percentage of the observed time, %) and frequency/minute of exploratory behaviours during an open-field test by Dutch Holstein and Norwegian cows from indoor and grazing systems.*

Behaviour	Dutch Holstein		Norwegian		s.e.d.	Significance		
	Indoor	Grazing	Indoor	Grazing		B	S	BxS
Walk while exploring (%)	7.5^{b}	4.3^{a}	7.8^{b}	6.8^{ab}	1.41	NS	NS	*
Walk while exploring (freq/min)	1.40^{b}	0.81^{a}	1.44^{b}	1.29^{b}	0.204	NS	*	*
Nose floor (%)	8.6^{b}	5.1^{a}	6.6^{ab}	7.6^{ab}	1.38	NS	NS	*
Nose floor (freq/min)	1.54^{b}	0.90^{a}	1.51^{b}	1.36^{b}	0.189	NS	**	**
Nose door (freq/min)	0.38^{b}	0.18^{a}	0.24^{a}	0.23^{a}	0.057	NS	*	*

* denotes significance at $P<0.05$ level; ** denotes significance at $P<0.01$ level.

Conclusion It can be concluded that Dutch Holstein cows show greater reactivity in an open-field test than Norwegian cows. Furthermore, management system had a greater effect on the behaviour of Dutch Holstein cows more than Norwegian cows.

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Changes in GIT digesta VFA as a result of fermentable carbohydrates in piglet diets

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Introduction The gastro-intestinal tract (GIT) microflora is thought to remain essentially stable in terms of the range of species present, but it is hypothesized that its activity can be influenced by the diet of the host. The addition of fermentable carbohydrates to an animal diet, may therefore affect the activity of the GIT microflora.

Materials and Methods The effect of fermentable carbohydrates was tested by the addition of either sugarbeet pulp (SBP=slowly fermentable), or a mixture of fructo-oligosaccharide (FOS=rapidly fermentable) and SBP, to a semi-purified weanling piglet diet (based on maize starch and fishmeal) with otherwise low levels of fermentable carbohydrates, and no added copper or antibiotics. A control diet was also included. Digesta samples were collected from five areas of the GIT, and VFA analyzed as an indicator of GIT fermentation. Three litters of weanling piglets (5 piglets/ litter at 4 weeks of age) were introduced to one of these diets immediately following removal from the sow. Individuals from the same litters were kept together to avoid microbial cross-contamination (as part of a larger experiment), but the procedure was repeated three times (nine litters in total), to separate the effects of diet and litter. All animals were kept at 24°C and digesta samples collected from piglets sacrificed on days 7 and 13 following introduction of the diet. Digesta samples were collected from three areas of the small intestine, and one each from caecum and colon. VFA were analyzed per piglet.

Results Means of VFA of caecal digesta are shown in Table 1 according to the factors: day, and animal diet, on Days 7 and 13 after piglets were offered the diet.

Table 1- VFA in caecal digesta (mmoles/g digesta)

	Acetic	***Propionic***	***Butyric***	***Total***	***BCR****	***pH***
Day 7						
Control	13.6	4.4	1.9	21.3	0.249	6.51
+ SBP	21.4	7.6	3.7	33.8	0.065	5.81
+FOS/SBP	27.4	8.8	4.6	42.9	0.099	5.75
Day 13						
Control	24.4	6.0	3.4	35.9	0.138	6.28
+ SBP	28.0	9.4	5.4	44.2	0.063	5.60
+FOS/SBP	27.6	7.4	4.1	41.4	0.120	5.85
Prob.						
Diet	**0.0085**	**0.0119**	**0.049**	**0.006**	**0.046**	**0.013**
Day	**0.0001**	0.354	0.211	**0.0005**	0.426	0.472
Diet ´ Day	0.354	0.123	0.919	0.383	0.297	0.883

* BCR= Branched-Chain Ratio [(iBut + iVal + Val) / (Acet +Prop + But)]

There were significant differences in the VFA concentration of caecal contents, whereby the presence of fermentable carbohydrates in the diet, led to higher concentrations, particularly for the mixture of added FOS and SBP on Day 7. Interestingly, this clear increase in total VFA was most marked on Day 7, but was less clear-cut by Day 13. BCR is an indicator of the amount of protein vs. carbohydrate fermented- the higher the value, the more protein fermentation (which leads to higher concentrations of branched-chain fatty acids). This work shows that the added carbohydrate diets had lower ratios in the caecum, from which is can be concluded that more carbohydrate was available in the caecum for those diets, compared with the control diets.

Conclusions
Results reported previously (Williams *et al*., 2000), showed increased microbial activity of the GIT microflora (tested *in vitro*) with age of the piglet after weaning. Given that VFA are primarily an end-product of fermentation these results seem to confirm that this increase in activity can also be shown *in vivo*. These results suggest that the weaner diet can be manipulated to influence microbial activity *in vivo*. This has important implications for feeding strategies for young piglets, particularly in relation to the use of anti-microbial growth promoters.

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Williams B.A., Bosch M., Schrama J.W. & Verstegen M.W.A. (2000) Changes in large intestine microbial activity as a result of changes in piglet diet. In: *Proceedings of the EAAP Meeting held in The Hague, August 21- 25*. Abst. P. 169

Changes in digesta NH_3 concentration related to fermentable carbohydrates in piglet diets

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Introduction In the absence of sufficient energy from a carbohydrate source, the GIT microflora can also use protein as a source of energy, by splitting amino acids leading to the formation of volatile fatty acids and ammonia (NH_3). Therefore, it is hypothesized that the addition of fermentable carbohydrates to an animal diet, could reduce the concentration of NH_3 of the gastro-intestinal tract (GIT) digesta, particularly in relation to the area where the fermentation takes place.

Materials and Methods The effect of fermentable carbohydrates was tested by the addition of either sugarbeet pulp (SBP=slowly fermentable), or a mixture of fructo-oligosaccharide (FOS=rapidly fermentable) and SBP, to a semi-purified weanling piglet diet (based on maize starch and fishmeal) with otherwise low levels of fermentable carbohydrates, and no added copper or antibiotics. A control diet was also included. Three litters of weanling piglets (5 piglets/ litter at 4 weeks of age) were introduced to one of these diets immediately following removal from the sow. The litters were kept together to avoid microbial cross-contamination (as part of a larger experiment), but the procedure was repeated three times (nine litters in total), to separate the effects of diet and litter. All animals were kept at 24°C and digesta samples collected from piglets sacrificed on days 1, 7 and 13 following introduction of the diet. Digesta samples were collected from five areas of the GIT, and NH_3 analyzed as an indicator of GIT protein fermentation.

Results Means of NH_3 of caecal digesta are shown in Table 1 according to the factors: day, position in the GIT (three areas of the small intestine- SmInt I, II and III), and animal diet.

Table 1- Analysis of variance for NH_3 in digesta for the main factors diet, day, and GIT position (mg/L).

Diet			***Day***			***GIT Position***		
	n	***NH_3***		**n**	***NH_3***		**n**	***NH_3***
CON	75	269^a	**1**	22	281^a	**SmInt1**	39	59^b
SBP/FOS	81	201^b	**7**	106	190^c	**SmInt2**	40	50^b
SBP	78	184^b	**13**	107	230^b	**SmInt3**	39	54^b
						Caecum	39	540^a
						Colon	40	542^a
Prob		0.0001			0.0001			0.0001
MSD		24.3			31.1			41.9

There were significant differences in the NH_3 concentration of digesta according to the main effects of diet, day and GIT position. The presence of fermentable carbohydrates (SBP/FOS and SBP) in the diet, led to lower concentrations of NH_3, particularly in the more caudal areas of the GIT (data not shown), where one would expect most fermentation to occur (i.e. caecum and colon). NH_3 concentrations generally decreased with age of the piglet, presumably with increasing activity of the microflora. NH3 concentrations remained low in all sections of the small intestine, with a dramatic increase in the caecum and colon.

Conclusions

An *in vitro* test for microbial activity reported previously, showed that the microflora of these same piglets was more active the longer the piglets had been exposed to the solid diet, following the change from a milk-only diet (Williams *et al*., 2000). However, these results also suggest that the addition of fermentable carbohydrates to the weaner diet, can also influence microbial activity *in vivo*. This finding that diet can shift the fermentation pattern from protein to carbohydrate as main substrate in the hindgut, has important implications for feeding strategies for young piglets particularly in relation to the use of anti-microbial growth promoters.

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Williams B.A., Bosch M.W., Schrama J.W. & Verstegen M.W.A. (2000) Changes in large intestine microbial activity as a result of changes in piglet diet. In: *Proceedings of the EAAP Meeting held in The Hague, August 21- 25*. Abst. P. 169

Energy and protein utilisation equations in lactating gilts

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Introduction Nutrition models of energy and protein utilisation have been developed for lactating pigs but there is little or no evidence in the scientific literature of the validation of such models. If there are systematic discrepancies in such models, then inappropriate supply of nutrients will either be an inefficient use of resources with oversupply or have an impact on piglet growth and subsequent reproductive performance of the sow with undersupply. In the current study, lactating gilts were fed isoenergetic diets differing in ileal digestible lysine : energy. Protein and energy utilisation were predicted using existing nutrition equations. The validity of the energy and protein utilisation equations was tested by determining if the predicted energy balance differed significantly from zero or if the predicted protein balance was not significantly less than zero.

Material and methods The isoenergetic (14.0 MJ DE/kg DM) diets consisted of similar ingredients, of which wheat, barley, soyabean, fish and maize gluten meals accounted for 0.97 of the diet compositions. The crude protein contents of the diets, which were labeled A, B, C, D and E, were 157, 184, 210, 236 and 262 g/kg and the ileal digestible lysine : DE ratios were 0.40, 0.58, 0.76, 0.94 and 1.12 g/MJ, respectively. The study consisted of 223 Large White gilts from the Edinburgh lean growth experiment and animals were randomly allocated to each diet. The selection lines covered a wide range of genotypes, many of which are relevant to current production systems. Five days prior to farrowing, gilts were moved to the farrowing house and were acclimatised to the diet. Between day 1 and day 21 post-farrowing, gilts were fed to appetite. Gilts were weighed and ultrasonically scanned on days 1 and 21 with litter size and individual piglet weights recorded. No cross-fostering was practiced. For each gilt, energy and protein balances from farrowing to day 21 were predicted from dietary inputs, maintenance requirements, mobilisation of body tissue and milk production using the equations of Whittemore and Morgan (1990). Predicted energy and protein parameters were analysed with residual maximum likelihood methodology with effects fitted for diet, genotype of gilt and the interaction, as necessary.

Results There were no statistically significant (P>0.05) differences due to diet for changes in gilt liveweight, ultrasonic measurements, litter weight gain or gilt food intake. In general, as dietary ileal digestible lysine : energy increased, lipid and protein loss increased as did energy required to excrete excess protein. There were consistent trends in all energy and protein traits, except for the higher energy and protein required for milk production for diet D, given diets C and E, which was due to the numerically larger litter sizes of gilts fed diets A and D (8.4 v. 7.8, s.e.d. 0.7). The correlation between litter weight gain and litter size at birth was 0.84 (s.e. 0.07), when only piglets surviving to 21 days were included in litter weight at birth. The energy balances of diets A, B, C and D were significantly less than zero (s.e. 40) and the protein balance with diet A was also significantly less than zero (s.e. 385). Expressing protein intake on the basis of dietary crude protein content resolved the negative protein balance but not the negative energy balance.

Table 1 Energy and protein utilisation of gilts fed different diets, based on "ideal" protein intake and on crude protein content of the diet (†).

Trait	Energy						Protein					
Diet	A	B	C	D	E	s.e.d.	A	B	C	D	E	s.e.d.
From dietary intake	1323	1331	1275	1291	1268	51	6796	9923	12428	15518	18178	512
From protein loss	-5	-5	-1	1	5	9	616	675	914	1078	1302	393
From lipid loss	22	29	88	118	148	50						
For maintenance	450	460	453	456	461	9	2559	2614	2573	2593	2619	50
For milk production	1031	1006	960	1070	908	82	7616	7436	7096	7907	6709	605
For excreting protein	1	14	44	72	122	6						
Balance	-141	-127	-98	-204	-70	56	-2748	540	3658	5990	10149	545
From dietary intake †							9945	11744	12830	14582	15911	513
For excreting protein †	12	30	49	61	95	6						
Balance †	-151	-143	-103	-192	-43	55	401	2361	4061	5054	7883	529

Conclusions Calculation of dietary specifications to provide sufficient energy and protein for lactating gilts based on the nutritional equations of Whittemore and Morgan (1990) is likely to result in an under-supply of energy for a wide range of diets. It is immaterial if the dietary protein intake is based on "ideal" protein or on the crude protein content of the diet as in both cases the predicted energy balances were significantly less than zero. The study demonstrates the sensitivity of nutritional equations to assumptions about dietary specifications and the problem of combining nutritional equations from different studies when modelling energy utilisation.

Acknowledgements
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The use of hyper-immunised egg as a source of prophylactic antibodies in the neonatal piglet

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Introduction Gastrointestinal infection caused by pathogenic bacteria and viruses are an important cause of diarrhoea and ill-thrift in human and animal neonates (Guerrant et al., 1986, Radostits et al., 1994). *Escherichia coli* (*E.coli*) and *Rotavirus* are both important causes of neonatal diarrhoea, in addition *E.coli* is an important factor in the post-weaning diarrhoea syndrome seen in early weaned piglets (Radostits et al., 1994). Neonates reared on maternal milk are protected by antibodies (IgA in humans and pigs, IgG in ruminants) which act passively in the gut against organisms which cause gastrointestinal disease. This study investigated the protective effect of egg antibodies (Lohmann Animal Health) against *E.coli* and *Rotavirus* challenge in neonatal piglets. The eggs were sourced from hens vaccinated against *E.coli* and *Rotavirus.*

Materials and Methods 15 piglets were caught at birth, deprived of colostrum and transferred to an SPF neonatal pig rearing unit. 15 litter-mates were allowed to suckle colostrum for 24 hours and were then transferred to the SPF unit. The piglets were fed on a sow milk substitute (SMS) for three to four days and then randomly assigned to dietary treatments of SMS, SMS plus 5% normal egg powder (NIM) or SMS plus 5% hyper-immunised egg (IMM). Three days later piglets were challenged simultaneously with $5x10^5$ PFU porcine *Rotavirus* and 10^8 to 10^9 of a marked strain of enterotoxigenic *E.coli* (K88). One group of littermates were not inoculated and were euthanased as controls at 0 hours infection. Litters were blocked by time and groups of infected littermates were euthanased at 24, 48, 72 and 96 hours respectively unless symptoms indicated early euthanasia on welfare grounds. Immediately following euthanasia lumen contents were collected at 25%, 50% and 75% along the small intestine for bacteriology and colon contents collected for ELISA detection of *Rotavirus.* The experiment was replicated three times, using different sows from the same source. All sows were immunised against enterotoxigenic *E.coli* (K88) two weeks prior to farrowing. Effects of dietary treatment on infection were compared by chi-squaren analysis. Bacterial counts were compared by a non-parametric statistical method (Kruskal-Wallis).

Results Some pigs were excluded from the experiment due to poor health prior to infection. Detection of *Rotavirus* or *E.coli* infection in the piglets was not affected by time following infection and therefore data from all time points were analysed together. Feeding hyperimmunised egg antibody (IMM) to both colostrum-deprived and colostrum-fed piglets significantly prevented infection with the marked strain of *E.coli* when compared with normal egg (NIM) or milk alone (SMS) ($p < 0.05$) (Table 1). However there was no significant effect of dietary treatment on *Rotavirus* infection (Table 2). Logarithmically transformed *E.coli* counts in the lumen were not significantly affected by time post-infection, by sow or by colostrum intake but were significantly lower in pigs fed the hyper-immunised egg ($p<0.05$).

Table 1 Effect of dietary treatment on infection with marked strain of *E.coli*

colostrum deprived	**IMM**	**NIM**	**SMS**	n
positive	**0**	**3**	**4**	7
negative	**6**	**1**	**2**	9
Total	6	4	6	16

colostrum fed	**IMM**	**NIM**	**SMS**	n
positive	**1**	**6**	**8**	15
negative	**10**	**6**	**4**	20
Total	11	12	12	35

Table 2 Effect of dietary treatment on infection with *Rotavirus* (based on ELISA from colon contents)

colostrum deprived	**IMM**	**NIM**	**SMS**	n
positive	**3**	**2**	**3**	9
negative	**3**	**2**	**3**	7
Total	6	4	6	16

colostrum fed	**IMM**	**NIM**	**SMS**	n
positive	**2**	**5**	**6**	13
negative	**9**	**7**	**6**	22
Total	11	12	12	35

Conclusions Egg antibody from hyper-immunised chickens can be used prophylactically to prevent and reduce gastrointestinal disease developing in neonatal pigs whether or not they have received colostrum. Further work would be required to show the optimum levels at which the egg could prevent disease in neonates and post-weaning.

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The effect of respiratory disease on various acute phase protein levels in the slaughter pig

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Introduction Respiratory health is an important aspect of pig production due to its effects on pig performance and welfare. Haptoglobin, an acute phase protein, has been identified as a sensitive indicator of infection with respiratory pathogens such as *Actinobacillus pleuropneumoniae* and has been suggested as a possible marker for non-specific surveillance of pig health status (Heergaard *et al*., 1998). Other acute phase proteins such as Major Acute Phase protein (MAP) and Serum Amyloid A (SAA) may also be of use in disease surveillance. However, little is known about the variation of these proteins and their associations with post-mortem signs of disease in the pig. This information could be of importance in monitoring herd health and in facilitating ante- and post-mortem inspection by identifying diseased animals. This study was designed to determine whether various acute phase proteins could be used to identify enzootic or pleuropneumonia in individual pigs or farms with increased prevalence of these diseases.

Materials and Methods Blood samples and lungs from 30 pigs (75-100 kg) from each of 17 farms were collected from the slaughterhouse. Serum samples were analysed for haptoglobin (haemoglobin binding assay), MAP (immunodiffusion), SAA (ELISA) and the negative acute phase protein (colorimetric assay). Each set of lungs were assessed for signs of enzootic pneumonia (EP) and pleuropneumonia (PL). A score for enzootic pneumonia was calculated by determining the percentage of consolidation for each lung lobe and multiplying that by a weighting factor based on the relative volume of each lobe. Tissue damage due to pleuropneumonia was calculated by estimating the percentage of each lobe missing due to adhesion of pleural membranes and scored as for enzootic pneumonia. Regression equations were calculated between the pneumonia scores and each acute phase protein for individual animals and for the farm means. Individual scores for pneumonia and the acute phase proteins and mean SAA score were transformed according to the equation (ln(x) + 0.01).

Results Mean farm EP score was significantly related to mean haptoglobin concentration (r^2=0.347, P<0.05 - see Figure 1). There were no significant relationships between the magnitude of pathological lesions of enzootic pneumonia or pleuropneumonia and haptoglobin when considering individual scores. There was no significant relationship between EP score and MAP, SAA and albumin concentration either at pig or farm level. There was no significant relationship between PL score and any of the measured acute phase proteins.

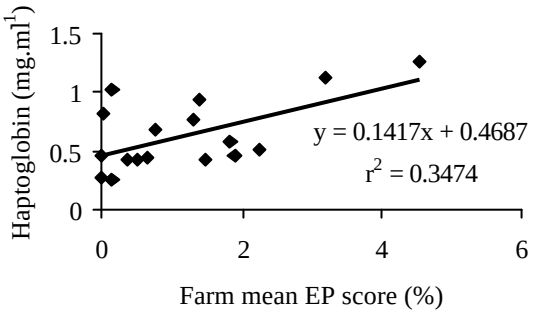

Figure 1 *The relationship between farm mean enzootic pneumonia score and serum haptoglobin*

Conclusions The results demonstrate that serum haptoglobin concentration is a useful indicator for the detection of enzootic pneumonia problems at farm level. The lack of significant associations between the measured acute phase proteins and respiratory disease in individual pigs may be due to the lesions seen in the slaughter pig more reflecting chronic rather than acute disease, as seen in studies with cattle (Horadagoda *et al.,* 1999). It is likely that the acute phase proteins were associated with other inflammatory conditions not categorised in this study. At this stage, it is unlikely that these acute phase proteins will be useful for ante- and post mortem detection of enzootic and pleuropneumonia in individual pigs.

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Major gene effect on serum insulin-like growth factor-1 concentration in pigs

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Introduction Insulin-like growth factor-1 has been positively associated components of growth in pigs, such as protein deposition. Therefore, serum insulin-like growth factor-1 concentration (IGF-1) was measured in lines of pigs divergently selected for daily food intake (DFI) or for efficient lean growth rate (LGS) to determine if there was a non-zero genetic association between IGF-1 and the selection criteria. If a genetic association did exist, then IGF-1 could be used as a physiological predictor of genetic merit in a breeding programme. The presence of a major gene effect on IGF-1 was also examined in the study.

Material and methods In each of the high and low DFI and LGS selection lines, 48 Large White boars and gilts were penned individually and performance tested from 30±3 to 90±5 kg with *ad-libitum* feeding. Blood samples were taken at 6 weeks of age (weaning), at the start and end of test and after 24 and 48 hours of fasting following the end of test. Serum IGF-1 concentrations, expressed as µg/l, were determined with a commercially available immunoenzymometric assay (Octeia, Boldon, England) using a monoclonal antibody raised against human IGF-1. The human and porcine amino acid sequences are identical and the assay excluded measurement of IGF-1 binding proteins. Between-selection line differences were estimated using residual maximum likelihood analysis. Segregation analysis determined if a gene with a large effect on IGF-1 was segregating within the selection lines and quantified its effect. The model assumed that the phenotype was genetically determined by an untyped major gene with two alleles and a polygenic component. The major gene was assumed to be completely additive. The polygenic variance component was not estimated, but fixed values of the heritability (0.1 to 0.7) were used in a series of analyses. The additive effect of the major gene, allele frequencies and the environmental variance were estimated assuming Hardy-Weinberg equilibrium.

Results There was a significant response in IGF-1 at 6 weeks of age with selection on DFI (156 v 103, s.e.d. 18 µg/l) but the magnitude of the response declined with time (Figure 1). In contrast, there was no response in IGF-1 at 6 weeks with selection on LGS but by 90 kg, IGF-1 in the high line was greater (212 v. 176, s.e.d. 10 µg/l) than in the low line.

For IGF-1 at 6 weeks of age, there was evidence of a major gene effect. The frequency and magnitude of the "positive IGF-1" allele was 0.60 (s.e. 0.01) and 56 µg/l (s.e. 6). The major gene accounted for 0.85 of the total genetic variance with the polygenic variance resulting in a heritability of 0.11 (s.e. 0.13).

At 30 kg, the magnitude of the major gene effect was 38 µg/l (s.e. 10) with an allele frequency of 0.54. The major gene still accounted for 0.81 of the total genetic variance and the heritability was 0.19 (s.e. 0.14).

The magnitude of a major gene effect for IGF-1 at 90 kg and subsequent times was not significantly different from zero. Heritability estimates for the three IGF-1 measurements were 0.43, 0.39 and 0.39 (s.e. 0.14).

For performance test traits (Table 1), the high DFI and LGS lines had similar growth rate (ADG; g/day) but differed in untrasonic mid-back backfat (BFAT; mm) and food conversion ratio (FCR) in a complementary manner to the low lines.

At 6 weeks of age, correlations between IGF-1 and ADG, BFAT, DFI and FCR were 0.10, 0.27, 0.16 and 0.07 (s.e. 0.07), respectively. At subsequent sampling times, phenotypic correlations between IGF-1 and performance test traits were always less than 0.2. The coheritability between IGF-1 at 6 weeks of age (0.18) and DFI was of the same order of magnitude as the phenotypic correlation, when determined from the direct and correlated responses to selection.

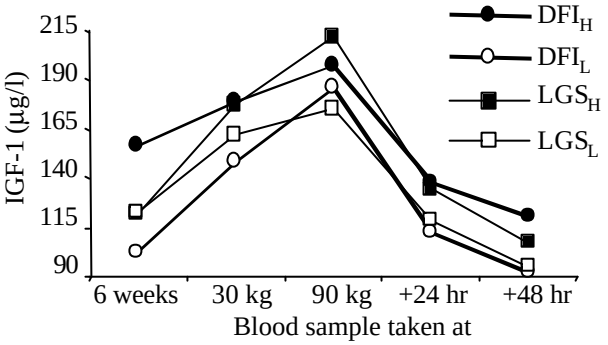

Figure 1 IGF-1 at different sampling times

Table 1 Performance traits of the selection lines

	DFI		LGS		
Trait	High	Low	High	Low	s.e.d.
ADG	881	816	904	816	24
BFAT	17.0	12.8	12.8	16.4	0.8
FCR	2.54	2.30	2.34	2.58	0.05

Conclusions The response to selection on DFI indicated that IGF-1 measured at 6 weeks of age may be a useful physiological predictor of genetic merit for daily food intake during test. The study detected a substantial major gene effect on IGF-1 measured at 6 weeks of age. Genotype data would be required to determine if there is a corresponding effect of the major gene on other traits. In a breeding programme, the perceived benefit of incorporating IGF-1 in a selection criterion will be over-estimated if an infinitesimal model is assumed, as genetic variation arising from the major gene effect will inflate the polygenic variance. The major gene effect could not be attributed to the encoding IGF-1 gene on chromosome 5 as DNA marker information would be required. In summary, based on the response in IGF-1 to selection on DFI and detection of a major gene effect on IGF-1, it would be pertinent for breeding companies to determine if a gene of large effect on IGF-1 was segregating within their nucleus populations.

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A non-invasive approach to determining extent of degradation in the rumen

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Introduction A non-invasive method is proposed for determining the extent of degradation in the rumen, based on the gas production technique and mathematical modelling. The exercise involves developing both a statistical model and a kinetic model (France *et al*., 2000). The statistical model shifts (or maps) the gas accumulation profile obtained using a faecal inoculum to a rumen gas profile, thus obviating the need for rumen sampling. The kinetic model determines the extent of degradation in the rumen from the shifted profile. It is presented as a generalised mathematical function, allowing any one of a number of alternative equation forms to be selected.

Statistical model The data used are from an experiment by Mauricio *et al*. (2000) in which matched and simultaneous *in vitro* gas production was measured with inocula derived from rumen fluid and from faecal matter using the same donor animals. The experiment used twelve forages differing in OM digestibility (range 548 to 807 g/kg), namely ammonia treated wheat straw, field cured hay (*Lolium perenne*), and ten artificially dried grasses (*Lolium perenne*) cut at different stages of growth. The gas production technique used was that described by Theodorou *et al*. (1994). The cumulative gas production profiles obtained for each forage using rumen liquor and faeces as the inoculum were plotted (data not shown). An equation was fitted to these profiles allowing estimation of parameters such as the lag time T (h) (France *et al*., 1993). The paired profiles differed in terms of lag time, and rise to and height of plateau, but their shapes showed similarity over time. Plots of gas accumulation for rumen liquor against gas accumulation for faeces at matched incubation times were plotted for each forage (data not shown) and a linear spline model with a single unknown break point (node) was fitted to each plot, giving 12 break points (one for each forage). The values of gas accumulation for faeces at the break point B_f (ml) correlate with the estimates of lag time T_f (h) obtained from the cumulative gas production profiles with faeces as the inoculum, and the line of best fit was:

$$B_f = 141.6(\pm 14.5) - 16.77(\pm 2.79)T_f, \quad r^2 = 0.779 \qquad (1)$$

All the points <u>below</u> their respective break point were pooled and the line of best fit relating gas accumulation using the rumen inoculum V_r (ml) to that using the faecal inoculum V_f (ml) was:

$$V_r = 8.07(\pm 2.96) + 2.411(\pm 0.106)V_f, \quad V_f < B_f \quad r^2 = 0.874 \qquad (2)$$

Similarly, all the points <u>above</u> their respective break point were pooled and the line of best fit was:

$$V_r = 110.8(\pm 2.56) + 0.761(\pm 0.019)V_f, \quad V_f > B_f \quad r^2 = 0.953 \qquad (3)$$

The line of best fit relating the residue remaining in the flask after 96 h incubation using the rumen inoculum U_r (g OM) to that remaining using the faecal inoculum U_f (g OM) was:

$$U_r = -39.4(\pm 40.0) + 0.881(\pm 0.134)U_f, \quad r^2 = 0.808 \qquad (4)$$

A gas production profile obtained using faecal inoculum can be shifted to a rumen gas profile by estimating its lag time T_f and applying eqn (1)-(3) to correct each observation.

Kinetic model

An expression for the extent of degradation in the rumen was derived as described by France *et al*. (2000). The scheme is applicable to any model for describing gas production profiles provided the model is monotonically increasing (e.g. linear, diminishing returns, sigmoidal). However, we recommend models based on a compartmental scheme which lends itself to mechanistic interpretation, in preference to purely empirical ones.

Discussion The method proposed converts gas production profiles obtained with a faecal inoculum into new profiles that resemble those obtained with a rumen inoculum. The results suggest that there is some biological or chemical basis for the break point, which requires further research. This work demonstrates that we can use alternative non-invasive sources of microbial and other degrading agents to determine extent of ruminal degradation. This will aid the study of ruminants in their natural environment and exotic species of herbivores in the wild.

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Comparison of methods for prediction of rumen fermentation patterns from diet composition.

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Introduction Previous attempts (Offer & Percival, 1998) have been made to develop a prediction system for rumen fermentation patterns from stepwise multiple linear regressions of the chemical constituents of the diet. These authors have also made comparisons between equations derived from diet wet chemistry and those developed from near infrared reflectance spectroscopy (NIRS). However, the potential of NIRS to predict the dynamics of rumen fermentation has not fully been explored using a wide range of forage treatments. Therefore the objective of this experiment was to develop equations from the chemical composition of the diet to predict rumen fermentation patterns and compare these with equations developed from undried and dried NIRS scans of the diets.

Materials and methods The rumen fermentation study was carried out as recorded by Brown *et al*. (2000), using 24 diets: 100% grass (g); 75% g: 25% maize silage (ms); 75% g: 25% fodder beet (fb); 75% g: 25% potato (p); 75% g: 25% whole crop wheat (wcw); 100% low digestibility silage (lds); 90% lds: 10% straw (s); 75% lds: 25% ms: 75% lds: 25% fb; 75% lds: 25% p; 75% lds: 25% wcw; 75% lds: 25% lucerne (l); 100% high digestibility silage (hds); 90% hds: 10% s; 75% hds: 25% ms; 75% hds: 25% wcw; 75% hds: 25% lu; 100% low dry matter silage (ldms); 100% high dry matter silage (hdms); 100% ms; 100% wcw; 100% hay (h); 50% h: 50% ms. During the study the chemical composition of the basal forages was determined twice weekly, and on rumen sampling days. Chemical analysis for volatile corrected oven dry matter, pH, ammonia nitrogen, gross energy, crude protein, volatile fatty acids and alcohol, ADF, NDF, ash, water soluble carbohydrate and starch were undertaken. NIRS scans of the undried forages were also determined in triplicate at these times. NIRS scans of the dried basal forages and dried mixtures of forages were also performed in duplicate. Scanning was undertaken using a Foss NIRSystems 6500 NIR spectrometer. Initial statistical analysis of the data was carried out using GENSTAT 5, REML procedure for the analysis of unbalanced data. Stepwise multiple linear regression (SMLR) equations were developed using the GENSTAT 5, forward selection procedure and cross validated using the leave-one-out-method, between the rumen data and the chemical composition of the diets. All NIRS calibrations were developed using modified partial least squares (MPLS) (Shenk & Westerhaus, 1991), with and without scatter corrections in ISI software (ISI NIRSystems Inc. Silversprings USA). The spectra were analysed using three mathematical treatments: 0,0,1,1; 1,4,4,1; and 2,10,5,1; where the first number represents the order of the derivative used, the second is the number of spectral points used, and the third and fourth are the number of data points in the smoothing segments. Cross-validations in the calibration set were obtained using the leave-one-out method.

Results Calibration and cross-validation results are shown in Table 1 (SEC = standard error of calibration, SECV = standard error of cross validation, R^2cv = R^2 of cross validation). Pace, Pprop, Pbut and Pval are the molar proportions of acetate, propionate, butyrate and valerate respectively.

Table 1: *Calibration and cross validation statistics for regressions based on chemical composition and NIRS scans of undried and dried diets.*

	Regressions based on chemical composition				NIRS calibrations based on undried scans				NIRS calibrations based on dried scans			
	SEC	R^2	SECV	R^2cv	SEC	R^2	SECV	R^2cv	SEC	R^2	SECV	R^2cv
pH	0.093	0.416	0.101	0.274	0.088	0.309	0.095	0.252	0.07	0.634	0.087	0.446
NH_3	2.00	0.688	2.19	0.628	1.13	0.901	1.219	0.882	1.23	0.877	1.51	0.812
Pace	13.1	0.805	16.0	0.648	5.02	0.965	7.82	0.916	4.36	0.974	11.8	0.812
Pprop	8.68	0.525	9.86	0.358	10.2	0.279	10.9	0.196	6.86	0.545	8.69	0.255
Pbut	6.83	0.859	8.97	0.719	6.21	0.859	8.38	0.739	5.40	0.894	7.57	0.791
Pval	4.27	0.755	4.79	0.677	1.94	0.903	2.30	0.876	1.38	0.955	1.74	0.93

As can be seen from the above table, NIRS undertaken on both undried and dried samples provided a better prediction for rumen fermentation patterns than regression equations based on the diet chemical composition, with the exception of proportion of propionate. The latter may have been due to a lack of range in the values.

Conclusions From these initial calibrations NIRS has the potential to predict rumen fermentation patterns for a range of forage diets.

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The influence of the pattern of peptide supply on microbial activity in the rumen simulating fermentor Rusitec

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Introduction Peptides and to a lesser extent amino acids accumulate in rumen fluid in the early post feeding period and rapidly decline thereafter (Broderick & Wallace, 1988). Numerous studies have demonstrated benefits to feeding peptides, in terms of increased microbial growth in the rumen (Newbold, 1999). However, given that peptides will only be available in the rumen for a short time after feeding it may be necessary to match supply of peptides and energy in the rumen to maximise the stimulation in microbial activity. The objective of this study was thus to investigate if microbial protein synthesis in rumen fluid would be enhanced by a synchronous provision of peptides and energy.

Materials and methods The study was carried out using the rumen simulation technique, Rusitec. Twelve vessels were supplied at the same time every day with 15 g of a basal diet of NDF prepared from sugar beet pulp supplemented with a commercial vitamins and trace element mix. Treatments were allocated at random to 4 vessels each and were: **ACI -** ammonia continuously infused 1.712 g/l NH_4Cl added to the infused saliva, **PAS-** Peptides added as a single shot; 612 mg/l NH_4Cl added to the infused saliva plus 1.285 g bactocasitone (Becton Dickson Microbiology Systems, MD, USA) added in 5ml of water to the vessels at the time of feeding,. or **PCI-** Peptides continuously infused; 612 mg/l NH_4Cl plus 2.06 g /l bactocasitone added to the infused saliva. $^{15}NH_4Cl$ (3.67 mg/ l) was added to all saliva's. After 21 d, total digesta (TD) and total (TB), liquid (LAB) and solid (SAB) associated bacterial samples where prepared as described by Carro & Miller (1999) The proportion of the TD N of microbial origin was estimated by dividing the ^{15}N enrichment (atom % excess) of the TD from each vessel by the enrichment of the TB. Daily microbial N production (mg/d) was estimated by multiplying total N production in TD by the proportion attributed to the microbes. Daily LAB production was calculated by multiplying the content of N in the LAB pellet by total weight of LAB recovered. The proportion of bacterial N derived from NH_3-N was estimated by dividing the ^{15}N enrichment of bacterial pellets by the enrichment in NH_3-N. Results were compared by one-way analyses of variance. Each vessel was considered as an experimental unit.

Results Free amino acids and peptides where detected, using fluorescamine, in the fermentor liquid for 4 h after feeding in the ACI treatment for 10 h in PAS, free amino acids/ peptides where detected in the vessels at all times with the PCI treatment. Treatments had no effect on dry matter degradation within the vessels and approximately 40% of the degradation occurred during the time no peptides where detected in the PAS treatment. Thus it appeared that a considerable difference was achieved in the synchrony of energy and amino peptide supply between treatments. Despite this there was no significant effect on total microbial N flow (g/d) between the treatments but there was a trend showing higher values with the peptides treatments Table 1. The flow of microbial as LAB was higher ($p<0.05$) in the PCI treatment showing a stimulation of this fraction of the bacterial population by the infusion of peptides. However LAB only accounted for 20- 30% of the total bacterial population. The proportion of microbial N derived from NH_3 was significantly lower in LAB from the PCI treatment ($p<0.05$, Table1).

Table 1 Effects of different forms and pattern of nitrogen supply on microbial nitrogen flow and the proportion of microbial N derived from NH_3-N in the rumen

	Ammonia continuously infused	Peptides continuously infused	Peptides added as a single shot	SED
Microbial N flow				
Total (g/d)	0.144	0.151	0.157	0.005
LAB (g/d)	0.030	0.059	0.039	0.005*
Proportion of microbial N derived from NH_3-N				
Total Bacteria	0.445	0.444	0.444	0.012
SAB	0.459	0.437	0.443	0.041
LAB	0.690	0.490	0.615	0.049*

little effect on the total microbial synthesis.
s in the free liquid are not reaching the SAB,

ı concentrations of ammonia, free amino acids
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ure system (RUSITEC). *British Journal of*

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Microbial activity in grass-fed *in-vitro* continuous cultures in response to infusion of graded levels of soluble sugars.

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Introduction Novel lines of high sugar ryegrass have been shown to increase the efficiency of N use and milk production in zero-grazed cattle (Miller *et al.*, 1999). An experiment was carried out to determine whether this was in part due to an increase in the efficiency of microbial protein synthesis in response to the amount and availability of water soluble carbohydrate (WSC) increasing the balance between energy and nitrogen supply to the rumen microbial population.

Materials and methods For two 10d periods an *in vitro* continuous culture system (Rusitec) as described by Czerkawski and Breckenridge (1977) was fed daily with fresh, chopped perennial ryegrass (var. AberElan; 15g DM/day, containing 22 and 249g/kg DM of total N and WSC, respectively). Artificial saliva was infused into 8 culture vessels either alone (control) or plus inulin and sucrose (80:20, w:w), to raise WSC inputs by 1.2, 1.5 and 1.8 times the level provided by the basal grass feed (2 vessels per treatment). A pre-experimental investigation determined that 84% of the WSC was released from perennial ryegrass in the first 14h post incubation in rumen fluid. Therefore to simulate WSC release, sugars were infused over a period of 14h followed by artificial saliva alone for the remainder of the 24h. $(^{15}NH_4)_2 SO_4$ was infused as a microbial marker. Samples of washed bacteria and effluent were taken after steady state had been reached on days 9 and 10 and microbial N determined by the method described by Carro and Miller (1999). All treatments were blocked according to period with level of sugar as the treatment structure and subjected to a general analysis of variance using Genstat 5 (Lawes Agricultural trust, 1997).

Results The effluent pH (initially 6.4) declined significantly ($P<0.05$) with increments of sugar infused to 6.3, 6.2 and 6.0 (se 0.05) respectively. Table 1 summarises the microbial parameters of the culture vessels. Additional sugar reduced ammonia-N concentration ($P<0.001$) and increased the efficiency of microbial synthesis ($P<0.001$), from 9.9 in the control to 10.8 and 12.7 g /kg organic matter (OM) apparently digested at levels 1.2 and 1.5, respectively. However at the highest sugar level efficiency fell to 7.1g N/kg OM digested. The digestion of OM decreased significantly with increasing sugar content ($P=0.004$).

Table 1 Microbial N production, apparent organic matter digested, ammonia N concentration and the efficiency of microbial N synthesis in a Rusitec system fed grass and infused with different levels of WSC.

	WSC infusion level					
	Basal	x 1.2	x 1.5	x 1.8	s.e.d	Significance
Microbial N (mg/d)	^{b}144.6	^{b}143.0	^{b}158.0	^{a}84.6	13.31	<0.001
OM apparently digested (g/d)	^{b}14.4	^{c}13.2	^{ac}12.5	^{a}12.0	0.53	0.004
Ammonia N (mmol/l)	^{b}3.8	^{c}2.2	^{a}0.9	^{a}0.5	0.31	0.001
Efficiency of microbial N synthesis (gN/kgOMAD)	^{a}9.9	^{a}10.8	^{b}12.7	^{c}7.1	0.79	<0.001

^{abc} Values not showing common superscript differ significantly; x1.2, x1.5, x1.8 = Basal WSC multiplication factors, OMAD = Organic matter apparently digested

Conclusions These results suggest that an additional WSC supply may increase the efficiency of microbial protein synthesis. The observed drop in microbial-N produced at the highest level of sugar inclusion is probably due to the decrease in pH in these vessels. The reduction of OM apparently digested indicates a change in substrate preference with, increasing soluble sugar supply leading to a reduction in the breakdown of structural components of the plant to provide energy and a greater reliance on the soluble sugars supplied.

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The influence of the temporal pattern of post-ruminal energy and protein supply on nitrogen metabolism in growing lambs

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Introduction The poor efficiency with which ruminants utilise nitrogen (**N**) for growth may be due to differences in the temporal pattern of energy and protein absorption following a meal. Efficiency is higher in non ruminant animals where energy (glucose) and protein is absorbed from the same site and at the same time following a meal. Indeed, when an asynchronous pattern of energy and protein supply is imposed on non ruminants, the efficiency of nitrogen utilisation is reduced (Barja *et al*., 1972). The present experiment compares the efficiency of N utilisation in growing lambs given different temporal patterns of post-ruminal energy and protein supply.

Material and Methods Six growing lambs (34.7 ± 6.2kg) were allocated to three 18d treatment periods in a 6×3 Latin square design. During each treatment period, lambs were fed dried grass pellets hourly to supply approximately 585kJ/kgW$^{0.75}$ per day (*i.e.*, 1.3× energy maintenance) and 1.95gN/kgW$^{0.75}$. After adaptation to diet (days 1-4), water was infused (26g/kgW$^{0.75}$/d) into the abomasum (days 5-11; *i.e.,* a 7 day covariate control period). From day 12-18, the water infusion was replaced by solutions of volatile fatty acids (**VFA**; triacetin and tributyrin supplying in total, 135kJ/kgW$^{0.75}$) plus casein (0.317gN/kgW$^{0.75}$). The total quantity infused was again 26g/kgW$^{0.75}$/d. The temporal pattern of VFA and casein infusion over a repeated 12h cycle varied with each of the three treatment periods. The three different temporal patterns were: *1)* synchronous pulses of VFA and casein (0-3h; synchronous pulse, **SP**), *2)* a 3h pulse of VFA (0-3h) with constant casein infusion (0-12h; asynchronous, **AS**) or *3)* a continuous infusion of VFA and casein (0-12h; synchronous continuous, **SC**). These infusion cycles were repeated twice per day from day 12-18 and were intended to reflect the pattern of absorption in the twice daily fed monogastric (SP), the twice daily fed ruminant (AS), and the continuously fed animal (SC). From day 7-9, and day 14-16, ^{14}C-urea was infused intravenously (35μCi/d), and urea entry rate determined from the specific radioactivity in urine on days 8 and 9, and on day 15 and 16. Urea excretion was determined from days 7-10 and 14-16. Blood was sampled (3× daily) on days 7-9 and 14-16 for IGF-I analysis, and at hourly intervals during one 12h infusion cycle on day 10, and 17 to determine insulin concentrations.

Results Infusion of casein and VFA increased both urea irreversible loss rate (**ILR**) (1.25 to 1.37gN/kgW$^{0.75}$, P<0.001) and urea excretion (0.65 to 0.73gN/kgW$^{0.75}$, P<0.1). The increment in urea ILR was significantly lower on treatment SP than on either of the other two treatments (SP<AS=SC, P<0.01; Table 1). Urea excretion followed a similar trend, however, these results were not significant, possibly due to the short collection time.

Table 1: Urea excretion and ILR (gN/kgW$^{0.75}$/d)

	Covariate Control (days 5-11)			*Nutrient Infusion*[1] *(days 12-18)*				*Sig. of Main Effects*	
Item	*SP*	*AS*	*SC*	*SP*	*AS*	*SC*	*SED*	*Infusion*	*Pattern*
Feed Intake	1.950	1.950	1.950	1.950	1.950	1.950			
Casein infusion	-	-	-	0.317	0.317	0.317			
Urea ILR	1.242	1.236	1.258	1.268	1.408	1.423	0.1621	P<0.001	-
D urea ILR/gN infused				*0.090*[a]	*0.603*[b]	*0.568*[b]	*0.1457*	-	*P<0.05*
Urea excreted in urine	0.620	0.667	0.650	0.660	0.758	0.772	0.0705	P<0.1	-
D urea excr./gN infused				*0.16*	*0.30*	*0.36*	*0.330*	-	*NS*

[1]*Within a row, values with different superscripts differ significantly (P<0.01)*

For the duration of one 12h infusion cycle, plasma insulin concentrations were increased to a greater extent by nutrient infusion on treatment SP ($AUC_{0\text{-}12h}$ 182-361μU×h/ml), than on either treatment AS or SC ($AUC_{0\text{-}12h}$ 182-273 and 193-282μU×h/ml, respectively; P<0.05). Although mean plasma IGF-I concentrations also increased significantly with nutrient infusion (10.0 to 12.6pmol/ml; P<0.001), infusion pattern did not consistently affect either the mean concentration, or the diurnal pattern of IGF-I.

Conclusion The timing of energy and protein absorption seemed to influence the metabolic fate of absorbed N. The positive response to synchronous absorption was only apparent when the nutrients were supplied in a pulsatile manner (compare treatments SP and SC). This may be due to the increased response in plasma insulin concentrations observed in treatment SP. These results may explain some of the differences in the efficiency of nitrogen utilisation between ruminant and non-ruminant animals.

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Effect of supplementation and stage of growth on the partitioning of nutrients by Hereford x Friesian steers fed on grass silage based diets

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Introduction Forage based feeding systems are often disadvantaged compared with those based on high cereal usage in terms of feed intake, live weight gain and efficiency of utilisation of dietary energy and protein. Furthermore, under some situations, particularly with animals fed on grass silage, cattle often have higher fat:protein carcass ratios than those fed other forage-diets. However, other factors such as age, genotype and physiological state may also influence nutrient partitioning. An experiment was conducted to investigate the effect of diet (based on silage alone or supplemented with additional energy and/or protein) and stage of development on the partitioning of nutrients between fat and lean deposition. Overall effects of diet on animal performance and carcass composition were reported by Scollan *et al*. (1999).

Material and methods Ninety two Hereford x Friesian steers were allocated on liveweight to one of 4 dietary treatments; grass silage fed either alone (diet S) or supplemented with fishmeal (diet FM; 150 g/kg silage DM intake but fed at equal ME intake to silage, or forage-concentrate (F:C) diets of silage and a barley/soya concentrate (80:20) at ratios of 70:30 or 30:70 (on a DM basis). Eight animals were slaughtered at the start of the trial to determine initial carcass chemical composition. Of the remaining 21 animals per group, 3 were slaughtered at liveweights ranging between 250 and 550 kg, at 50 kg intervals. Animals were individually fed and diets were offered *ad libitum* (except for diet FM see above) along with 100 g/d of a commercial mineral and vitamin premix. At slaughter, half carcasses were minced for the determination of fat and protein content. The composition of the silage was 271.9g freeze dry matter/kg with a total-N and estimated ME of 26.5 g/kg DM and 11.8 MJ/kg DM, respectively. The total-N and ME content were 31.1 and 108.4 g/kg DM and 13.5 and 14.1 MJ/kg DM for the concentrates and fishmeal, respectively. General regression was used to test the effects of diet and stage of development and interactions on all measured parameters.

Results Effect of diet and slaughter liveweight on carcass fat and protein content is given in Figure 1. The amount of carcass protein was very similar across diets and slaughter weights, although there was some indication that it was higher in steers fed the high concentrate diet (30:70 F:C) at 250 kg, but lower at 550 kg, compared to animals fed on silage only (Figure 1a). Carcass fat content (kg) was similar between animals on all diets at liveweights < 300 kg, However, at > 400 kg the carcasses of animals fed on concentrates contained significantly more fat ($P < 0.01$) compared to those fed on silage or FM (Figure 1b).

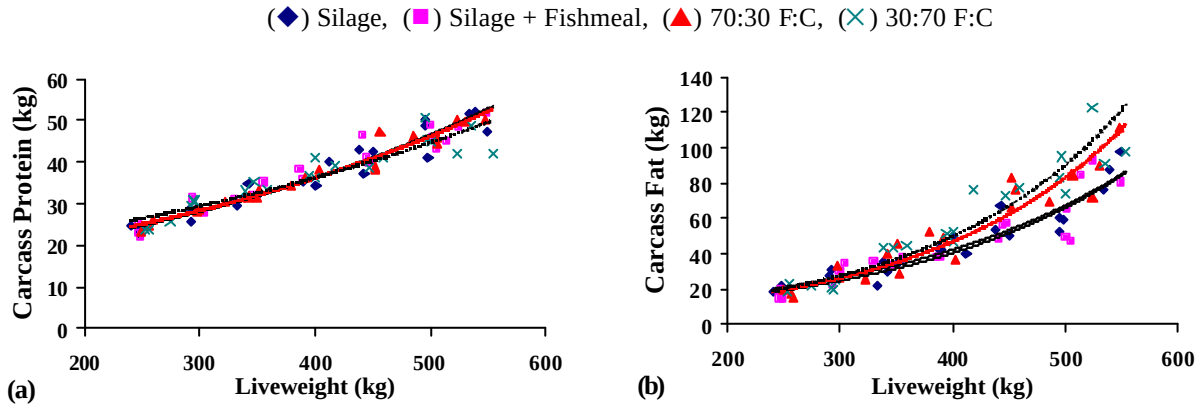

Figure 1. Carcass protein (a) and fat (b) against liveweight of Hereford x Friesian steers fed on grass silage supplemented with additional energy and/or protein.

Conclusions Deposition of carcass protein was remarkably linear across slaughter weights and not different between diets, although there was an indication of an increase and a reduction on the high concentrate diet (30:70 F:C)at 250 and 550 kg, respectively. This may indicate a reduction in mature protein mass of the animals fed on this diet. However, the animals fed on silage supplemented with concentrates contained higher amounts of carcass and total body fat, especially at higher liveweights (>400 kg; Figure 1b). As concluded by Scollan *et al*. (1999) good quality grass silage will support high levels of performance without the need for additional concentrate supplementation. The latter may contribute towards increased fat deposition within the animal.

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Effects of pre-weaning food presentation on response to solid feed in piglets post-weaning

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Introduction Piglets commonly show a setback in growth and a low intake of solid feed on weaning at three to four weeks of age. Outdoor reared piglets, however, have been found to spend more time eating solid feed than indoor reared piglets in straw yard weaning pens (Webster and Dawkins 2000, Cox and Cooper 2001), even though outdoor piglets do not conventionally receive creep feed prior to weaning. Outdoor piglets are, furthermore, frequently observed chewing the roll nuts provided to outdoor sows. In this study, therefore, we investigated the effect of two aspects of outdoor rearing on the feeding behaviour of indoor reared piglets. These aspects were maternal facilitation of feeding and large pellet size.

Materials and Methods The study was carried out on a commercial pig unit with sows (Camborough line 12) housed on either extensive or intensive systems. In the outdoor system, sows farrowed in straw filled arks and both sow and piglets had access to pasture. In the intensive system, sows farrowed in crates with a heated creep area. For the experimental study, indoor litters were exposed to one of four treatments. Normal, conventional feeding of creep feed (SCA Start Rite 88) near creep area; PCR, creep feed placed near sows' trough; SPF, creep feed mixed with sow rolls (Fisher Feeds SSO rolls) near creep area; SPRF, rolls near sow and creep feed near creep area. In all four treatments the piglets received their daily ration (approximately 250g) at the same time as the sows were fed in their trough (Fisher Feeds LSI pellets). Four sows (n = 16) and their litters (n = 170) from each treatment were observed on the first day of creep feeding (14 ± 3 days of age) and the day before weaning (24 ± 3 days). Observations were for 15 minutes from addition of fresh feed to the pen. Each litter was weaned individually and observed for 30 minutes following movement to straw-yard housing with access to a pellated feed (SCA Start-Rite 88) in a trough. In addition four litters were selected from the outdoor population for this post-weaning observation. The percentage of time feeding was calculated for each litter in each observation and analysed using one-way ANOVA at each age with Tukey's pair-wise comparisons used to identify differences between the treatments.

Results On the first day of exposure to solid feed PCR piglets performed more feed directed behaviour than the conventionally fed piglets ($P < 0.001$), but SPR and SPRF piglets showed no increase in feeding behaviour (Table 1). By the day before weaning, however, all three experimental treatments showed increased food directed behaviour relative to conventional feeding regime ($P < 0.05$ for each). All three experimental treatments also showed an increase in food directed behaviour following weaning, relative to conventional conditions ($P < 0.05$), though none of the treatments showed as much food directed behaviour as the outdoor piglets ($P < 0.05$).

Table 1. Percentage of time (mean ± SE) feeding on by indoor piglets in the fifteen minutes following first addition of creep (Day 12) and on day prior to weaning (Pre-wean) and in half an hour following weaning for indoor and outdoor piglets.

Treatment	Normal	PCR	SPR	SPRF	Outdoor	F : P
First Day						
Creep	10.9 ± 6.0	35.2 ± 7.8	7.5 ± 2.4	5.9 ± 1.9		6.96 **
Sow	N/A	N/A	2.1 ± 2.1	6.4 ± 2.4		N/A
Total	10.9 ± 6.0	35.2 ± 7.8	9.6 ± 4.3	12.2 ± 3.5		4.59 *
Pre-Wean						
Creep	15.2 ± 2.9	44.5 ± 6.5	25.9 ± 7.8	13.2 ± 3.9		7.01 **
Sow	N/A	N/A	13.2 ±5.5	21.4 ± 2.4		N/A
Total	15.2 ± 2.9	44.5 ± 6.5	39.1 ± 1.5	34.6 ± 3.2		10.1 ***
Post Wean						
Total	2.41 ± 2.0	14.2 ± 5.4	11.8 ± 5.8	10.1 ± 3.0	21.9 ± 4.0	4.55 *

Conclusion

Both pellet size and maternal facilitation increased the piglets' interest in solid feed in prior to weaning and immediately following weaning, though none of our treatment litters performed as much food directed behaviour as the outdoor reared piglets. Both factors may therefore play a role in increasing intake of creep feeds, which would be desirable as higher intake of creep feed pre-weaning may reduce the problems of low feed intake and the setback in growth associated with early weaning.

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Testing two theories of food intake using growing pigs: the effect of a period of feeding on a high bulk food on the subsequent intake of foods of different bulk content

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Introduction The objective of this experiment was to provide a severe test of the two frameworks currently available for understanding and predicting voluntary food intake. Framework 1 predicts that an animal will eat at a level that will allow potential performance to be achieved subject to its capacity to deal with a constraint, such as the bulk content of the food, not being exceeded. In framework 2 intake is seen as that which will allow some biological efficiency, such as the ratio of net energy intake per litre of oxygen consumed, to be maximised (Tolkamp and Ketelaars, 1992). The frameworks differ in their prediction of the effect that a period of prior feeding on a high bulk food (severely limiting) will have upon the subsequent intake of foods of differing bulk content. Framework 1 predicts that the intake of a low bulk food, that is non limiting, but not that of a moderate bulk food, that is limiting, will be increased under such circumstances. Framework 2 predicts that intake will be increased regardless of the type of food being fed as long as the Metabolisable Energy of that food is utilised more efficiently.

Materials and methods Forty pigs (Manor Meishan, PIC), weighing 7.5 (s.d. 1.07)kg were individually penned. The experiment had two periods, P1 and P2: P1 (12-18kg) and P2 (18-32kg). Three foods were used, a control food (C) based on micronised wheat (13.4 MJ DE and 243g CP per kg fresh food), and two high bulk foods containing 50% (M) or 70% (H) sugar beet pulp. The pigs were fed *ad libitum* and the temperature maintained at 18°C. The treatments were: T1, food C in P1 and P2; T2, food M in P1 and P2; T3, food H in P1 and C in P2; T4, food H in P1 and M in P2. T3 and T4 were used to compare the predictions of the two frameworks. Intakes and live-weights were recorded daily. The data were analysed as a completely randomised design in Minitab using a General Linear Model. The three feeding treatments were used as factors in the model and intake, LWG and FCE as the variates.

Results In P1 there was a highly significant effect of treatment on intake and live-weight gain ($P<0.001$). Both were decreased as the level of SBP inclusion in the food increased. As the growth rate on M was 54% and on H was 32% of that on C both these foods were limiting (Table 1). In P2 growth on T2 (MM) was not significantly less than that on T1 (CC), therefore food M was not limiting in P2. There was a highly significant ($P<0.001$) interaction for intake in P2 (Table 2). The pigs that changed from H to C, T3, increased intake by 412gd^{-1} compared to those that were fed C throughout, T1, ($P<0.001$). In contrast the pigs that were changed from H to M, T4, increased intake by only 95gd^{-1} compared to the pigs on T2 fed M throughout (ns). The live-weight gain of pigs changed from H to C, T3, was 152gd^{-1} ($P<0.001$) higher than that of the pigs that were fed C throughout, T1. In contrast the gain of the pigs on T4 (HM) was only 57gd^{-1} (ns) higher than that of the pigs on T2 that were fed M throughout. This interaction just failed to be significant ($P=0.056$).

Table 1:The performance of pigs fed one of three foods differing in SBP content in Period 1.

Treatment (%SBP)	Food Intake (gd^{-1})	Gain (gd^{-1})
C (0)	732	564
M (50)	612	301
H (70)	536	183
s.e.d	24	22
Effect		
Treatment	***	***

Table 2: The effect of being fed one of three foods differing in SBP content in Period 1 on the subsequent performance of pigs fed one of two foods differing in SBP content in Period 2.

Food (%SBP)		Food Intake (gd^{-1})	Gain (gd^{-1})
Period 1	Period 2		
C (0)	C (0)	1093	717
M (50)	M (50)	1359	683
H (70)	C (0)	1505	869
H (70)	M (50)	1454	740
s.e.d		60	34
Effect			
Food Period 1		***	***
Food Period 2		*	**
Interaction		***	ns

Conclusions The contrast between frameworks required the use of foods that would limit growth (M and H) compared to the control food, C. Although M did not prove to be a clearly limiting food in Period 2, the data provide evidence that under conditions of compensation foods of moderate bulk, such as M, may become limiting. Pigs that were changed from H to C increased both food intake and liveweight gain by considerably more than pigs that were changed from H to M. The evidence is in closer agreement with the predictions set by Framework 1 than by Framework 2. The pigs compensating when changed from H to C were less efficient than those on C throughout, suggesting that the gain was fatter.

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Creep feed consumption and individual food intake characteristics of group housed weaned pigs

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Introduction The provision of creep feed to suckling pigs is considered to stimulate early food intake as well as health post weaning. However, Barnett *et al.* (1989) found no effects of creep feeding on post-weaning performance. Research by Pajor *et al.* (1986) indicated that there is a high variability in creep feed intake both among and within litters. This variability in creep feed intake is probably the main cause of disagreement on the effects of creep feed provision. The objective of the present study was to assess the effect of creep feed consumption on individual food intake characteristics and performance of group-housed weaned pigs. Chromic oxide was added to the creep feed to identify piglets that consumed food during the suckling period. In the piggery, IVOG®-feeding stations were used to measure individual food intake after weaning.

Materials and Methods During the suckling period 14 litters (149 piglets) were fed a commercial creep feed (12.71 MJ Net Energy/kg, 15.2 g lysine/kg) supplemented with 1% of chromic oxide. Another 5 litters (48 piglets) were not given access to creep feed ("No-feed"). Piglets were weaned at 28 days after birth. Ten, six and one day prior to weaning faecal samples from all the piglets were taken using faecal loops. A green colour of the faeces indicated that the pig had eaten creep feed (see Barnett *et al* , 1989). Pigs that showed three times green coloured faeces were considered as "eaters". Pigs that never showed green coloured faeces were considered as "non-eaters". At weaning 22 piglets of each type ("No-feed", "Non-eaters" and "Eaters") were selected based on bodyweight, litter origin and gender. These 66 pigs were assigned to six pens equipped with IVOG®-feeding stations (Insentec B.V., Marknesse, The Netherlands) for weaned pigs (Bruininx *et al*., in press). "Eaters", "Non-eaters" and "No-feed" pigs were equally divided over all six pens. After weaning a prestarter (day 0-13) and a starter diet (day 14-34) were provided as described by Bruininx *et al.* (in press). Performance traits and the individual food intake characteristics: latency time (interval between weaning and first feed intake) and initial food intake (intake during the first 24 h following first food intake) were determined for all piglets. Data were analysed using the Residual Maximum Likelihood Procedure in Genstat (1993) with fixed effects of pen, gender and creep feed characterization. Bodyweight at weaning was included as a covariate. Survival like, Kaplan-Meier curves were constructed for latency time as affected by creep feed intake (Bruininx *et al.* in press).

Results The average performance traits and the initial food intake for the three creep feed types are presented in Table 1. Averaged over the first 13 days after weaning, eaters ate more ($P<0.05$) than the no-feed pigs whereas ADFI of the non eaters was intermediate. Moreover, ADG of the eaters was higher ($P<0.05$) than ADG of Non eaters and No feed pigs. Averaged over the total 34 day period the effect of creep feed intake on post weaning ADFI was less pronounced ($P=0.11$), whereas ADG of the eaters was the highest ($P<0.05$). Initial food intake was not affected ($P>0.1$) by food intake prior to weaning.

Table 1 Performance and food intake characteristics following weaning

	Eaters	Non-eaters	No feed	SEM
Number of piglets	22	22	22	
Day 0-13				
ADFI	266[a]	226[ab]	208[b]	17.5
ADG	188[a]	141[b]	137[b]	14.8
Day 0-34				
ADFI	535	491	499	21.6
ADG	372[a]	324[b]	316[b]	16.0
Initial food intake	37.5	17.6	20.6	7.72

[ab] Means within a row with different superscript are significantly different ($P<0.05$)

[1] Initial food intake = amount of feed consumed during the 24 h following the first food intake (g per kg metabolic bodyweight)

The pattern of the survival like Kaplan-Meier curves for the latency time of the eaters differed of that of the non-eaters ($P=0.06$) and No-feed ($P<0.05$) pigs respectively. About 50% of the eaters started with food intake within 4 hours after weaning whereas 50% of the Non-eaters and No-feed pigs needed respectively 6.7 and 6.9 hours.

Conclusions The results of the present study show that creep feed intake by suckling pigs stimulate early post weaning food intake as well as post-weaning performance. However, the minimum amount that a piglet has to eat prior to weaning to obtain an advantage in post-weaning performance remains to be established.

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Braude Scholarship 2000 The effect of voluntary food intake on the postweaning growth of the pig

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Characteristically, voluntary food intake in the young pig in the days after weaning is low and very variable. Such low feed intakes can lead to reduced digestive efficiency and ultimately poorer physical performance. This is due to the digestive tract requiring a continuous supply of nutrients to maintain gut integrity and digestive capacity.
The presence of this check in a young pigs growth immediately after weaning is a well documented problem of commercial significance. It is multi faceted and has been found to encompass many issues such as stocking density, mixing of litters, feeder type and space and the formulation and presentation of the feed.
The Braude scholarship enabled me to travel across Europe to visit various research institutes and commercial companies in the Netherlands, Belgium and Poland including, ID-Lelystad, Institute for Animal Health, Netherlands, Rosmalen Institute for Pig Research, Netherlands and the experimental farms of the Provimi Group in Veldriel, Netherlands and Central Soya, Chelmno, Poland who were all engaged in trying to find solutions to the postweaning problem. The research they were involved in was as diverse as the problem itself appears to be.

This paper will summarise some of the main findings of the study tour.

If as stated above the consumption of sufficient quantities of a solid feed in the immediate postweaning period contributes significantly to the health and welfare of the young pig then if we are to increase the feed intake of such animals we must understand how the pig adapts to a solid diet and how this occurs over time. It is common however when conducting large scale trials to measure the feed intake of weaner pigs on a group or pen basis. Data on the individual feeding patterns and intakes of the individual could help us to understand why some pigs adapt better to weaning than others. E.M.A.M. Bruininx at the Research Institute for Pig Husbandry, Rosmalen, Netherlands used individual feeding stations to monitor feeding behaviour in 3 weight classes of weaned pigs. It was found that although the heaviest pigs at weaning had higher ADFI and ADG ($P<0.05$) than the lighter pigs they took longer to make their first visit to the feeder postweaning, perhaps due to previous exposure to solid feed (Bruininx *et al* 2000). Is it therefore these heavier pigs that need to be the focus of our research?

In contrast to this, Wim Boersa at ID Lelystad, Netherlands is taking a different approach to maintaining gut integrity by looking at the possibility of using Lactic acid bacteria as a replacement for in feed antibiotics. This is due to the bacteria's potential as a probiotic, contributing to disease resistance. Many strains of lactic acid bacteria exist however and only some are potentially beneficial to the young animal. Commercially animal feeds containing probiotics are available but only through smaller specialist companies. At present the immunologists at Lelystad are developing a system using cell cultures to predict which strains could be useful, once this work is complete it will be possible to determine the effectiveness of the various strains without the need for large scale trials, which it is hoped would lead to the larger scale use of bacteria as probiotics by feed manufacturers.

In terms of commercial research, at institutes both in the Netherlands and in Poland this seemed to concentrate more on the physical form of the diet and/or the presentation of the diet to the animal. A particular area of interest was the use of rooting substrates to increase the voluntary intake of solid foods preweaning which would hopefully lessen the stress of weaning and increase feed intake immediately postweaning.

There still seems to be as many questions to answer as have been answered with regards to what motivates the young pig to eat in the initial hours postweaning. Study tours such as those funded by the Braude scholarship can only serve to increase the rate of exchange of information between institutes, which must lead to a better understanding of such a complex problem

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Genetic evaluations and parameter estimates for dairy cow fertility in the United Kingdom

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Introduction Selection for milk production alone would lead to a genetic decline in cow fertility (e.g. Kadarmideen et al., 2000) thus incorporation of fertility in selection seems desirable. The Dairy Information System (DAISY) is a comprehensive recording scheme for insemination and health events and hence the data quality is more reliable for genetic analysis of cow fertility. The objective of this study was to analyse DAISY data to estimate sire breeding values and genetic parameters for various fertility measures based on insemination and calving events on daughters.

Materials and Methods After editing, the data set consisted of 38716 multiple lactation records (up to parity 5) on 16687 cows in 134 herds, daughters of 684 sires. Fertility measures, computed using calving and insemination dates, were interval traits (in days): calving interval (CINT), calving and first service (CAFS), calving and conception (days open: DOPN) and first and last service (IFLS), or pregnancy traits: success or failure of conception to first service (COFS) and number of services for a conception (NSPC). Data editing and validation rules for all fertility measures were as described in Kadarmideen et al. (2000). Heritabilities (h^2) and permanent environmental variance (c^2) and all correlations were estimated via multi-trait repeatability models using ASREML software (Gilmour et al., 1998). In all analyses, milk yield was included as an additional trait to account for its correlated effect on fertility: delayed inseminations (CAFS) of high yielders and early culling due to low milk yield and hence absence of re-calving date (CINT). A sire model was used with relationship among sires included in the **A** matrix, which had 837 animals. After model checking with various fixed effects, the final model for all traits included herd-year-season of calving, age at calving and parity as fixed effects and sire, permanent environmental effects of cow, and residual effects as random. Estimated variance components were then used in multi-trait BLUP to estimate transmitting abilities of sire (sire PTA).

Results and Discussion Phenotypic mean and SD, h^2 and c^2 for fertility traits with a range for sire predicted transmitting abilities (PTAs) estimated from multi-trait BLUP evaluation are given in Table 1. The estimates of h^2 were similar (~0.03) for CINT and DOPN and were also the highest of all traits analysed. The estimates of c^2 were significantly different from zero and ranged from 0.034 to 0.055, suggesting the non-genetic effects carried across cow parities for all traits. The standard errors were 0.01 for both h^2 and c^2 and for all traits.

Table 1. Mean, SD, h^2, c^2 and range (min-max) of sire PTAs estimated from multi-trait BLUP

Traits	Mean (SD)	h^2	c^2	Sire PTA
CINT	380 (46)	0.031	0.045	-5.52 - +8.74
CAFS	71.7 (22.5)	0.024	0.034	-2.48 - +3.15
COFS	0.55 (0.50)	0.011	0.038	-0.06 - +0.04
IFLS	26.3 (41.6)	0.018	0.049	-0.42 - +6.24
DOPN†	98.0 (45.7)	0.030	0.041	-6.21- +6.81
NSPC	1.77 (1.11)	0.014	0.055	-0.09 - +0.14

†Estimates for DOPN are from univariate analysis

The wide range of sire PTAs for each trait in Table 1 suggests the existence of genetic variation among sires and hence the possibility of sire selection. Genetic and phenotypic correlations (r_g and r_p) are given in Table 2. DOPN was dropped from multi-trait analyses as it had both r_g and r_p close to unity with CINT. Absolute r_g between many fertility traits were high, as expected. Both the direction and magnitude of r_g agrees with previous findings (e.g. Kadarmideen et al., 2000). These estimates of r_g suggest that, for example, genetic selection for reduced CAFS would result in reduced CINT and increased COFS.

Table 2. Genetic correlations above and phenotypic correlations below diagonal (with s. e) between 5 fertility traits. Each estimate is from a multi-trait analysis with milk yield as an additional trait.

Trait	CINT	CAFS	COFS	IFLS	NSPC	MILK
CINT	-	0.77 (0.10)	-0.85 (0.10)	0.91 (0.04)	0.81 (0.08)	0.33 (0.10)
CAFS	0.38 (0.01)	-	-0.41 (0.24)	0.53 (0.20)	0.23 (0.23)	0.33 (0.11)
COFS	-0.62 (0.004)	0.05 (0.01)	-	-0.87 (0.09)	-0.82 (0.09)	-0.16 (0.15)
IFLS	0.90 (0.003)	-0.06 (0.01)	-0.69 (0.003)	-	0.98 (0.06)	0.32 (0.12)
NSPC	0.77 (0.004)	-0.06 (0.01)	-0.76 (0.002)	0.86 (0.002)	-	0.30 (0.14)
MILK	0.20 (0.01)	0.12 (0.01)	-0.14 (0.01)	0.16 (0.01)	0.15 (0.01)	-

Conclusions This study has provided preliminary sire PTAs and genetic parameters for many fertility traits based on available insemination and (or) calving records on daughters. These parameters were estimated after accounting for possible biases on fertility measures due to selection on milk yield. Genetic variation found among sires for many fertility traits suggests that routine sire selection for daughter fertility is possible in the United Kingdom.

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Selection indexes using calving interval, condition score and milk yield in dairy cattle

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Introduction Increasing genetic merit for production has been associated with a decline in dairy cow fertility. In order to sustain lactation it appears that appreciable amounts of body condition are being mobilised, which may impinge on fertility. Body condition score (BCS) of first lactation heifers is recorded by Holstein UK and Ireland (HUKI) as part of its national type classification scheme. BCS may be a useful selection criterion for improving fertility. Calving dates and hence calving interval (CI) are generally very reliably recorded, but the usefulness of CI as a selection criterion is hampered for a number of reasons, one being that only the most fertile cows have two consecutive calving dates. This is a serious issue that still needs to be addressed. Our aim here was to investigate if there is genetic covariation between BCS and CI after adjustment for milk yield and to investigate selection responses in all three traits when selection is for each trait in turn and how responses are affected by restrictions imposing no genetic change in one of the traits. Restricting a trait to no change when it is correlated to a trait under selection may be important in developing customised indexes to satisfy specific requirements.

Materials and methods Genetic parameters were calculated using the same data set and models as Pryce et al. (2000). Genetic parameters were estimated using a trivariate analysis of 44674 records on milk yield and BCS and 19042 CI record (Table 1).

Table 1 Genetic variances (diagonal) and covariances for CI, BCS and Milk

	CI	BCS	Milk
CI	51.2	-2.8	3764
BCS		0.65	-323
Milk			626200

The genetic variances for, and covariances between, CI and BCS were adjusted for milk yield to calculate the partial genetic correlation. Responses to selection in the different traits were calculated using selection index theory for CI, BCS and Milk. It was assumed that selection would be achieved through a progeny test of 100 daughters per sire with measurements on these 3 traits. Phenotypic (P) and genetic (G) (co)variance matrices obtained in the trivariate analysis were used to calculate optimal index weights (b) with economic values (e) in the breeding goal being either (i) 1 for CI, 0 for milk, BCS , (ii) 1 for BCS, 0 for CI, milk, or (iii) 1 for Milk, 0 for CI, BCS. The selection index weights were then given by b= P^{-1} G e and correlated responses (CR) in each trait were calculated by $CR = \frac{b^T \cdot G}{\sqrt{(b^T \cdot P \cdot b)}} i$ where i is the standardised selection intensity (assumed to be equal to 1, to give results per SD change in index). Restricted indexes were constructed following (Cameron, 1997).

Results After genetic adjustment for milk yield the genetic correlation between BCS and CI was –0.22, so a thin cow will have a longer calving interval than a fatter cow with the same genetic potential for milk. However the genetic correlation between BCS and CI prior to adjustment for milk yield was –0.48 (0.09) indicating that differences in genetic potential for milk make the the relationship between BCS and CI stronger. With single trait breeding goals, selection for milk will result in a longer CI and lower BCS (Table 2; +4.43 days and -0.40 respectively per SD change in the selection index). Selecting for milk alone while restricting changes in CI had the most impact on progress in yield, and selecting for milk but restricting changes in BCS will still result in an increase in CI (+3.21 days).

Table 2 Responses to single goal selection for CI, BCS and Milk using both unrestricted and restricted indexes

Goal	CI			BCS			Milk		
Trait restricted	None	BCS	Milk	None	CI	Milk	None	CI	BCS
CI (d)	-4.26	-3.42	-2.62	-3.15	0	-1.07	4.43	0	3.21
BCS (1-9)	0.24	0	0.08	0.77	-0.56	0.66	-0.40	-0.13	0
Milk (kg)	-327	-216	0	-383	134	0	768	390	653

Conclusions The genetic relationship between BCS and CI is only partly due to genetically thinner cows having a propensity to produce more milk. Selection for yield alone will result in a further declines in BCS and CI but progress in yield can continue to be made with no change in CI.

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A comparison of the Holstein Friesian and Norwegian cattle breeds for milk production at two levels of nutrient intake

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Introduction Breeding programmes for Holstein Friesian (HF) animals have been based on improved milk production with little emphasis on functional traits such as fertility. This has resulted in a rapid increase in milk production potential of the national dairy herd but unfortunately this has been at the expense of issues such as longevity, especially due to poor reproductive performance. In contrast Norwegian (NC) dairy cattle have been bred via a multi-trait selection procedure for 25 years and there is evidence that fertility has improved during that period. These differences in selection procedures for the two breeds may have major effects on overall herd output and profitability within the grass-based systems of milk production employed in Northern Ireland. The present study is part of an overall study comparing the effects of HF and NC genotypes on food intake, animal performance, nutrient utilisation, behaviour, health, fertility and longevity. The objective of the present paper is to present the effects of breed on food intake and utilisation, and animal performance when offered two levels of nutrient inputs based on grass silage-based diets.

Materials and Methods Thirty-two in-calf HF heifers (PIN (00) £44) and thirty-two in-calf NC heifers (total merit index = 10.1) were selected in Holland and Norway respectively. These heifers were representative of the top 1% and 5% of the HF cattle in the UK and NC cattle in Norway respectively. The heifers had a mean age at calving of 25.5 and 25.8 months for the HF and NC breeds respectively and arrived at the Institute approximately one month prior to calving after being reared in their country of origin. The mean calving dates were 16 February and 10 February and post-calving weights were 502 and 473 for the HF and NC cattle respectively. Post calving the animals were blocked into pairs within each breed and allocated at random to either a low or high input system, based on grass silage for the full lactation. The low and high levels of nutrient input had forage:concentrate ratios of 70:30 and 40:60; and 80:20 and 50:50 for the first and second 100 day intervals of lactation. The silage offered during the first 200 days of lactation was harvested from the primary growth of a perennial ryegrass sward between 17 and 19 May, and ensiled after a 24 hour wilt without additive treatment. The concentrate consisted of 230, 225, 300 and 245 g/kg fresh weight of barley, wheat, sugar beet pulp and soyabean respectively. All cows received the equivalent of 160 g/day of a mineral vitamin pre-mix. The diets were offered as total mixed rations through Calan gates linked to a system of automatic cow identification and weigh cells.

Results The silage had a pH of 3.9 and concentrations of dry matter (DM), ammonia nitrogen (N) and crude protein of 300 g/kg, 104 g/kg N and 133 g/kg DM respectively. The effects of cow genotype, reared in their country of origin, and input system on food intake and animal performance are presented in Table 1. The HF genotype had significantly higher food intake (P<0.001), yields of milk (P<0.001) and fat plus protein (P<0.001), concentration of fat (P<0.001) and milk energy output/kg DMI (P<0.001) relative to the NC genotype. The NC cattle gained more condition (P<0.001) relative to the HF genotype. Increasing concentrate input decreased silage DM intake (P<0.001) and increased total DM intake (P<0.001), the yield of milk (P<0.001), fat plus protein (P<0.001), protein concentration (P<0.001), live weight change (P<0.05) and condition score (P=0.08). Level of nutrient intake had no effect (P>0.05) on the concentrations of fat or lactose and milk energy output/kg DMI. There were significant interactions between cow genotype and level of nutrient intake for the yields of milk (P<0.01) and fat plus protein (P<0.001).

Table 1 The effects of cow genotype and input system on food intake and animal performance during the first 28 weeks of lactation

	Level of nutrient intake (NI)							
	High		Low			Significance		
Genotype (G)	HF	NC	HF	NC	Sem	G	NI	GxNI
Food intake (kg DM/d)								
Silage	8.1	7.4	10.4	9.8	0.19	**	***	NS
Total	17.4	15.8	13.8	13.1	0.29	***	***	NS
Animal performance								
Milk yield (kg/d)	25.2	21.1	18.9	17.7	0.49	***	***	**
Milk composition (g/kg)								
Fat	42.7	40.2	42.7	39.2	0.85	***	NS	NS
Protein	34.4	34.2	30.8	30.3	0.47	NS	***	NS
Lactose	49.3	48.9	49.8	49.4	0.35	NS	NS	NS
Fat plus protein (kg/d)	1.96	1.57	1.41	1.25	0.029	***	***	***
Milk energy MJ/kg DMI	4.7	4.2	4.5	4.3	0.10	***	NS	NS
Live weight change (kg/d)	0.33	0.40	0.01	0.10	0.042	NS	***	NS
Condition score change	-0.33	0.33	-0.38	-0.03	0.113	***	NS	NS

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

Genetic Evaluation of Dairy Bulls For Energy Balance Traits Using Random Regression

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Introduction The term 'negative energy balance' (NEB) is used to describe the condition in early lactation when the energy available from food intake is lower than that of the energy used by the cow for milk output, maintenance and activity. Current selection objectives may be favouring cows that are genetically predisposed to mobilise body tissue. This may have consequences for fertility since cows appear to resume reproductive activity only after the nadir of NEB has passed (Veerkamp *et al.*, 2000). Extreme NEB may be considered generally undesirable, as it is a precursor to health and fertility problems. The aims of this study were: 1) to predict genetic merit for sires for traits contributing to energy balance; 2) to combine those breeding values into an overall energy balance evaluation for bulls using either daily energy flux or body state changes.

Materials and Methods Data for 298 heifers were extracted from the database of Langhill records collected since 1990. Traits considered were daily measurements of milk yield (MY), condition score (CS) and liveweight (LWT) measured weekly and feed intake (FI) measured 4 days each week. A pedigree file was constructed from the complete Langhill database. There were 40 sires and 206 dams with progeny records in the data and 82 grand sires and 189 grand dams in the pedigree file. Variance component estimation was performed using a random regression animal model using the DxMRR statistical package. The random regression model fitted included the trait being evaluated , the fixed effects of genetic line (2 groups), feed group (2 groups) and measurement group (year and week of measurement). The covariates used were percentage Holstein genes (linear) and age at calving in months (linear and quadratic). Orthogonal polynomials were used to model both the animal genetic effect and the permanent environmental effect and measurement errors were allowed to vary throughout lactation. The four traits were analysed separately and energy balance was calculated using the Effective Energy System of Emmans (1994) from daily breeding values for either milk yield and feed intake or condition score and liveweight.

Results and Discussion. The mean genetic energy balance curve calculated from feed intake and production output for all bulls with at least 10 daughters in the data is given in Figure 1. Energy balance for the same bulls calculated from body state changes is given in Figure 2. These curves show a difference of around 40 days in the return to positive energy balance suggesting that long-term body state changes may lag behind daily intake and output measures. However, the similarity between parameters for each curve indicates that CS and LWT may be useful in calculating energy balance where measurements of individual cow feed intakes are unavailable.

Figure 1. Genetic energy balance curve calculated from energy intake and expenditure.

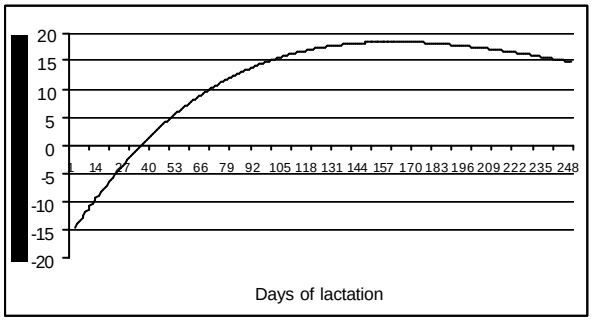
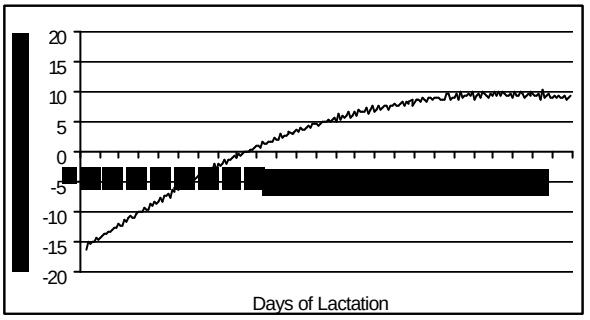


Figure 2. Genetic energy balance curve calculated from body energy changes.



Conclusions Random regression techniques can be used to calculate breeding values for traits influencing energy balance over time. Breeding value profiles for energy balance for dairy bulls can be calculated from traits describing body tissue mobilisation as well as from feed intake and milk output. It may be possible to use nationally recorded CS and conformation data to predict liveweight and therefore calculate breeding values for energy balance for bulls at the national level.

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Multi-trait selection indexes for sustainable improvement of UK hill sheep

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Introduction. Profitability of hill sheep enterprises depends on both maternal and lamb traits, and selection programmes should include all aspects of performance. However, the relative contribution of different traits to overall profitability depends on the type of hill farm and, specifically, the severity of the environmental constraints on the farm (Conington *et al*., 2000). The objective of this paper is to derive and evaluate selection indexes for holistic and sustainable genetic improvement of hill sheep on 'intensive' and 'extensive' hill farms by considering carcass, maternal and 'sustainability' traits simultaneously.

Material and Methods. Selection indexes were derived for two categories of hill sheep farms: i) intensive (INT) where all surplus lambs not required for breeding are 'finished' for slaughter, and ii) extensive (EXT) where all lambs not required for breeding are sold as stores, and where there are constraints on hill pasture quality and limited availability of improved pasture for twin lamb rearing. Ten goal traits were included in the overall breeding objective: ewe mature live weight, ewe longevity, number of lambs reared, number of lambs lost, maternal component of weaning weight, fleece weight, weaning weight (as a trait of the lamb), carcass weight, carcass conformation score and carcass fat class. Ultrasonic measurements of fat and muscle depth, and weaning weight , were included in the index as measurements made on the live animal. Selection index calculations require genetic parameters for all traits and economic values for the goal traits. Genetic parameters were estimated from a dataset obtained from Scottish Blackface sheep on two SAC experimental hill farms including 3,962 lamb records, 4,410 repeated and 1,534 single ewe records. A total of 98 sires were represented, 36 of which were used on both farms. Variance and covariance components were estimated from univariate and bivariate animal model analyses, using Variance Component Estimation (VCE). Initial economic values for each goal trait used in the indexes were those reported by Conington *et al*. (2000), and differed between the INT and EXT scenarios. Economic values were multiplied by the expected number of discounted gene expressions over a 15-year time horizon, the net present value (NPV) coefficient, to get NPV economic weights. The NPV coefficient is the same for all lamb performance traits (1.6164) and for all maternal traits (1.3323). Those for wool and mature size are 1.3769, and for longevity 0.3032. Selection indexes were then constructed, incorporating typically available information from relatives such that they mimicked multi-trait BLUP evaluations. These indexes were evaluated to determine the expected genetic change in each trait with selection, the net economic gain and the overall accuracy of selection. Sensitivities of the index to changes in the NPV economic weights proportionally 0.5 above and below the base values were investigated.

Results. Heritabilities (h^2), means, phenotypic standard deviations (σ_p), NPV economic weights and expected annual progress in the traits in the index are shown in Table 1. The average discounted returns per generation from using the extensive and intensive indexes are £220 and £350 per 100 ewe flock respectively, and the corresponding accuracies were 0.40 and 0.41. In general, improvements in maternal traits are expected, with smaller changes in carcass quality traits. All weight traits are expected to increase. Responses are lower for the more extensive farm systems. Generally, changes in economic values resulted in less than 0.10 proportional changes in expected progress.

Table 1. Genetic parameters, NPV economic weights (/100 ewes) and genetic responses (?G / annum) according to farm type.

Trait	h^2	Mean	σ_p	NPV Econ. Weight (INT)	NPV Econ. Weight (EXT)	**? G** (INT)	**? G** (EXT)
Mature size (kg)	0.47	59.4	4.85	-16.7	-14.3	0.654	0.608
Longevity (days)	0.08	1756	378	2.06	1.6	6.4	7.1
Lamb loss (lambs/ewe)	0.03	0.24	0.10	-42.6	-29.4	0	0
No. lambs weaned (lambs/ewe)	0.07	1.35	0.64	36.1	22.6	0.023	0.020
Average weaning weight (kg)	0.10	29.2	5.33	72.1	66.9	0.135	0.141
Fleece weight (g)	0.62	182	48	1.7	1.7	21	26
Lamb weaning weight (kg)	0.26	27.8	3.55	88.9	70.9	0.371	0.341
Estimated subcutaneous Fat %	0.17	11.1	1.56	-32.2	N/A	-0.007	N/A
Conformation score (units†)	0.09	3.00	1.13	127.5	N/A	-0.006	N/A
Carcass weight (kg)	0.33	16.8	1.99	123.3	N/A	0.121	N/A
Muscle depth (mm)	0.30	17.6	2.1	N/A	N/A	N/A	N/A
Ave Fat depth (mm)	0.25	2.00	0.69	N/A	N/A	N/A	N/A

† Threshold units on the underlying normal distribution scale.

Conclusions. This paper shows that carcass and maternal traits can be genetically improved simultaneously in hill environments. Overall, progress in goal traits will be lower in the more extensive environments. With the exception of lower responses to the number of lambs reared, greater emphasis is placed on maternal traits for more extensive, lower production-level farms.

Acknowledgements We thank SERAD, MLC and BWMB for funding.

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Confirmation of the presence of a major gene for fecundity in Thoka Cheviot sheep by segregation analyses

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Introduction The putative Thoka gene, with large effects on fecundity, originated in Icelandic sheep. The gene was introduced to the UK in 1985 through a programme of crossbreeding and established in Cheviot sheep (Russel *et al*., 1997). Ewes have been retained in the flock as putative Thoka gene carriers if they have lambed in each of the first three years and had at least two sets of twins. Progeny tests on a separate population of ewes have been used on two occasions to identify rams believed to carry the gene. Despite this complex breeding programme, the actual segregation of a gene for fecundity has yet to be unambiguously demonstrated in this flock. The purpose of this study is to use complex segregation analysis to demonstrate the existence of this gene, estimate the size of its effect and frequency of the favourable allele within the population.

Material and Methods Complex segregation analysis is a statistical technique which may be used to test for the presence of a polymorphic gene segregating in a population of known pedigree (Hill and Knott, 1990). Phenotypic observations are partitioned into an effect due to background genes, an effect due to the hypothesised segregating gene and an error component. The output from the analysis includes the size of the effect of the gene, the genotype status of each animal (homozygote, heterozygote carrier and non-carriers) and variance components for the background genetic and residual terms. The analysed dataset comprised of litter size records collected over 13 years, with up to 5 parities per ewe, from the flock of Thoka-Cheviot ewes believed to carry the gene. The total dataset comprised of 982 litter size records on 333 ewes. The complete pedigree for this population contained 806 animals. Segregation analyses were performed using a Markov chain Monte Carlo method, implemented using Gibbs Sampling, which provides posterior probability distributions for each parameter. The genetic model assumed that the gene was purely additive, as the absence of homozygous carrier females meant a dominance effect could not be estimated. The Gibbs Sampler had a burn-in period of 10,000 iterations, after which realisations for each parameter were drawn every 50 iterations. A total of 10,000 realisations were drawn, giving precise posterior probability distributions for each parameter.

Results The posterior probability distribution for the size of effect of the Thoka gene is shown in Figure 1. There is extremely strong evidence for the presence of a fecundity gene within the flock, with the mean increase in litter size between carrier and non-carrier ewes being 0.63 lambs (s.e. 0.073). The estimated gene frequency within the flock was 0.21 (s.e. 0.089). This frequency is consistent with the mating design. Additionally the analysis correctly predicted the genotype of the two founder rams, demonstrating the potential value as a tool for detecting carrier ewes. A summary of the variance components and other output statistics is given in Table 1. The polygenic heritability for litter size in this flock, removing the effect of the Thoka gene, was fixed at a previous estimate of 0.1.

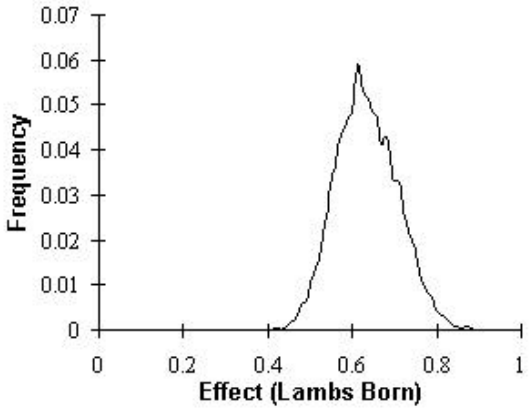

Figure 1 Posterior distribution of the additive major gene effect

Table 1 Means and standard errors of the additive gene effect, gene frequency, additive genetic (polygenic) variance (s^2_A), error variance (s^2_E) and true heritability (h_t^2) calculated from 10,000 realisations

	Mean (se)
Additive Gene Effect	0.63 (0.073)
Gene Frequency	0.21 (0.089)
s^2_A	0.039 (0.0021)
s^2_E	0.353 (0.0187)
$^{\#}h_t^2$	0.310 (0.0504)

$^{\#}$true heritability calculated by including the variance due to the major gene effect with the polygenic variance component.

Conclusions Complex segregation analyses have been used to unambiguously show the presence of a fecundity gene in a flock of Thoka Cheviot ewes, and estimate the size of its effect. The challenge now remains to combine segregation analyses with genetic markers to find the chromosomal location of the gene and eventually the gene itself.

Acknowledgements The Thoka experimental programme was funded by SERAD

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Relationships between muscularity indices and carcass traits in Suffolk, Charollais and Texel lambs

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Introduction Shape of the carcass is considered important commercially and is usually assessed using a subjective score for conformation. Carcasses of higher conformation are perceived to have higher lean to bone (L:B) ratios and give joints of better shape at a weight, characterised as shorter and having a greater thickness of muscle. Some of these benefits have been shown, but so has a positive association between conformation and fatness. Purchas *et al*. (1991) proposed that muscularity indices could be used as an alternative to the conformation score. The objectives of this study were to investigate the relationships between muscularity, shape of joints and composition within breeds and the relationships between different muscularity indices. Knowledge of the latter relationships is important to determine how many indices are required to adequately describe carcass muscularity.

Materials and Methods Data were available for 100 Suffolk (50 males and 50 females), 40 Texel and 20 Charollais lambs, which originated from the ESCA flock at SAC, ANTUR flock at IRS and two commercial pedigree flocks respectively. All lambs were housed indoors, in single breed–sex groups from approximately 8 weeks of age and fed *ad libitum* on a high quality pelleted food (188g CP and 11.7 MJ ME per kg DM). One fifth of lambs within each breed-sex were slaughtered at each of 14, 18 and 22 weeks of age, and the remainder at 26 weeks. Live weight (Lwt) was recorded prior to slaughter. After slaughter, carcasses were chilled (24hrs), split and the length of the side (SL), leg circumference (Lcirc) and inside leg length (InsL) measured. *L. dorsi* (LD) width (A) and depth (B) were measured at the $12^{th}/13^{th}$ rib. LD area was approximated as A*B*0.8. The left side was then dissected, and the weight of three muscles surrounding the femur (3M), total muscle weight (TM), total bone weight, and femur length (FL) recorded. Three muscularity indices were defined, one in the loin ASL=A/SL, one in the hind leg $3MFL=\sqrt{(3M/FL^3)}$, and one for the whole carcass $TMSL=\sqrt{(TM/SL^3)}$. Data were analysed using regression techniques. Lwt was continuously distributed across age groups for each breed-sex, which allowed a linear regression on Lwt to be fitted. Coefficients were not different between the Suffolk males, females and the Charollais ($P>0.05$), therefore a common slope was fitted for these groups. The same was true when regression on a muscularity index was added to the model (multiple regression). Partial correlations between indices (adjusted for Lwt) were not different between the Suffolk males, females and Charollais lambs ($P<0.05$), therefore, a pooled estimate was calculated for these groups.

Table 1. Partial regression coefficients (s.e.) for muscularity indices when fitted with Lwt in a multiple regression †

	ASL		3MFL		TMSL	
	Suff/Char	Texels	Suff/Char	Texels	Suff/Char	Texels
Lcirc	**-0.09 (0.17)** ^{ns}	**0.68 (0.25)** **	1.63 (0.52) **	2.69 (0.53) ***	**0.73 (0.60)** ^{ns}	**2.51 (0.54)** ***
InsL	**0.27 (0.17)** ^{ns}	**-0.35 (0.24)** ^{ns}	-1.90 (0.51) ***	-2.12 (0.53) ***	-0.36 (0.60) ^{ns}	-1.76 (0.55) **
LD Area	3.33 (0.25) ***	3.06 (0.36) ***	2.55 (1.33) ^{ns}	4.10 (1.35) **	6.68 (1.40) ***	3.23 (1.26) *
Lean%	2.16 (0.36) ***	1.92 (0.52) ***	5.43 (1.32) ***	3.00 (1.35) *	**8.60 (1.35)** ***	**3.17 (1.22)** *
Fat%	-2.16 (0.36) ***	-1.18 (0.63) ^{ns}	**-3.98 (1.62)** *	**1.17 (1.66)** ^{ns}	**-8.21 (1.70)** ***	**0.55 (1.54)** ^{ns}
L:B	**0.04 (0.04)** ^{ns}	**0.25 (0.06)** ***	**0.64 (0.11)** ***	**1.13 (0.12)** ***	**0.63 (0.13)** ***	**1.02 (0.12)** ***

† superscripts show significant differences from zero; coefficients in bold differ between the breed groups ($P<0.05$)

Results Associations between indices located within a joint and measures representing the shape of that joint (Lcirc and InsL in the leg, LD area in the loin) were significant and in a favourable direction (Table 1). Indices were all positively associated with Lean%. Coefficients for Fat% were negative (Suff/Char) or non-significant (Texels). All indices, with the exception of ASL for the Suff/Char, were positively associated with carcass L:B. Partial correlations between each index was moderate to high in the Texels but lower for the Suff/Char group (Table 2).

Table 2.
Partial correlations between the indices

	ASL	3MFL	TMSL
ASL	-	**0.52**	0.56
3MFL	**0.14** ^{ns}	-	**0.74**
TMSL	0.53	**0.38**	-

Suff/Char below diagonal, Texels above; ^{ns}, not different from zero ($P<0.05$); Estimates in bold are different between groups ($P<0.05$)

Conclusions Muscularity of a joint is associated with improvements in the shape of that joint. Increases in muscularity at a Lwt also tended to be associated with improvements in composition. Muscularity through the carcass may be described adequately in the Texels using a single index such as TMSL, but separate indices for the leg and loin may be required for the Suffolk and Charollais breeds.

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The inheritance of traits describing early lamb performance in Scottish Blackface sheep

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Introduction Ewe prolificacy, lamb survival and early lamb growth are economically important traits, playing a major role in determining the profitability of hill sheep farms (Conington *et al*., 2000). In currently advocated selection strategies these traits are usually expressed as traits of the dam, however, relatively low heritabilities limit the genetic progress achievable (Conington *et al*., 2001). An alternative approach is to treat these traits, where appropriate, as traits of both the ewe and lamb. This study investigates reparameterisation of these traits as joint expressions of both lamb and ewe genotype, and considers the impact of the results on currently-advocated selection strategies.

Materials and Methods Records on litter size, lamb survival and lamb weights at birth, 4- and 8- weeks, and gestation length were collected on a flock of Blackface sheep at Roslin Institute's Upland farm (Blythbank) from 1988 to 1999. The flock was closed in 1988 and parentage was recorded for all animals. The complete dataset comprised 167 sires, 1181 dams, 2289 litters and 3683 lambs. Genetic parameters were estimated using the ASREML package (Gilmour, 1996), fitting an animal model including all known pedigree relationships between sheep. Litter size was analysed as a trait of the ewe, but gestation length, lamb weight at birth, 4- and 8-weeks of age, and lamb survival to 4- and 8-weeks of age were analysed fitting both a direct genetic (i.e. lamb) and maternal genetic component. The outputs from these analyses include the heritability, describing the lamb genetic component, and the maternal genetic component, analogous to considering the measurement as a trait of the ewe. Furthermore, for gestation length and lamb live weights, a covariance between maternal and direct genetic effects was fitted to determine the antagonism, if any, between direct and maternal effects. Selection index theory was used to evaluate the implications of the finding.

Results Heritabilities and maternal genetic effects for all traits are shown in table 1. Litter size was only analysed as a trait of the dam. Both lamb and dam genotype affected most traits strongly, with maternal genetic components for lamb survival and litter size similar to those reported by Conington *et al*. (2001), when analysing these as traits of the ewe. Lamb survival was much more strongly affected by lamb genotype than by dam genotype. This is in agreement with inferences from behavioural observations on the same flock in which genetic differences were observed in lamb behaviours, but not maternal behaviours, associated with lamb survival (Dwyer *et al*., 2000). These results imply that lamb survival should be included in selection indices as a trait of both the lamb and the dam, utilising survival data on half-sibs of candidates for selection, instead of simply as a trait of the dam. This will have a number of advantages: (i) genetic improvement will be realised immediately rather than only after the daughters of selected rams begin lambing, (ii) the rate of genetic improvement will be greater and (iii) there will be a greater number of expressions of genetic improvement. Selection index calculations predicted a 2.5 fold increase in genetic gain for lamb survival, with the number of expressions of survival increased by a factor of 1.21 in a 15-year period.

Table 1. *Heritabilities (h^2) and maternal genetic effects (m^2) for early performance traits*

	Gestation Length	Birth Weight	Weight at 4 Weeks	Weight at 8 weeks	Survival to 4 weeks	Survival to 8 weeks	Litter Size
h^2	0.23	0.14	0.14	0.15	0.35	0.27	N/A
s.e.	0.04	0.04	0.04	0.06	0.05	0.05	
m^2	0.35	0.28	0.19	0.19	0.07	0.07	0.10
s.e.	0.03	0.03	0.04	0.04	0.02	0.02	0.02

The estimated correlations between direct and maternal genetic effects were, gestation length: –0.26 (s.e. 0.10), birth weight: –0.51 (s.e. 0.10), 4-week weight: –0.16 (s.e. 0.30), 8-week weight: –0.20 (s.e. 0.32). These results indicate a tendency for an antagonism between maternal and lamb genetic effects. Thus, selection which increases lamb growth rate, hence birth weight and dystocia incidence, will tend to be offset by maternal effects which counteract this increase in birth weight. This will limit the expected rate of increase in birth weight and dystocia.

Conclusions Early performance traits have been shown to be affected by both the lamb and the dam genotype. Lamb survival is more strongly influenced by lamb genotype than maternal genotype, in agreement with inferences from behavioural observations. Treating lamb survival as a trait of the lamb in selection indices will result in increased rates of genetic progress which are realised sooner. A genetic antagonism between lamb and maternal components of birth weight will limit increases in birth weight, and hence dystocia, if selection increases lamb growth rates.

Acknowledgements We gratefully acknowledge MAFF for funding.

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Gene frequency estimation from a biased sample of individuals

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Introduction With individual genes being identified that have an important effect on performance and fitness in livestock it is likely that such genes will be included in selection programmes. However, in order to devise sensible strategies to achieve this, knowledge of the frequency of the gene of interest is required. In practice, it is possible that estimates for gene frequency are based on genotype testing of only a subset of the population. The question then arises as to what conclusions can be drawn about the population gene frequency particularly in the likely scenario where the sample genotyped is not chosen at random. A procedure was developed by Van Arendonk *et al*. (1989) to have genotype information on as many individuals as possible given financial limits in the numbers genotyped. In this procedure the genotypes of individuals are predicted using pedigree information and the rules of Mendelian inheritance. What is less clear is the value of the additional information in predicting the population gene frequency. The objective of this study was to assess to consequence of sampling procedures on estimates of gene frequency when additional genotype information is or is not obtained by predicting genotypes on individuals themselves not tested.

Materials and methods Stochastic simulation was used to model a population with overlapping generations. A single locus with two alleles (A and a) was simulated where the initial frequency of A (p) was either 0.25 or 0.5. The genotype of the base population of 50 males and 50 females was obtained from a uniform distribution with probability p of an allele being A. In each subsequent year, 5 sires and replacement dams were chosen at random from the 50 animals of each sex available. These were mated hierarchically with each mating pair producing two individuals with one of each sex. By sampling one allele at random from each parent, the genotype of the progeny was created. Sires were used over one year, and dams over four. Ten generations were simulated (approximately 25 years), and replicated 1000 times. At the end of each replicate a proportion of animals was sampled (10, 30 or 50% of the population) and their genotype taken as known. Two sampling scenarios were applied, one involving random sampling and the other where sampling was biased towards a particular genotype. The bias applied was that the AA genotype was sampled twice as often as it would have been at random. This meant that the expected value for the frequency of the A allele was 0.632 for an initial frequency of 0.5 and 0.343 for an initial frequency of 0.25. Genotypes were then predicted for those individuals not sampled using the procedure of Van Arendonk *et al*. (1989).

Results In Table 1 the gene frequency estimated when a proportion of the population is sampled either at random or in a biased way (favouring the AA genotype by two-fold) is shown. With random sampling, the gene frequency for the sample closely approximated that for the population, even when a relatively small proportion of 10% was genotyped. Unsurprisingly, when the sample is biased, the sample gene frequencies simply reflect that bias. The extent of the bias was lessened when taking account of the predicted genotypes although the frequency of A continued to be overestimated by at least 10%. Deviations in the sample mean from their expected values occur because it is not always possible to sample enough individuals of a particular genotype in order to fully apply the bias. This is because genetic drift led to fixation of an allele in some replicates (as would be expected in populations of finite size).

Table 1 Population, sample and predicted genotype means by generation ten under random and biased sampling.

	Percent	Means under Random sampling			Means under Biased sampling		
Frequency A	sampled	Population	Sample	Predicted†	Population	Sample	Predicted†
0.25	10	0.250	0.263	0.252	0.250	0.294	0.276
	30	0.249	0.240	0.249	0.249	0.300	0.278
	50	0.253	0.267	0.247	0.250	0.295	0.278
0.5	10	0.497	0.500	0.511	0.506	0.596	0.568
	30	0.502	0.493	0.490	0.495	0.606	0.560
	50	0.499	0.511	0.498	0.498	0.593	0.569

† Mean of sample and predicted genotypes for population. Standard errors averaged 0.01.

Conclusions By using predicted genotypes, the effects of bias in a sample on the estimate of the population gene frequency can be reduced, although the estimate remains biased. The practical difficulty is that the presence or extent of bias is likely unknown where genotyping is done commercially. If a truly biased sample has been taken, then it likely should not be used to estimate the gene frequencies for the population or breed concerned. It is possible to envisage that this type of bias could occur if a genotype associated with a clinical disease were sampled from group of predominantly ill animals, or when individuals perceived to have 'favourable' genotypes are chosen for testing based on ancestral genotype information.

Acknowledgements SAC receives financial support from the Scottish Executive Rural Affairs Department.

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Analysis of PrP genotype in relation to performance traits in Suffolk sheep

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Introduction At a time of high public awareness of food safety issues, particularly with respect to transmissable encephalopathies, and a government policy to eradicate scrapie, there is increasing pressure for breeders to select for scrapie resistance in sheep. Genetic variation has been identified at the PrP locus that confers differing degrees of susceptibility to scrapie. In the Suffolk breed this variation is confined to amino acid variations at codon 171, resulting in two alleles R and Q. Animals with the genotype RR and RQ show a greater degree of resistance to scrapie than animals with genotype QQ (Hunter, Moore, Hosie, Dingwall and Greig, 1997).
Genotyping of pedigree animals has now become routine for many breeders and RR animals are favoured. It is, however, unknown whether there is a relationship between PrP genotype and performance traits. The objective of this study was to analyse the relationship between PrP genotype at codon 171 and the performance traits included in the Lean Index (Simm and Dingwall, 1989), which is used for selection in the Suffolk breed.

Materials and Methods Three member flocks of the Suffolk Sire Referencing Scheme provided data for the study. They consisted of individual animal records for 8-week weight (8wt), scan weight (Swt) at approximately 20 weeks and ultrasonic fat (FD) and muscle depth (MD) and the Lean Index score. The goal of the Lean Index is to improve the weight of lean in the carcase while limiting the increase in fat weight. The index is calculated from the estimated breeding values (EBVs) for scan weight, fat depth and muscle depth. PrP genotype at codon 171 was also available for all animals included in the study. A total of 300 animal records were available, representing both older breeding animals and lambs, which were the progeny of 36 sires.

Least squares analysis of variance was used to analyse the data. In addition to genotype the fixed effects included in the analysis, where significant, were: sex of the individual, age of dam, flock of birth and rearing type (single *versus* multiple). For scanning traits (weight, fat depth, and muscle depth), the age at scanning was included as a covariate.

Results The distribution of scrapie genotypes among the animals included in the study is summarised in Table 1.

Table 1 Distribution of PrP genotypes

Number of records	Total	Genotype at codon 171		
		RR	RQ	QQ
Flock A	76	30	34	12
Flock B	49	20	29	0
Flock C	175	103	62	10
Total	300	153	125	22
(proportion)		(0.51)	(0.42)	(0.07)
Number of sires	36	30	27	11

Table 2 Mean (s.e.) performance of different PrP genotypes

Trait	Sig[1]	Genotype at codon 171		
		RR	RQ	QQ
8wt	n.s.	29.2 (0.38)	30.1 (0.39)	29.1 (0.84)
Swt	n.s.	64.1 (0.80)	64.3 (0.80)	63.1 (1.56)
MD	n.s.	32.2 (0.31)	32.0 (0.31)	31.9 (0.61)
FD	n.s.	4.2 (0.16)	4.2 (0.16)	4.2 (0.30)
Index	n.s.	183 (5.9)	184 (6.1)	167 (11.3)

[1]n.s.: $p>0.05$

The differences between the means of the three genotypes, shown in Table 2, were small and were not statistically significant for any of the four traits studied or the Lean Index.

Conclusions The data provided no evidence of a relationship between PrP genotype at codon 171 and the performance traits studied in this population of Suffolk sheep. It is, therefore, unlikely, that selection for scrapie resistant genotypes would compromise selection for high Lean Index.

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Acknowledgements This study would not have been possible without the kind co-operation of members of the Suffolk Sire Referencing Scheme and was funded by the Meat and Livestock Commission.

The effect of stocking density, group size and boar presence on the behaviour, aggression and skin damage of sows mixed in a specialised mixing pen at weaning

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Introduction The mixing of unfamiliar sows at weaning leads to aggression whilst dominance hierarchies within the group are established (Kay *et al*, 1999). The objective of this study was to determine whether the presence of a boar would reduce the incidence of aggression and level of skin damage of newly mixed sows. The overall aim of the project was to improve welfare by designing a suitable strategy for mixing groups of newly-weaned sows

Materials and methods Forty groups of multiparous sows were exposed to one of eight treatments arranged in a 2x2x2 factorial design with two levels of boar presence, two levels of group size (six or seven animals) and two levels of stocking density ($3.5m^2$ or $4.1m^2$ per animal). Groups of five, six or seven unfamiliar sows were mixed at 09:00 on Day 1 (weaning) into a rectangular pen, removed on Day 2 at 08:30 to individual feeding stalls and returned to the pen at 09:00. Sows were observed directly from 09:00 till 12:00 on Day 1 and video tape records were taken continuously for 28 hours from 09:00 on Day 1. All aggressive incidents were recorded and categorised into three classes: brief (knock, snap or bite), one-sided fight (no retaliation by defender) or two-sided fights (defender retaliates). The pens were divided into marked grid references, at 1.5m intervals, to allow for the calculation of flight and chase distances of sows involved in aggressive incidents. Skin damage scores were taken as the total number of lesions over four body areas; head and shoulders, flanks, hind quarters and vulva, and were recorded before weaning, at 12:00 on Day 1 and 13:30 on Day 2. Resulting data were analysed using repeated measures ANOVA with group as the experimental unit.

Results Group size and stocking density was found to have no effect on the behaviour, aggression, or skin damage of groups of mixed sows and did not interact with the main effects of boar presence. The total number of aggressive incidents, per hour, decreased over the 28h period ($P<0.001$, Figure 1) with the majority of incidents occurring within the first four hours. The presence of a boar within the group reduced the number of aggressive incidents, the flight distance and the duration of incidents but was found to have no effect on chase distance (Table 1). Boar presence also resulted in a lower level of skin damage. Scores of 23.5 vs 34.1 were recorded on Day 1 for present vs absent respectively (s.e.d. 4.55; $P<0.05$). Day 2 scores were 47.4 vs 63.8, present vs absent respectively (s.e.d. 6.45; $P<0.05$). No difference was found in the level of damage to the vulva for scores recorded on either Day 1 or Day 2.

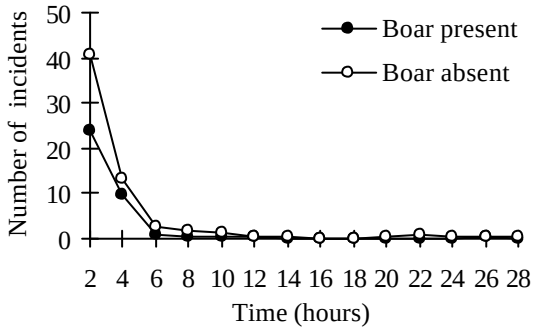

Figure 1 Mean number of aggressive incidents per hour over total mixing period (s.e.d. 1.616).

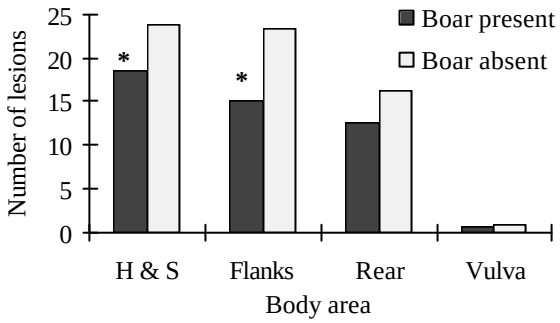

Figure 2 Mean level of skin damage per sow (Day 2) using score at weaning as a covariate (s.e.d. 6.45).

Table 1 Mean observations per hour for sows mixed in pens in the presence or absence of a boar

	Boar		Group size		Stocking density		
	Present	Absent	6	7	M	H	s.e.d.
N° of incidents	2.54	4.49**	3.52	3.51	3.39	3.65	0.554
Flight distance (m)	1.76	2.77*	1.89	2.64	2.13	2.40	0.563
Chase distance (m)	0.91	1.53	0.95	1.49	1.09	1.36	0.408
Duration (s)	3.50	9.00*	4.20	8.30	6.60	5.90	3.040

Conclusions The results suggest that the presence of a boar in a specialised mixing pen can reduce the level of aggressive incidents and subsequent skin damage in groups of newly-weaned sows.

Acknowledgements This work was funded by MAFF.

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The effects of gestation on behaviour, heart rate and heart rate variability of gilts

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Introduction In recent years, there has been increased interest in the development of non-invasive methods to assess the welfare status of an animal. The objectives of this study were to determine the suitability of using heart rate variability analysis as a measure of autonomic activity in pigs during various behaviours and to determine whether these measures were influenced by stage of gestation.

Materials and Methods The subjects were nine group-housed, PIC Camborough gilts. For 4 weeks preceding the experiment, the gilts were habituated to human contact and wearing a heart rate monitor. The experiment began when the gilts were still maiden. Thereafter, testing order was fixed to coincide with conception and stage of gestation (weeks 1,3,5,7,9,11 and 13). The gilts were served using a combination of natural mating and artificial insemination. On the day before testing a gilt was moved into the test pen and allowed 22 hours to acclimatise to her surroundings prior to being tested the following afternoon. During this acclimatisation period, behaviour was recorded continuously. Just prior to the 1-hour testing period, the gilt was fitted with heart rate monitor set to record interbeat intervals (IBI). Two monitors were used sequentially in each test with both recording 30 minutes of data. After the first monitor was fitted the gilt was left undisturbed for 10 minutes. Behaviour and interbeat intervals were then recorded continuously in the absence of the experimenter. After testing all equipment was removed and the gilt was returned to her social group. Behaviour, posture, and location were recorded during the acclimatisation period using 5-minute scan samples, and during the test period using continuous focal sampling. Heart rate data was edited manually. Single or double interbeat intervals were considered anomalous if they differed by more that 20% of the beats immediately surrounding it. And replacement intervals were calculated. Segments of data that contained more than 2 collateral error points were not edited and excluded from the analysis. Frequency- and time-domain analyses were performed for 5-, 30- and 60-minute epochs as well as for 5-minute epochs of specific behaviours. Subsequent data analysis was carried out using the general linear model repeated measures procedure. Post hoc comparisons were carried out using Tukey's honestly significant difference test to determine which means differed.

Results Overall, there was a decrease in general activity levels with increasing stage of gestation. The proportion of observations where the gilts were observed rooting ($F=7.2$, $P<0.001$), standing ($F=7.70$, $P<0.001$ – see Figure 1) and walking ($F=5.8$, $P<0.001$) all decreased with increasing stage of gestation. Initially, all gilts spent more time lying sternally than lying laterally. Increasing gestation resulted in a decrease in lying sternally ($F=2.6$, $P=0.022$) with a concomitant progressive increase in the time spent lying on their side ($F=6.3$, $P<0.001$). Despite a decrease in general activity over gestation, mean heart rate increased by more than 15% ($F=4.64$, $P<0.01$ – see Figure 2).

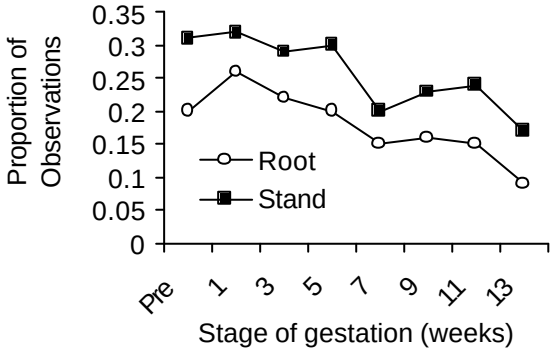

Figure 1: Gestation related changes in the proportion of observations gilts were rooting and standing

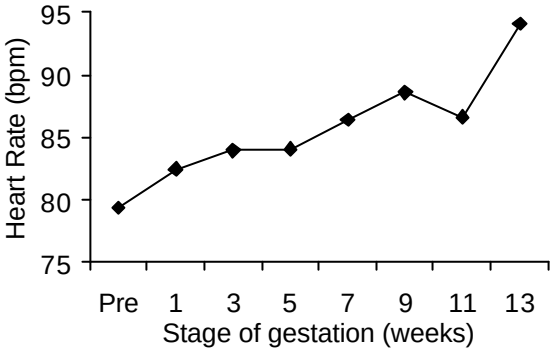

Figure 2: The effects of stage of gestation on mean heart rate

Gestation resulted in a 70-75% decrease in total heart rate variability illustrated by a decline in the total power ($F=9.73$, $P<0.001$) of the power spectral densities generated by Fast Fourier Transform. This decline in total power was reflected in all components of the spectral density and a very strong second order polynomial relationship was found between gestation induced changes in total power and mean heart rate ($R^2=0.89$, $P<0.001$). Sympathetic activity increased with stage of gestation ($F=3.94$, $P<0.001$).

Conclusions The methods for calculating HRV and the parameters used in human research are also suitable for assessing autonomic nervous activity in pigs and thus have potential as non-invasive tools to help in the assessment of pig welfare. There are gestation-induced changes in behaviour, mean heart rate, and autonomic regulation of cardiac activity, with overall activity decreasing, mean heart rate increasing and heart rate variability decreasing.

Acknowledgement We thank Richard Hodgkinson and staff of DMU Lincolnshire Farms Ltd Pig Unit for their help in this study.

Influence of group size on the performance and behaviour of 4 to 10 week old pigs

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Introduction Increasing the group size of weaned pigs can lead to more efficient use of resources by producers. However research with finishing pigs suggests that increasing group size can also lead to increased aggression and reduced performance (Spoolder *et al*., 1999). The objective of this study was to assess the effect of group size on the performance and behaviour of weaned pigs.

Materials and method In a randomised block design, one thousand, two hundred and eighty Large White x Landrace pigs were allocated to one of five group sizes from weaning at 4 weeks of age until 10 weeks of age. Eight blocks were used, each containing a group of ten, twenty, thirty, forty and sixty pigs. Each group was balanced for gender and weight. Pigs in each group were divided arbitrarily into heavy, medium and light categories on the basis of weaning weight and marked accordingly. The pigs were housed in combined Stage 1/Stage 2 accommodation with plastic slatted floors, thermostatically controlled ventilation and a space allowance of 0.38 m^2 per pig. One four-space feeder and separate drinking bowl was provided per ten pigs. All pigs were weighed individually at weaning and at 10 weeks of age. Variation in weight within groups was calculated as the difference in weight between the heaviest and lightest 20 per cent of the group at 10 weeks of age. Feed intakes were recorded weekly and feed conversion ratios calculated. One feeder in each group was videotaped for a continuous 24 hour period during the first, third and fifth week of treatment. The tapes were scanned at 20 minute intervals and the number of small, medium and large pigs at the feeder was recorded. In addition, aggressive behaviours in the form of fights, headthrusts and displacements from the feeder were recorded for 30 seconds at each sampling interval. Performance data were analysed by covariance analysis. Weaning weight was the covariate in the analysis of feed intake, feed conversion and growth rate, and variation in weight at weaning was the covariate in the analysis of variation in weight at 10 weeks. Analysis of variance was used to compare behavioural data between treatments. A chi-squared test was used to assess whether the number of small, medium and large pigs observed at the feeder differed from expected values. Data were analysed using Genstat 5.

Results Performance results are given in Table 1. There were no significant differences between treatments in the growth rate of medium or large pigs, or in overall growth rate, feed intake or feed conversion values. Variation in weight within groups was significantly greater in groups of ten than in larger groups ($P<0.05$). This was related to the fact that small pigs in groups of ten tended to show lower growth rates than small pigs in larger groups ($P=0.1$).

Table 1 *Performance of pigs between 4 and 10 weeks of age and variation in weight between heaviest and lightest 20 per cent of pigs in group at 10 weeks of age*

	Group size						
	10	20	30	40	60	s.e.m.	Significance
Feed intake (g/d)	770	746	788	774	808	38.9	
FCR	1.42	1.43	1.50	1.51	1.52	0.039	
DLWG (g/d)							
Small	449	510	517	515	513	20.2	0.1
Medium	577	540	519	546	536	16.8	
Large	598	566	537	564	571	18.4	
All	543	540	524	544	540	13.7	
Weight variation at 10 weeks of age (kg)	14.9[a]	12.6[b]	11.9[b]	12.7[b]	11.3[b]	0.70	*

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

There were no significant differences between treatments in the incidence of aggressive behaviour. The number of small and medium pigs observed at the feeder was higher and the number of large pigs observed at the feeder was lower than expected values in each treatment. This was significant ($P<0.01$) in all treatments except in groups of thirty pigs.

Conclusions These results suggest that the group size of weaned pigs can be increased from ten to sixty animals without any adverse effects on performance or welfare as measured by aggression at the feeder. Increasing the group size to more than ten animals appears to facilitate feeding by smaller animals and reduce within-group variation in weight. This may be due to the greater choice of feeders in larger groups which allows smaller pigs to feed away from larger animals. The greater incidence of small and medium pigs at the feeder in all treatments may suggest a slower rate of eating among these animals.

Acknowledgements The authors gratefully acknowledge funding from the Northern Ireland Pig Producer Research Levy and the Department of Agriculture and Rural Development for Northern Ireland.

Reference

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The effects of prior experience of straw and depth of straw bedding on the behaviour of growing pigs

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Introduction UK legislation dictates that all pigs shall have access to straw or other material or object suitable to satisfy their behavioural needs (Welfare of Livestock Regulations, 1994). However, it is unknown how much straw must be provided to be behaviourally rewarding, and whether pigs' prior experience of straw can impact upon their subsequent behavioural needs. Therefore, the aim of the current experiment was to investigate these issues by exposing pigs which did, or did not, have prior experience of straw to four levels of straw bed depth.

Materials and methods Thirty-two groups of 10 male and female growing pigs (initial live weight=27.2±2.49kg) were each exposed to one of eight experimental treatments such that each treatment was replicated four times. A 2 x 4 factorial design was used to investigate the effects of two levels of prior straw experience (N: no prior experience of straw and S: straw based housing from birth until selection for trial), and four levels of straw provision (N: none, M: 0.92±0.16kg/group/day, S: 10.92±0.52kg/group/day and D: 21.84±1.04kg/group/day). Observations of pig behaviour were made using two methodologies over a period of 10 weeks using a dedicated ethogram of straw-directed behaviours developed in a previous experiment. First, the behaviour of three focal animals in each group was sampled using direct *ad libitum sampling* for 10 minutes each week. Second, pig activity and straw- and penmate-directed behaviour was recorded over a 24h period in weeks one, five and nine using *time sampling* with a 10 minute interval. The experimental data were analysed using repeated measures ANOVA.

Results Groups of pigs spent a larger proportion of their active time interacting with penmates in the barren environment compared to their straw bedded counterparts (0.13, 0.09, 0.08, 0.07 for N, M, S and D groups respectively; SED=0.009; P<0.001). With prior experience of straw, the groups bit other pigs more in barren environments than groups of pigs with no such prior experience (Figure 1). When pigs had no prior experience of straw, the expression of tail-biting was elevated for three weeks after being moved into growing/finishing accommodation (Figure 2; $P<0.05$), although there were no statistically significant interactions between prior experience and straw depth.

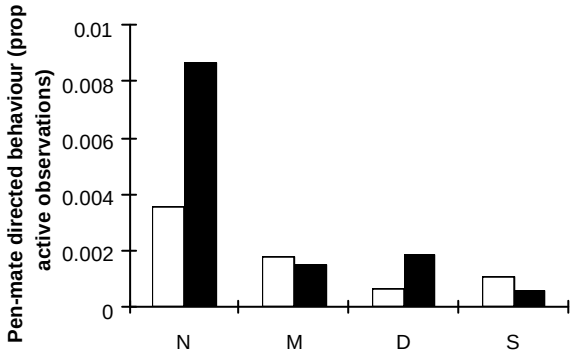

Figure 1: The interaction between prior experience and straw bed depth on the proportion of active behaviour which was directed towards pen-mates. Values represent means for groups with (■), or without (□) prior experience of straw (SED=0.001).

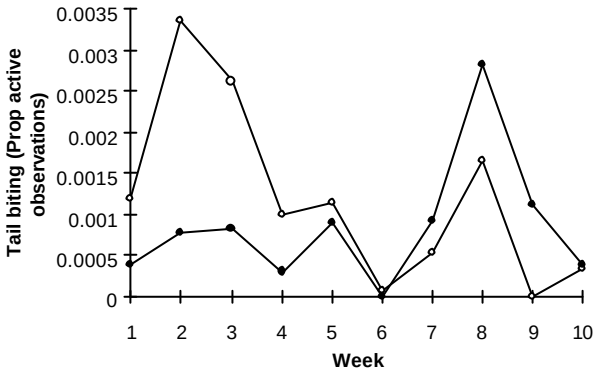

Figure 2: The interaction between prior experience and week on the proportion of active behaviour which was spent tail-biting. Values represent means for groups with (•), or without (o) prior experience of straw (SED=0.0009).

Conclusions These results suggest that moving pigs from previously strawed accommodation to unstrawed accommodation increases the occurrence of adverse pen-mate directed behaviour, and that when pigs have had prior experience of straw, even a small quantity of straw in the growing/finishing accommodation may serve to ameliorate the negative effects of the environmental challenge.

Acknowledgements This work was funded by MAFF.

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Validation and development of a behavioural test to predict the predisposition of growing pigs to perform harmful social behaviour such as tail biting

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Introduction Harmful social behaviour is behaviour that is directed at pen mates which, if persistent, causes injury. It includes tail, ear and flank biting. The aim of this work was to determine if pigs that are predisposed to harmful social behaviour can be identified early in life using a test. In this experiment a test that measured the chewing behaviour of pigs on an artificial tail was developed from Fraser (1987), as one of a wider battery of tests under investigation in the overall project. The validity of the test was evaluated by correlating the rope directed behaviour in the test with harmful social behaviour in the home pen.

Material and Methods A total of 166 pigs from 41 first cross LW/LR dams mated with 25 different sires and were studied from birth to slaughter (weeks 1 to 20). There were a total of 4 farrowing periods at three-week intervals. Pigs were weaned at 4 weeks. After weaning the pigs were put into groups of 8, consisting of 2 litters with 4 pigs (2 boars and 2 gilts) from each litter. Half of each litter within each group were experimental (1 boar and 1 gilt) and half were nonexperimental (1 boar and 1 gilt). The pigs were reared in standard commercial housing and received a standard commercial diet. The resident pen behaviour of each of the experimental animals was observed over a 5-minute period once each week between 1300 and 1630h from week 1 to 20. The frequency of occurrence and duration of harmful social behaviours, including, tail biting, ear biting and pig nosing displayed by the focal animal was recorded using a hand-held Psion. The rope chew test was carried out when pigs were 4 weeks of age. Pigs were tested individually for 10 minutes in the creep area of the farrowing pen. Two 0.5m pieces of pliable rope, one soaked in a 5% concentration of Sodium Chloride and the other plain, were hung over the edge of the creep partition so that they were approximately 10 cm off the ground. The exploratory, rope-directed and other behaviours of the piglet were video recorded. The data were analysed using Genstat, version 5. Pearson's product-moment correlations were calculated between behaviours in the rope chew test and the resident pen behaviour in subsequent weeks, and between different behaviours within each situation.

Results The time spent tail biting in the resident pen was positively correlated with the time spent performing other harmful social behaviour; nosing pig ($r=0.275$, $P<0.01$), ear biting ($r=0.182$, $P<0.05$) and genital anal nosing ($r=0.209$, $P<0.05$). There were also positive correlations between the frequency of tail biting and the number of times the behaviours, nosing pig ($r=0.411$, $P<0.01$), belly nosing ($r=0.370$, $P<0.01$), ear biting ($r=0.364$, $P<0.01$) and genital anal nosing ($r=0.265$, $P<0.01$) were performed. These harmful social behaviours were significantly positively correlated with a number of behaviours in the rope chew test (Table 1).

Table 1 *Correlations between rope directed behaviours in the rope chew test and harmful social behaviours in the resident pen (RP) in the weeks subsequent to the tail chew test*

Harmful social behaviour in RP	Rope directed behaviours in tail chew test				
	Sniff salty rope	Chew salty rope	Manipulate plain rope	Contact plain rope	Rope directed behaviour
Nosing pig (duration)	0.269**	0.256**			0.201*
Ear biting (duration)	0.245**	0.393**	0.223*		0.248**
Genital anal (duration)	0.252**				0.252**
Belly nosing (frequency)				0.331**	0.331**
Genital anal (frequency)			0.258**		0.258**

* $P<0.05$; ** $P<0.01$

Conclusions The results of the present experiment suggest that rope directed behaviour observed in the rope chew test at 4 weeks of age is predictive of a pig's predisposition to perform harmful social behaviours. Tail biting was consistently positively correlated with other harmful social behaviours and these behaviours were positively correlated with rope directed behaviours. It is therefore concluded that pigs that perform high levels of rope directed behaviour in the tail chew test, may be more likely to perform harmful social behaviour in the resident pen in subsequent weeks.

Acknowledgements The authors gratefully acknowledge funding from Ministry of Agriculture, Fisheries and Food.

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Body tissue changes in Scottish Blackface ewes during one annual production cycle

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Introduction Fat and muscle reserves are mobilised during winter by breeding hill ewes, living in harsh environments, as they conceive and carry their lambs (Russel *et al*., 1968). Tissue depletion and repletion were studied in Scottish Blackface ewes through one productive cycle using computed tomography (CT). The aim was to use this non-destructive method to quantify patterns of change in carcass fat, carcass muscle and internal fat in ewes of differing age and reproductive status.

Materials and Methods Ewes were scanned on five occasions: pre-mating in 1997, pre-lambing, marking (mid-lactation), weaning and pre-mating in 1998. Only animals (n=143) that reared the same number of lambs as they gave birth to were included in the dataset. For each animal at each event cross-sectional CT scans were taken at five anatomical sites – ischium, hip, 5th lumbar vertebra, 2nd lumbar vertebra and 8th thoracic vertebra. A total of 3575 CT images were analysed to yield areas of carcass fat, carcass muscle and internal (non-carcass) fat. Prediction equations were derived for weights of these three tissues from a separate calibration dataset (n=30 ewes; RSD = 368g, 836g and 714g for carcass fat, muscle and internal fat respectively). Animals were of two age groups - 110 gimmers (2 years old) mated for the first time (8 barren, 73 single- and 29 twin-bearing), and 33 older ewes mated for the second time (1 barren, 19 single- and 13 twin-bearing). Least square means were produced for predicted total carcass fat, carcass muscle and internal fat at each scanning event using the restricted maximum likelihood procedure in Genstat v.4.1 (Lane and Payne, 1996). Ewe age, number of lambs born, and their interaction were fitted as fixed effects.

Results Results are summarised in Figure 1. The pattern of tissue mobilisation of single- and twin- bearing ewes did not differ significantly, due to preferential feeding of twin-bearing ewes through pregnancy and lactation. Therefore, results were combined for all ewes with lambs. From pre-mating to pre-lambing a similar weight of fat was lost from carcass and internal depots in pregnant ewes, but muscle changes were negligible. From pre-lambing to marking (early lactation) loss of carcass fat weight was similar to the loss during pregnancy, but loss of internal fat was much less, since this depot had been extremely depleted by lambing. Muscle weight loss was similar to that of carcass fat during this period. Loss of fat during pregnancy comprised a much larger proportion of internal fat than carcass fat (67% vs. 22%). During early lactation internal fat was reduced by a further 12% (of pre-mating weight) versus 25% for carcass fat. On this basis, internal fat was more labile during pregnancy and carcass fat more labile during early lactation.

In late lactation all three tissues were repleted with absolute gains of muscle exceeding those of the two fat depots, which were similar. However, relative to tissue size, internal fat gains were greater than those of the two carcass tissues, which were similar. Post-weaning, muscle gains were negligible while carcass fat gains were greater than those of internal fat. These patterns of gain provide further evidence that internal fat is the more labile store of energy.

Barren ewes had significantly higher levels of fat and muscle, from pre-lambing onwards, than ewes bearing lambs (P<0.05). Gimmers had less carcass fat than older ewes (P<0.05) from pre-lambing onwards and less internal fat (P<0.05) at marking and weaning, although both age groups showed similar patterns of tissue change. Muscle weight did not differ significantly between age groups at any scanning event.

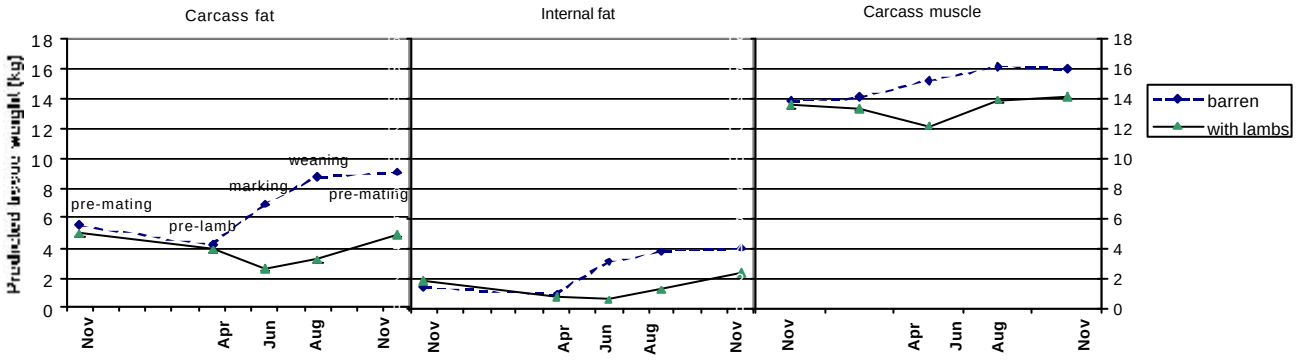

Figure 1 *Changes in predicted body tissue weights through one annual production cycle*

Conclusions Internal fat is a more labile store of energy than carcass fat or carcass muscle. This conclusion leads to two questions: (i) Where animals partition energy differently between carcass and internal depots, or where they differ in the differential depletion of these energy stores, is this related to their ability to produce milk and nurture lambs? (ii) Does the relative size of different fat depots influence the degree to which they are mobilised?

Acknowledgements We thank MLC, SERAD and BWMB for funding and Kirsty McLean for data collation.

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Annual depletion and repletion of carcass fat depots in Scottish Blackface ewes

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Introduction To what extent different fat depots of the carcass are more or less useful to the animal as readily labile energy stores is not clear. A knowledge of how ewes partition and use fat within the carcass is vital in order to estimate the impact of selection to reduce subcutaneous fat on other carcass fat depots. This study was designed to describe changes in the different carcass fat depots over one annual production cycle.

Materials and Methods Data were available for 126 Scottish Blackface ewes which had each been CT (computer tomography) scanned five times over one year as part of a larger study looking at changes in carcass muscle, total carcass fat and internal fat. CT scans from the latter study were subjected to further image analysis to separate subcutaneous fat (SCF) from intermuscular fat (IeMF) and measure tissue areas. Two cross-sectional scan positions per sheep were analysed, one through the caudal ischium and one through the 8th thoracic vertebra. Weights of SCF and IeMF in the half–carcass were predicted from the fat areas measured. Intramuscular fat (IaMF) in the half-carcass was predicted from the density of muscle in these scans (Young, unpublished data) and predicted muscle weight.

The five scanning events corresponded to pre-mating (day 1), late pregnancy (day 128), mid-lactation (day 208), weaning (day 290) and pre-mating (day 367). Thus changes in four periods were considered, namely pregnancy, early lactation, late lactation and post-weaning.

Each ewe gave birth to and reared either zero, one or two lambs. Data were analysed using GLM procedures. A simple model was fitted including number of lambs reared (either 0, 1 and 2 or 0 and 1+) and age of ewe (2 or 3).

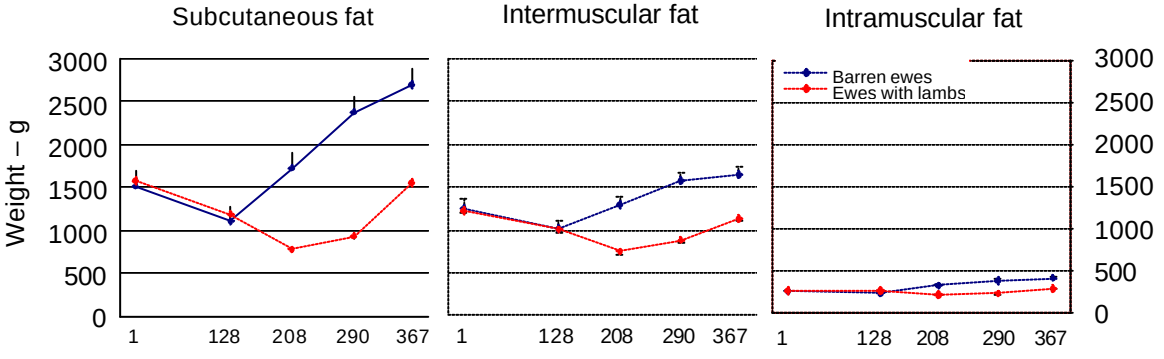
Results Carcass fat depots were differentially mobilized during pregnancy and early lactation (Figure 1).

For each fat depot, differences between ewes with one lamb and those with two lambs were small and largely non-significant throughout the period studied. Consequently data for ewes with lambs were grouped. Lack of a difference between ewes with different numbers of lambs was attributed to management. Ewes with lambs were preferentially fed in late pregnancy and during lactation.

From pre-mating until late pregnancy there was no significant difference in fat weights between barren ewes and those with lambs. However, barren ewes had higher weights for all fat depots than ewes with lambs for all scan events from day 208 onward ($P<0.05$). In contrast, ewes with lambs continued to mobilize fat during early lactation. SCF was most severely depleted (50%), followed by IeMF (39%) and IaMF (19%) ($P<0.05$).

In ewes with lambs, repletion began in late lactation and accelerated post-weaning leading to each depot fully recovering the starting weight by day 367. Barren ewes exceeded their starting fat weights to varying degrees by day 367 (SCF, +77%; IeMF, +31%; IaMF, +62%).

Figure 1. Barren ewes compared to ewes with lambs. *Plot of least square means(with standard errors) for predicted half-carcass fat weights at five times during annual production cycle.*



Conclusions Subcutaneous fat is the largest and most lab epot. However, a considerable amount of fat is also lost as intermuscular fat. In contrast, intramuscular fat is not an important lipid store because although it shows a similar pattern of change to the other fat depots, both the relative and absolute changes are small. Condition scoring, which is most closely related to subcutaneous fat is a good indicator of total fat change in the carcass.

Preferential feeding can offset the greater depletion in carcass fat commonly seen in ewes rearing multiple lambs compared to those rearing one lamb.

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The effect of midwinter shearing on rumen digesta kinetics in highly prolific crossbred ewes

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Introduction The effective rumen degradability (ERD) of dietary protein is used to quantify the amount of dietary protein entering the small intestine of ruminants. Midwinter shearing of prolific ewes increases rumen degradation rate (Kd) of dietary protein (Šebek and Everts, 1999). However, these differences in Kd may be compensated by a different rumen passage rate (Kp), since cold exposure is often associated with increases in Kp. Kp can be estimated by use of an undegradable marker. This method, although reliable for fluids seems less reliable for solids. The objective of the present experiment was to quantify the effect of midwinter shearing on ERD in highly prolific ewes. As an alternative to the use of Kp, ERD of crude protein (CP) and of protein-free-organic-matter (OM_CP) were estimated from periodical changes in rumen volume partitioned as rumen degradable (Xd) and rumen undegradable (Xu) components.

Materials and methods 10 mature barren ewes of a prolific crossbred were fitted with a rumen cannula and housed indoors. The ewes were offered 980 g dry matter (DM) of grasshay and 350 g DM of concentrates daily. They were fed at 8.00 h and 20.00 h. The treatments (unshorn and shorn) were applied consecutively to each animal. In shorn ewes measurements were performed during the fifth week after shearing at a mean daily ambient temperature of 3.7 (s.d. 3.4) ºC. During 5 days the rumen was emptied once daily at either 1, 2, 4, 8 or 12 hours after morning feeding. The sampling procedure was randomised across sampling times. Partitioning of rumen content (Xd and Xu) was based on *in situ* rumen incubation of samples of rumen content. One rumen content sample was washed to estimate the soluble fraction Xs and two samples were incubated *in situ* for 336 hours to estimate Xu. Xd was calculated as Xd=100–Xu–Xs. Each sample of rumen content was incubated in the same animal from which it had been obtained. ERD was calculated from measured periodical changes in Xd and Xu of rumen content using a model described by Aitchison et al. (1986). Total disappearance of Xd from the rumen fitted best as $RC_t = A + B * \exp^{\{-K*t\}}$; where t=time after feeding (h), RC_t= rumen content of Xd at t=t, A=estimated rumen content of Xd at t=∞, B=estimated amount of disappeared rumen content of Xd at t=∞, K=estimated fractional rate of disappearance of Xd from the rumen (h^{-1}). Differences between treatments were examined within ewes with a Student t-test.

Results In shorn ewes degradation was more enhanced (larger Xd fraction and smaller Xu and Xs fractions) than in unshorn ewes (Table 1). Combined with a smaller rumen pool size in shorn ewes these differences resulted in an increasing difference in rumen content of Xd with unshorn ewes (Figure 1).

	CP			OM_CP		
	Us	S	SED	Us	S	SED
Intake (g/day)	198	215	8*	801	878	36
Rumen pool (g)	214	178	10*	587	492	24*
ERD	0.49	0.54	0.072	0.36	0.49	0.061
Xd	0.45	0.48	0.013*	0.52	0.56	0.007*
Xu	0.10	0.10	0.002	0.26	0.25	0.006*
Xs	0.45	0.42	0.015	0.22	0.20	0.006*

* = P<0.05

Table 1 Mean results unshorn (U) and shorn (S) ewes

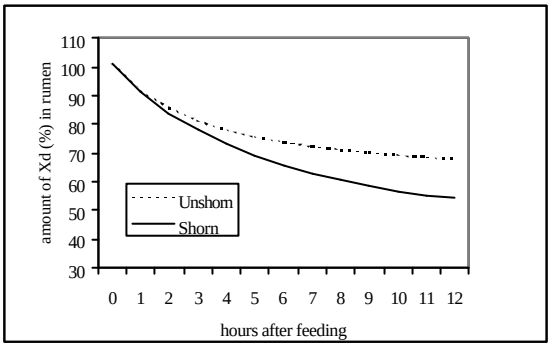

Figure 1 Disappearance of Xd_CP from the rumen

Shorn ewes degraded larger amounts of nutrients. This difference was supported by the higher estimated ERD for CP. This will result in a lower amount of feed protein reaching the small intestine. The larger amount of degraded OM_CP indicated increased growth of rumen microbes. Furthermore, cold exposure and smaller rumen pool sizes have been associated with an increased efficiency of microbial growth. Therefore it may be expected that shorn ewes have more microbial protein entering the small intestine which may compensate for the reduced duodenal flux of feed protein.

Conclusions The results of the present study demonstrate that midwinter shearing changes rumen digesta kinetics in ewes. Due to these changes less feed protein reaches the small intestine of shorn ewes. The results indicate that the reduction in feed protein entering the small intestine may be compensated by an increase in microbial protein synthesis. Therefore, the amount of protein reaching the small intestine of shorn ewes can not be estimated without assumptions for the efficiency of microbial protein synthesis. Protein evaluation for sheep should take account of these differences between unshorn and shorn ewes.

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Effects of protein source, formaldehyde treatment and rumen-protected methionine on the metabolism and performance of pregnant and lactating ewes fed straw

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Introduction Fishmeal is a suitable protein source for pregnant and lactating ewes, providing higher levels of undegradable protein than vegetable protein sources, with an improved biological value (Robinson, 1987). Vegetable protein sources may however be improved by formaldehyde treatment to reduce protein degradability and by the addition of rumen-protected amino acids. The objective of the current experiment was to compare the effects of feeding concentrates containing fishmeal with concentrates containing soya-bean meal, formaldehyde treated soya-bean meal and formaldehyde treated soya-bean meal with rumen-protected methionine.

Materials and methods At 103 days of gestation, 44 twin-bearing Charollais x Lleyn, Charollais x Cambridge, Friesland x Lleyn and Suffolk x Mule ewes were allocated to treatment by breed, age, weight and condition score. The four dietary treatments differed in the main protein source contained in the concentrate; soya-bean meal (S; 100g/kgDM), formaldehyde treated soya-bean meal (TS; 100g/kgDM: Sopralin™, Trouw Nutrition, UK), formaldehyde treated soya-bean meal with added methionine (0.75g/kg: Smartamine™ M, Rhône Poulenc, France; TSaa; 100g/kgDM); or fishmeal (F; 80g/kgDM). All concentrates were designed to be isonitrogenous (210gCP/kgDM) and isoenergetic (13.1 MJ/kgDM) and to produce an ERDP:FME ratio of greater than 11.5g/MJ in the concentrate during pregnancy. The ewes were individually penned and fed 0.6kg of concentrate at -6 weeks increasing to 1.1kg at lambing and 1.6kg from lambing to +4 weeks. Straw refusals were recorded and fresh offered at proportionally 1.25 of the previous calculated intake. Weekly blood samples were analysed for ß-hydroxybutyrate (BHB). Lamb birth and weekly live weights were recorded, along with initial colostrum and 21-day milk yields. Nitrogen degradability coefficients for the concentrates were determined by the method of Ørskov and McDonald (1979) using 4 rumen cannulated wether sheep. The experiment was analysed using analysis of variance.

Results Ewes fed F and TSaa had higher pre-partum straw intake ($P<0.01$) than ewes fed either S or FS and tended to have higher post partum intakes than ewes fed S ($P=0.063$; Table 1). Feeding diets containing formaldehyde treated soya-bean meal (TS) resulted in an improvement in calculated pre-partum and post partum metabolisable protein (MP) supply compared with diets containing untreated soya-bean meal (S). Further increases in calculated pre-partum MP supply were achieved by the addition of rumen-protected methionine (TSaa). No effect of treatment on litter birth weight was observed. However, ewes fed diet F had lambs with lower growth rates than those fed Tsaa ($P<0.05$). No effect of treatment on either 12-16 hour colostrum yield or on 21-day milk yield was observed. Ewes fed TSaa had higher 12-16 hour colostral yields of fat and protein ($P<0.05$) than those fed F, whilst ewes fed S and had higher pre-partum ($P<0.05$) and post partum ($P=0.051$) plasma BHB concentrations than ewes fed F or TS.

Table 1 *Calculated pre and post partum MP supply (g/d) for the total diet fed and ewe performance and metabolism*

	F	S	TS	Tsaa	s.e.d.	Sig.
Mean pre-partum straw intake *(g fresh weight/day)*	627	471	456	623	60.9	**
Mean post partum straw intake *(g fresh weight/day)*	802	553	667	827	108.3	NS
Calculated pre-partum MP supply	103	87	97	106	3.0	***
Calculated post partum MP supply	199	188	211	215	3.8	***
12-16 hour colostrum secretion rate *(g/h)*	78	98	102	101	12.1	NS
12-16 hour colostrum fat yield *(g)*	8.6	12.1	11.9	14.2	1.93	*
12-16 hour colostrum protein yield *(g)*	4.9	6.9	7.2	9.0	1.27	*
21 day milk secretion rate *(g/h)*	99	106	117	106	8.2	NS
Litter birth weight *(kg)*	8.04	8.90	8.22	8.93	0.465	NS
Lamb growth rate (0-28d) *(g/d)*	241	261	257	272	10.2	*
Mean pre-partum plasma BHB conc. *(mmol/l)*	0.54	0.97	0.62	0.75	0.124	*
Mean post-partum plasma BHB conc. *(mmol/l)*	0.58	0.82	0.56	0.73	0.103	NS

Conclusions Ewes fed concentrates containing fishmeal produced lambs with lower growth rates. Increases in calculated MP supply were achieved by addition of formaldehyde to soya-bean meal, whilst further addition of protected methionine increased pre-partum straw intake and 12-16 hour yield of colostral fat and protein. Fishmeal can be successfully replaced by untreated or formaldehyde treated soya-bean meal in concentrates fed to pregnant and lactating ewes.

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Effects of grass or concentrates on muscle fatty acids and flavour in 2 sheep breeds

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Introduction In previous research the benefits of grass feeding in lambs for healthiness (fatty acid composition) and flavour have been demonstrated (Fisher *et al*., 2000). This work examined a very short period of feeding grass or concentrates in a hill breed (Beulah) and a breed noted for meat production (Texel).

Materials and methods Forty female lambs, half pure Dutch Texel and half Beulah Speckled Face, were weaned at 3 months of age and used to examine the effects of breed and production system on carcass and meat quality (2 x 2 factorial design). Ten lambs of each breed were fed hay and a standard concentrate diet containing 160g/kg crude protein and 10 were grazed on improved upland grazing. After 7 weeks the lambs were slaughtered at the University of Bristol. Loin (*m.longissimus)* joints were conditioned at 1°C for 7 days after which steaks were displayed in modified atmosphere packs under retail conditions for measurement of lipid oxidation (shelf life), a muscle sample was used for fatty acid analysis and eating quality was assessed by the taste panel after grilling chops to 72°C internal temperature.

Results The lambs gained only 6.5kg body weight and produced 13kg carcasses (Table 1). The Beulahs had fatter carcasses than Texels which had a lower concentration of fatty acids (marbling fat) in muscle and higher proportions of the PUFAs 18:2, 18:3 and 20:5. Grass-fed animals had higher proportions of the n-3 PUFA (18:3 and 20:5) and conjugated linoleic acid (CLA) but less 18:2. Lipid oxidation after 7 days display was similar in the two breeds but animals fed concentrates had much greater oxidation than those fed grass. There were few differences in flavour scores between the breeds (Table 2) but several differences between the production systems, with grass-fed animals having lower abnormal lamb flavour and higher overall liking.

Table 1. Weight gain and carcass data. Muscle fatty acid concentration (g/100g muscle) and composition (g/100g fatty acids)

	Breed				Production system			
	Texel	Beulah	s.e.d.		Grass	Concentrates	s.e.d.	
Live wt gained (kg)	6.1	7.0	0.93	NS	5.4	7.7	0.93	*
Carcass wt (kg)	14.4	12.2	0.58	***	12.6	14.0	0.58	*
Fat class (0-20)	5.2	8.5	0.66	***	6.1	7.5	0.66	NS
Conformation (1-5)	4.4	2.0	0.16	***	3.0	3.4	0.16	*
Total fatty acids	1.5	2.5	0.16	***	2.2	1.9	0.16	*
18:2 n-6	8.3	4.9	0.50	***	5.4	7.9	0.50	***
18:3 n-3	2.3	1.7	0.11	***	2.6	1.4	0.11	***
20:5 n-3	1.7	1.2	0.12	***	1.6	1.3	0.12	*
CLA	1.3	1.7	0.13	**	1.8	1.2	0.13	***

Table 2. Lipid oxidation at 7d of display (mg malonaldehyde/kg meat) and scores for flavour descriptive terms (0-100)

	Breed				Production system			
	Texel	Beulah	s.e.d.		Grass	Concentrates	s.e.d.	
Lipid oxidation	0.42	0.80	0.20	NS	0.21	1.01	0.20	***
Lamb	24.6	28.1	2.74	NS	28.9	23.9	2.74	NS
Abnormal lamb	22.0	24.0	2.59	NS	19.9	26.1	2.59	*
Metallic	4.7	6.2	1.52	NS	3.4	7.5	1.52	**
Rancid	6.7	8.2	1.46	NS	5.9	9.1	1.46	*
Livery	4.1	6.5	1.46	NS	2.8	7.8	1.46	***
Soapy	8.8	11.0	1.77	NS	7.3	12.6	1.77	***
Overall liking	18.2	20.2	1.70	NS	21.7	16.7	1.70	**

Conclusions There were important difference in meat yield, composition and eating quality between the breeds and production systems after the short growth period. Texels and grass-fed lambs had high proportions of n-3 PUFA in muscle. Texels also had a high proportion of 18:2 n-6, similar to lambs fed concentrates. However, the differences in flavour between production systems were much greater than those between breeds. The results suggest that lower oxidative breakdown of muscle lipids in grass-fed animals is important, perhaps due to greater consumption of natural antioxidants. Grass feeding also increases CLA levels which, along with n-3 PUFA, are beneficial in human nutrition.

Acknowledgement J S Hewerdine was supported at Bristol as an MLC scholar. MLC also funded the project.

Reference

Fisher, A.V., Enser, M., Richardson, R.I., Wood, J.D., Nute, G.R., Kurt, E., Sinclair, L.A. and Wilkinson, R.G. 2000. Fatty acid composition and eating quality of lamb types derived from four diverse breed x production systems. Meat Science 55, 141-147.

Vitamin E supplementation and meat quality in lambs

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Introduction Colour deterioration and lipid oxidation are major factors limiting the shelf life and acceptability of meat. Vitamin E slows down these processes and meat from animals fed supranutritional amounts of vitamin E has an increased shelf life. However, vitamin E supplementation in sheep has produced variable results (Enser *et al*., 1999). This work shows the effects of supplementing vitamin E on the concentration in plasma over a 63 day feeding period and on meat quality.

Materials and methods Five groups of 8 Suffolk × Charollais wether lambs (mean liveweight 24.8 ±1.6 kg), which came off grass, were individually penned and randomly allocated by liveweight to a dry pelleted diet based on wheat, molassed sugar beet pulp, soyabean meal and rapeseed meal supplemented with either 30, 60, 120, 250 or 500 mg/kg DM ? -tocopheryl acetate. Diets were fed *ad libitum* following a 5 day adaptation period. Blood samples were obtained weekly to monitor plasma vitamin E. After slaughter samples (m. *semimembranous*) were taken for muscle vitamin E content. Vitamin E analysis was conducted by HPLC. For colour assessment during display and oxidative stability 15mm thick leg steaks were aged for 6 days in vacuum at 0°C, repacked in modified atmosphere (O_2:CO_2, 0.75:0.25) and displayed under light (cool white fluorescent illumination with 700lx, 16hr on/8hr off) at 4°C for 6 days. M. *semimembranosus* colour was determined daily using CIELAB L*a*b* colour space and oxidative stability was determined as thiobarbituric acid reacting substances (TBARS) after 3 and 6 days of display**.**

Results Vitamin E levels were low at the start of the trial and decreased during the first 2-3 weeks at all levels of supplementation (Figure 1). Except for the 30mg supplement, plasma levels subsequently increased but only on the 500mg supplement were final values higher than those at the start of the trial. However, both plasma ($p<0.001$) and muscle ($p<0.001$) vitamin E levels were highly correlated with the dietary vitamin E levels in slaughter samples. Colour during display at day 6 was slightly better ($p<0.05$) at the 250mg and 500mg supplementation level but TBARS were significantly affected ($p<0.001$) by the vitamin E level on both display days (Table 1)

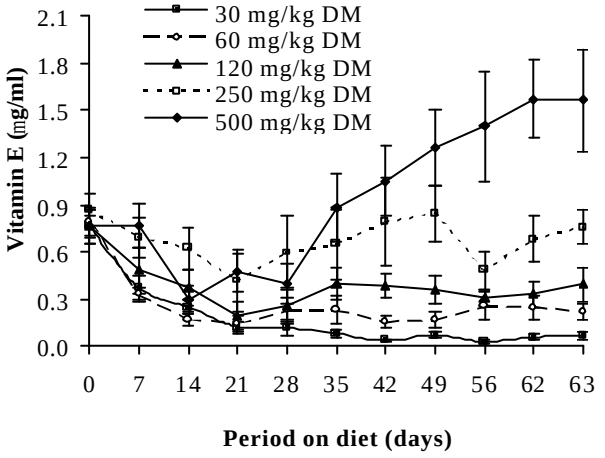

Figure 1 *Plasma vitamin E levels during the feeding period in relation to vitamin E supplementation level*

Table 1 *Influence of dietary vitamin E level on plasma and muscle vitamin E concentrations at slaughter and lipid oxidation (means and standard errors of the mean)*

	30mg	60 mg	120mg	250mg	500mg
Plasma vit. E(µg/ml)	0.06	0.22	0.39	0.76	1.56
(SEM)	(0.02)	(0.05)	(0.10)	(0.11)	(0.32)
Muscle vit. E(µg/g)	0.67	1.01	1.39	2.33	3.41
(SEM)	(0.06)	(0.06)	(0.14)	(0.22)	(0.38)
TBARS Day 3[1]	1.21	0.94	0.28	0.10	0.05
(SEM)	(0.21)	(0.14)	(0.07)	(0.03)	(0.01)
TBARS Day 6[1]	2.43	1.98	0.56	0.19	0.07
(SEM)	(0.39)	(0.26)	(0.15)	(0.04)	(0.01)

[1] mg malonaldehyde/kg muscle

Conclusions Lambs fed on a complete dry pelleted feed require at least 500 mg α-tocopheryl acetate/kg DM to produce muscle concentrations in excess of 3.3 µg/g required for optimum meat quality (Faustman *et al*., 1989). The causes of the low deposition of dietary α-tocopherol under these dietary conditions remain to be established.

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Effects of diet and time on feed on phospholipid fatty acid composition and beef meat flavour

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Introduction The polyunsaturated fatty acids (PUFA) of the intramuscular phospholipids are the most significant lipids in the development of cooked beef flavour (Mottram and Edwards, 1983). In lamb, differences in n-6 and n-3 fatty acid composition have been shown to be a major factor influencing consumer perception and acceptability (Sañudo *et al*, 2000). Altering meat PUFA, especially n-3 fatty acids, to improve its value in human nutrition (COMA, 1994) may have effects on flavour and acceptability. The aim of this work was to evaluate differences in flavour perception when intramuscular fatty acid composition is changed in beef meat.

Material and methods Twenty eight Charolais cross steers, initial live weight 548 kg, were fed for 60 or 90 days before slaughter a diet containing 125 g/kg whole linseed (n-3 source) or 280 g/kg full fat soya (n-6 source). At 48 h post mortem, the loin joint was dissected and 2-cm thick steaks of m. *longissimus* were obtained, frozen immediately and kept at –20 ^{o}C for fatty acid analysis (GC) of the phospholipid fraction and for flavour volatile analysis. The rest of the joint was conditioned for 10 days prior to being vacuum packaged and frozen to await sensory analysis. After thawing at 4 ^{o}C for 24 h, 2-cm thick chops were prepared and grilled at 160 ^{o}C to an internal temperature of 74 ^{o}C. A 10-member trained taste panel assessed beef flavour intensity on a 0-100 line scale. Dissected muscle was grilled and 5g transferred into a conical flask kept at 60 ^{o}C and swept with nitrogen at 40ml per min for 60 minutes to transfer volatiles onto a Tenax trap. Volatiles were analysed by gas chromatography/mass spectrometry. Analysis of variance was applied to the 2x2 factorial analysis design by GLM procedures of the SAS package.

Results Phospholipid fatty acid composition was influenced by diet with most differences occurring by 60 days (Table 1). Linseed fed animals contained more n-3 fatty acids and soya fed animals had a higher content of n-6 fatty acids, although differences in length of feeding were significant only for n-6 fatty acids ($p<0.05$). However, no significant differences were found in beef flavour intensity between linseed and soya fed animals or between periods on feed. There were few correlations with flavour compounds. Only the correlation between the content of total n-3 fatty acids and the proportion of sulphur-containing flavour compounds ($r=0.54$, $p<0.05$) was significant, possibly due to the higher instability of n-3 *versus* n-6 fatty acids providing appropriate precursors. Only alcohols among the group of volatile compounds showed significant differences between diets ($p<0.01$) and times on feed ($p<0.05$), but with a significant interaction between both effects ($p<0.001$) since with soya fed animals these decreased with time on feed while for linseed fed animals they increased.

Table 1. *Polyunsaturated n-3 and n-6 fatty acid composition of m.*longissimus *phospholipids (mg/100g muscle), sensory beef flavour score (0-100 scale) and the proportion (x100) of headspace volatiles in beef from steers fed linseed or soya for two times on feed (ToF, 60 and 90 days).*

	n	Linseed		Soya		sed	Significance		
		60 d	90 d	60 d	90 d		Diet	ToF	D*T
n-3 fatty acids	*7*	65.90	72.62	45.74	39.46	1.28	***	n.s.	**
n-6 fatty acids	*7*	96.42	107.04	139.65	154.10	2.95	***	*	n.s.
Beef flavour	*7*	24.0	27.6	26.7	22.0	2.86	n.s.	n.s.	*
Alcohols	*5*	2.69	5.18	3.11	2.63	0.21	**	*	***
Aldehydes	*5*	48.34	53.52	49.34	58.88	2.14	n.s.	n.s.	n.s.
Ketones	*5*	20.92	18.17	19.93	19.17	1.87	n.s.	n.s.	n.s.
N-containing	*5*	1.06	1.16	1.18	1.65	0.35	n.s.	n.s.	n.s.
S-containing	*5*	13.43	8.73	11.91	5.29	4.22	n.s.	n.s.	n.s.

n = number of animals per group; n.s.= no significant; * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$

Conclusion It is possible to produce a healthier beef meat high in n-3 fatty acids without significant changes in flavour assessed by a trained taste panel. Although n-3 polyunsaturated fatty acids are more susceptible to lipid oxidation than the n-6 PUFA, the higher total PUFA content (n-3 + n-6) in soya could explain the lack of significant diet effects. However, further studies should be done to understand the complexity of the chemistry of flavour and the multiple interactions of all meat components, including lipids, sugars and amino acids.

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Effects of breed, sex, degree of maturity and nutritional management on eating quality of lamb meat

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Introduction Quality of lamb meat is a topic of great importance to both retailers and consumers and has received considerable attention over the past few years (*Wood et al. 1999*). First of all, the theme is important for the future of sheep production. Sheep producers operate in competitive market and their survival is threatened because sheep meat is continually facing challenges to maintain/increase its market share. Second, it relates to the emergence of specific market desires. In this respect, it has been suggested that lamb meat, which meets the demands of individual markets, should be the objective if sheep production is to remain competitive (*Sanudo et al. 2000*). The objective of the current study was to investigate the effects of post-weaning nutrition and degree of maturity (or live weight at slaughter) on the eating quality of lamb meat produced by three dairy Greek breeds of sheep.

Materials and methods Lean samples of the leg joints of 69 carcasses of lambs of three indigenous dairy Greek breeds of sheep, the Boutsko (B), Serres (S) and Karagouniko (K) breed, were assessed in a series of three taste panel tests (TPT). For TPT 1, 24 lambs (4 intact males and 4 females of each breed) were used. They were reared in individual pens on a concentrate ration fed *ad libitum* and a small amount of Lucerne hay. The lambs used (27 intact males only) for TPT 2 were also individually penned but were fed on three different levels of concentrate (High, H, medium, M and Low, L) and *ad libitum* on Lucerne hay. The third TPT was performed by using 18 intact male lambs that were finished on irrigated sown pasture *(Lolium perenne + Trifolium repens)* after being group fed indoors for 63 days on three levels of concentrate (denoted also as H, M and L) together with *ad libitum* Lucerne hay. Lambs used in TPT 1 were assigned to be slaughtered at one of 4 standard proportions of mature weight (PMW; i.e. 0.30, 0.45, 0.60 and 0.90) for each breed; at three fixed live weights (TSLW; i.e. 23, 28 and 33 kg), common for all breeds for TPT 2 and at two fixed proportion of mature weight (PMW; i.e. 0.48 and 0.54) for each breed in TPT 3. The leg joints were oven cooked at 200°C until an internal temperature of 75°C was reached. Lean cubes, 2 x 2 x 2 cm in size, subject to taste panel evaluation were cut from *Musculus adductore* (n=9), *Musculus femoris* (n=5) and *Musculus semitendinosus* (n=6). Ten panellists were involved in TPT 1 and twenty in TPT 2 and TPT 3, respectively. Eating quality was evaluated in an eight-point scale (1 to 8) with larger scores indicating a more favourable rating. Four attributes were rated: Flavour, Juiciness, Tenderness, and Overall Acceptability. Data were analysed separately for each TPT by using an analysis of variance (ANOVA) model accounting for possible differences between the panellists.

Results Overall scores of all eating quality characteristics, between the three breeds, was reasonably high indicating good acceptance of meat from the lambs assessed; they were much heavier than the traditional ones in Greece. In TPT 1 panellists preferred the samples of female lambs (5.15 vs. 5.69 s.e.d. 0.093 for male and female lambs, respectively). Scores of eating quality characteristics, across sexes, decreased as the live weight at slaughter increased (6.07, 5.93, 5.64 and 4.08, s.e.d. 0.092, $P<0.001$; 5.23, 5.14, 4.99 and 4.80, s.e.d 0.128, $P<0.01$; 6.18, 6.04, 5.92, 5.71, s.e.d. 0.106, $P<0.106$ for Flavour, Tenderness and Overall Acceptability respectively for PMW of 0.30, 0.45, 0.60 and 0.90). None of the interactions with breed were statistically significant. In TPT 2, previous feeding treatment of lambs affected significantly the characteristics of their eating quality with respect to Juiciness ($P<0.001$), Tenderness ($P<0.01$) and Overall Acceptability ($P<0.05$). The average scores across breeds and TSLW were: 5.04, 5.56, 5.39 (s.e.d. 0.111, $P<0.001$) for Juiciness; 5.42, 5.77, 5.79 (s.e.d. 0.128; $P<0.01$) for Tenderness and 5.61, 5.93, 5.86 (s.e.d. 0.130; $P<0.05$) for H, M and L treatment, respectively. The samples of lambs fed on M levels of concentrate were rated with the highest scores with the exception of those from lambs that were slaughtered at 28 kg where the highest scores were observed when lambs were fed on L levels of concentrate. In TPT 3 there were no major differences between breeds in any category. Samples of lambs slaughtered at heavier weights (PMW: 0.54) were more acceptable, in relation to Flavour (5.29 vs. 5.63, s.e.d. 0.140), Juiciness (4.92 vs. 5.44, s.e.d. 0.122), Tenderness (4.98 vs. 5.61, s.e.d. 0.138) and Overall Acceptability (5.15 vs. 5.63, s.e.d. 0.118) than those from less mature lambs (PMW: 0.48).

Conclusions The results indicated that manipulation of post-weaning nutrition of lambs from Greek dairy breeds of sheep, based on locally produced feeds and irrigated sown pasture, can be used to produce carcasses with enhanced eating quality characteristics. The amount of concentrate in the diet and the degree of maturity of lambs at slaughter seem to have the most pronounced effects on the eating quality of lamb meat.

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On the diet selection of sheep: effects of adding urea to foods with different protein contents

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Introduction The basis of diet selection for protein in ruminants remains unclear. Tolkamp *et al*., (1998) proposed a hypothesis to account for the conflicting findings. They proposed that ruminants select their diet on the basis of effective rumen degradable protein (eRDP) rather than for MP yield. As the protein supply of ruminants comes largely from microbial protein, the idea that ruminants select a diet that meets the requirement for eRDP, which is captured and utilised by the microbes to produce microbial protein, is not an unreasonable one. The objective of this experiment was to test the hypothesis that both inadequate and abundant eRDP levels would be avoided by growing sheep when given choices of foods.

Materials and Methods. Three basal foods were formulated. D had deficient (50g eRDP/kg fresh matter (FM)), H adequate (100g eRDP/kg FM) and P excess (130g eRDP/kg FM) protein in relation to requirements. The fermentable metabolisable energy (fME) content of all 3 foods was 9.4MJ/ kg FM. 6 more foods were made by adding 12.5 (1) or 25 (2) g urea/kg FM to each of the 3 basals. Urea was used as a source of eRDP as it allows a change in the eRDP content of the food with no appreciable effect on the food ingredients. 96 female, Texel x Greyface sheep weighing 30 (s.d. 3.4)kg and individually penned were randomly allocated to 1 of 12 groups. Groups 1 to 6 (n=6) were offered a single food (D, D2, H, H2, P or P2) throughout the experiment (10 weeks). Groups 7 to 12 were given a choice (n=10) between 2 foods (6 weeks). One food was either D, H or P and the other food was the same basal food with either 12.5 or 25g urea/kg added (e.g. D v D1 or D v D2). The position of the foods was changed half way through the choice period to test if food position had an effect of diet selection. All choice fed sheep had the chance to experience separately the 2 foods allocated for 2 week periods prior to the choice being given. The liveweights of the sheep were measured on the first day of the experiment and weekly thereafter. The data for daily food intake and diet selection (expressed as the proportion of total food intake taken as the urea supplemented food) were analysed as a factorial design. Initial live weight was used as a covariate.

Results Adding urea to a food deficient in calculated eRDP (D) resulted in an increase in food intake ($p<0.05$) and live weight gain ($p<0.01$). Adding urea to a food calculated to satisfy the eRDP requirements (H and P) had no beneficial effects on intake (Fig. 1) or live weight gain. Sheep offered foods D, D2, H, H2, P and P2 had live weight gains of 149, 218, 278, 263, 325 and 299 (s.e.d. 21) g/d ($p<0.001$) respectively. Food intake of the choice-fed sheep was 1568, 1757, 2061, 2069, 1981 and 1988 (s.e.d. 119) g/d ($p<0.001$) for sheep offered DvD1, DvD2, HvH1, HvH2, PvP1 and PvP2 respectively. Across all 6 choice-fed treatments there was a highly significant preference ($p<0.01$) for the food with the urea supplementation (Fig. 2) with only 0.36 (s.e. 0.03)kg/kg of the selected diet as the non-urea supplemented food. The preference for urea was greater where the higher level was used. These preferences were unaffected by the protein contents of the foods used. When the position of the foods were changed the sheep followed the foods and therefore diet selection was not affected by food position.

Fig. 1. The daily food intake by single fed sheep offered basal foods differing in calculated eRDP content (D, H or P) and urea supplementation (0 or 25g urea/kg).

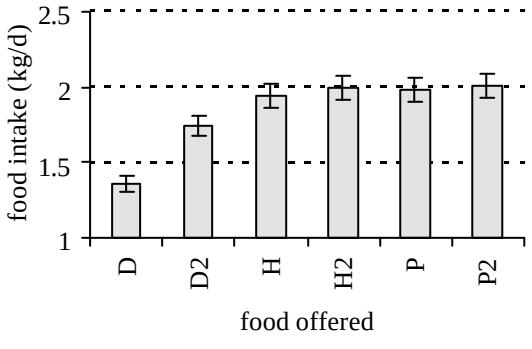
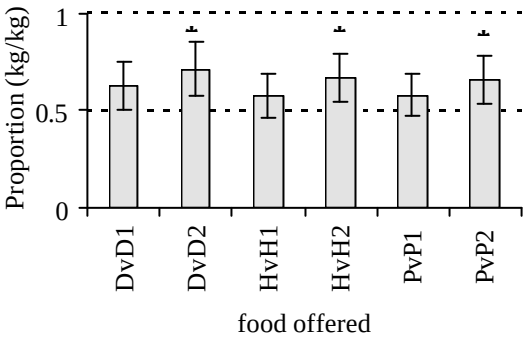


Fig.2. The proportion of urea supplemented food selected (kg/kg) by sheep offered a choice between an unsupplemented food (D, H or P) and the same food supplemented with 12.5 (1) or 25 (2) g urea/kg. *denotes that the diet selected differs significantly from random.



Conclusion Regardless of the eRDP content of the food to which urea was added the sheep selected for urea. The results do not support the hypothesis that sheep will avoid excess eRDP when given a choice and suggest that eRDP may not be the relevant dimension in all cases of diet selection in ruminants.

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On the diet selection of sheep: sodium bicarbonate modifies the effect of urea on diet selection

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Introduction It was hypothesised that both inadequate and abundant levels of effective rumen degradable protein (eRDP) would be avoided by ruminants given a choice of foods (Tolkamp *et al*, 1998). However, we have found that sheep do not appear to avoid the excess consumption of eRDP expected when they were offered a choice of foods that differed in eRDP content through the addition of urea to one of the foods (James *et al*., 2001). A possible reason could be due to the fact that the foods offered had low fibre contents. The sheep may have selected for the urea supplemented food in an attempt to buffer the rumen. The buffering ability of the urea could help to minimise the disruption of the rumen such as a fall in pH caused by the low fibre foods. The objective of this experiment was to test the hypothesis that the preference for the urea supplemented food would be reduced by the addition of sodium bicarbonate (buffer).

Materials and Methods. A basal food (H) calculated to meet the requirements of the sheep (100g eRDP/kg fresh matter (FM), 86g MP/kg FM and 9.4MJ fME/ kg FM) was formulated. Five more foods were made by adding 12.5 or 25g urea/kg FM to H and 20g sodium bicarbonate (SB)/kg to H and both the urea supplemented foods. Fifty six female, Texel x Greyface sheep weighing 35 (s.d. 4.3)kg and individually penned were randomly allocated to 1 of 8 groups (n=7). Groups 1 to 4 were offered a single food: H ,H+25g urea/kg, H+SB or H+25g urea/kg+SB for 4 weeks. Groups 5 to 8 were offered the choice H vs. H+12.5g urea/kg, H+SB vs. H+12.5g urea/kg+SB, H vs. H+25g urea/kg or H+SB vs. H+25g urea/kg+SB for 4 weeks. Food refusals were weighed and fresh food offered at 0830h daily. The live weights of the sheep were measured on the first day of the experiment and weekly thereafter. The data for daily food intake and diet selection (expressed as the proportion of total food intake taken as the non-urea supplemented food) were analysed as a 2x2 factorial design (urea*SB). Initial live weight was used as a covariate.

Results. There was no significant effect of single food treatment on food intake or live weight gain. Sheep consumed 2065, 2163, 2174 and 2225 (s.e.d. 118)g/d and gained 308, 332, 358 and 319 (s.e.d. 33)g/d when offered H ,H+25g urea/kg, H+SB or H+25g urea/kg+SB respectively. While not significant, the preference for the food supplemented with urea was greater when 25g urea/kg was added to one of the foods offered as a choice rather than when 12.5g urea/kg was added. Adding SB to both foods significantly ($p<0.01$) decreased the preference for the food supplemented with urea (Table 1). Choice between foods offered had no significant effect on food intake or live weight gain. Sheep gained 382, 363, 333 and 422 (s.e.d 64)g/d when offered the choice H vs. H+12.5g urea/kg, H+SB vs. H+12.5g urea/kg+SB, H vs. H+25g urea/kg or H+SB vs. H+25g urea/kg+SB respectively.

Table 1. The proportion of urea supplemented food (Food 2) selected and food intake by sheep offered a choice between H and H supplemented with urea (12.5 or 25g urea/kg), with or without SB supplementation (20g SB/kg).

Food 1		Food 2		Proportion of Food 2 selected	Food intake
Urea (g/kg)	SB (g/kg)	Urea (g/kg)	SB (g/kg)	(kg/kg)	(g/d)
0	0	12.5	0	0.480	2076
0	20	12.5	20	0.291	2089
0	0	25	0	0.695	2104
0	20	25	20	0.396	2217
			s.e.d	0.109	128
			Urea	NS	NS
			SB	**	NS
			Urea*SB	NS	NS

Conclusions Supplementing both foods offered as a choice with sodium bicarbonate altered the diet selection in agreement with the hypothesis, i.e. sheep avoided an excess of eRDP supplied by urea when sodium bicarbonate was supplied as an alternative buffer. This suggests that one of the objectives of the diet selection of ruminants could be to maintain the pH of the rumen within a certain physiological range and that sheep are prepared to consume an excess amount of eRDP supplied by urea in order to achieve this.

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The interactive effects of novel food flavours and food composition on the diet selection of sheep

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Introduction Animals have predispositions towards the organoleptic properties, such as flavours, of the foods available to them. These predispositions can influence the feeding behaviour and diet selection of animals and prevent from, or enable them to select a diet that meets their nutrient requirements, in both short - and longer - run (Forbes and Kyriazakis, 1995). In this experiment, we investigated whether predispositions of sheep for novel food flavours could affect their diet selection when offered a choice between two foods with different nutrient content. The specific hypotheses tested were that such predispositions are: (i) influenced by the nutritional quality of the food that are associated with, and (ii) affected by the current nutritional state of the animal.

Materials and Methods Forty-eight Texel x Greyface female lambs (41.3, sd 5.34 kg) were used. Two food (H and L with different CP (137 and 72 g CP/kg DM respectively) and similar ME supply (9.2 and 8.8 MJ/kg DM respectively) were formulated as pellets. Half of the sheep (n=24) were offered food H (group HP) and the other half were offered food L (group LP) *ad libitum* for 56 days. At the end of this preliminary period, which established animal state, sheep weighed 51.0 and 45.4 (P<0.001; s.e.d. 1.49) kg respectively. Subsequently, sheep were assigned to one of five choice feeding treatments balanced for animal state and live weight. The main experiment consisted of two Periods (I & II) each lasting 14 days. During Period I sheep from both states were offered either a choice between foods H and L or a choice between these foods with the addition of one of two flavours, Garlic (G) or Orange (O). The following choice treatments were considered: H *v.* L (n=4), HG *v.* L (n=5), H *v.* LG (n=5), HG *v.* LO (n=5) and HO *v.* LG (n=5). In Period II the order of association between foods and flavours in each choice treatment was reversed. Diet selection was expressed as a proportion of food H selected by sheep on each choice treatment over total intake. Data were analysed for both Periods and animal state by using a 2x2 analysis of variance (ANOVA).

Results When comparisons were made between the two Periods diet selection was significantly affected by Period x choice and Period x choice x state interactions (P<0.001 for both) indicating that it changed significantly with time between the two groups of sheep. Within the two Periods (Table 1) diet selection was significantly different between treatments (P<0.05 for both Period I & II) indicating that the addition of flavours to foods modified diet selection. When there were no flavours added (treatment L-H), diet selection did not differ significantly from random (0.5). In treatments with one flavoured food there was initially a preference for the unflavoured food, however, this preference declined with time. Where both foods were flavoured there was almost a dominant effect on diet selection by the O flavour that was a non-preferred flavour. In particular, such effects occurred when the O flavour was added to food L. The developed preferences as observed during Period I were disrupted after the reversal of the flavour-food associations in Period II. The total mean food intake between the five treatments did not differ significantly either in Period I (1995, 2156, 2120, 2293 and 1963 s.e.d. 166.8 g/day) or in Period II (2207, 2438, 2275, 2347 and 2159 s.e.d. 177.5 g/day) respectively.

Table 1 Means of the proportion of food H (g of H food consumed/g total food intake) selected by sheep of groups LP and HP, for Period I and Period II; associations between foods and flavours were reversed in Period II

Period I					Period II				
Choice	Group				Choice	Group			
treatment	LP	HP	Mean		treatment	LP	HP	Mean	
H *v.* L	0.50	0.46	**0.48**		H *v.* L	0.57	0.56	**0.56**	
HG *v.* L	0.59	0.58	**0.59**		H *v.* LG	0.63	0.54	**0.58**	
H *v.* LG	0.72	0.53	**0.63**		HG *v.* L	0.69	0.65	**0.67**	
HO *v.* LG	0.55	0.52	**0.54**		HG *v.* LO	0.67	0.67	**0.67**	
HG *v.* LO	0.73	0.71	**0.72**	(s.e.d. 0.081)	HO *v.* LG	0.51	0.37	**0.44**	(s.e.d. 0.076)
Mean	**0.62**	**0.56**	(s.e.d. 0.049)		Mean	**0.62**	**0.56**	(s.e.d. 0.046)	

Conclusion These findings contribute to a better understanding of how animals learn about foods available to them as a choice. It appears that the initial feeding responses of sheep towards novel foods could be affected by predispositions for specific food flavours. However, subsequent diet selection depends mainly of the induced post-ingestive consequences of foods even for initially non-preferable flavours.

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The ability of the horse to associate orosensory characteristics of foods to their post-ingestive consequences in a choice test

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Introduction The ability of animals to select appropriate levels of nutrients for growth or maintenance has been demonstrated in pigs ((Kyriazakis et al. 1990) and lambs (Glimp, 1971; Early and Provenza, 1998). However, there has been little research on the diet selection abilities of the horse, and it is often assumed, due to their many feeding related illnesses, that horses may not be well adapted to control intake or select their best diet. The aim of this study was to investigate the horse's preference for cue flavours when associated with different energy concentrations and hence investigate the horse's ability to associate flavours with post-ingestive consequences.

Materials and Methods Twelve adult horses of mixed breed were used. Two types of feed pellets were used, one high energy (H) and one low energy (L) with 11.3 and 9.3 MJ DE/kg respectively. A basal mix (B) consisting of one part H to one part L was also used (10.3MJ DE/kg). 15ml of Mint (M) or Garlic (G) were added as cue flavours. The horses were presented with a choice of MB or GB for 29 meals (original test). The time allowed for a meal was calculated based on the individual horse's intake rate. Food was removed after this time and weighed. The horses were ranked and paired for mint preference and randomly assigned to group A or group B. Horses in group A were then given a choice of ML or GH energy. Horses in group B were given a choice of GL or MH. This was repeated for 29 meals (H v L 1). The basal choice of MB or GB was then repeated for 10 meals (Basal 2). After a short break of approximately one week the basal test was repeated for a further 40meals (Basal 3). The pairing of energy and flavour were then switched so group A was given a choice of GL or MH and group B was given a choice of ML or GH, for 30 meals (H v L 2). A final basal test then was repeated for 30 meals (Basal 4). For each meal, mint intake as a proportion of total food intake was calculated. Data was analysed using a repeated measure ANOVA with horse nested within group and test as factors. Tukeys post-ANOVA pairwise comparisons were used to investigate differences in preference between tests.

Results In the original basal test an overall preference for mint was found, (0.65 ±0.012). In the first high versus low test, group A showed a decrease in preference for mint (by 0.21 ±0.03 $p<0.001$). Group B showed an increase in preference for mint (by 0.10 ±0.03, $P<0.02$). For the second basal test the mint preference of group A remained lower than the original (difference of 0.15 ±0.04, $p<0.05$). Group B's preference for mint dropped but not significantly. In the third basal test Group B's mint preference was lower than in the high versus low test (difference of 0.19 ±0.03, $p<0.001$).In the second high versus low test, group A showed an increase from the previous basal test (by 0.25±0.03, $p<0.001$), while group B showed a significant decrease (by 0.22 ±0.03, $p<0.001$). On return to basal group A showed a decrease in mint preference (by 0.24 ±0.03, $p<0.001$) and group B showed an increase (by 0.10 ±0.03, $p<0.05$).

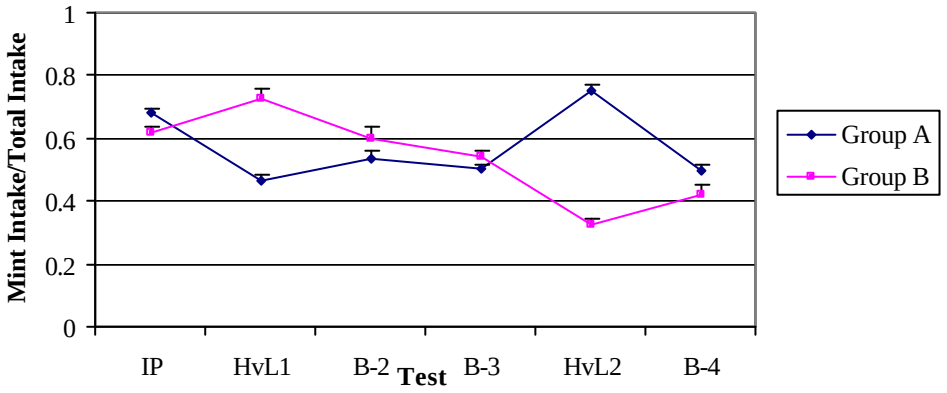

Figure 1: Mean mint intake as a proportion of total food intake at each test for each group.

Conclusions The results suggest that horses can select a high energy diet over a low energy diet and that horses can form associations between foods and their nutritional composition. The results provide evidence that post-ingestive consequences influence diet selection and horses can change their preference for foods as their associated energy levels are altered.

Acknowledgements We would like to thank WALTHAM Centre for Pet Nutrition for funding this PhD studentship and De Montfort University Equine Yard for providing assistance with the horses.

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Effect of resource density on the use of spatial memory by foraging sheep

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Introduction Sheep can use spatial memory to locate preferred food items in a background of less preferred items and forage more efficiently if the preferred patches are aggregated (Edwards *et al*. 1994). However, if a constant proportion of available patches contain food, foraging efficiency is not affected by the total number of patches (Edwards *et al*. 1996). The objective of this study was to test the hypothesis that spatial memory will be used to a lesser extent as the proportion of patches containing food increases. Not only is the animal faced with a potentially more complex task as the proportion of food patches increases but the cost:benefit ratio of using spatial memory is less. If 10 of 100 potential foraging sites contain food, an animal with perfect spatial memory could locate 5 of these patches in only 5 visits, whereas random searching requires 50 visits. If 50 patches contain food, 5 visits are required with perfect spatial memory but only 10 visits using random searching. Thus the benefit of using spatial memory over random search is reduced from 5:1 to 2:1.

Materials and methods A 10 x 10 grid of food bowls was laid out at 4m spacings within a 50 x50 m bare earth arena. On day 1 a group of 5 non-pregnant, non-lactating Welsh Mountain ewes that had not received concentrate for at least 3 years were offered a pelleted proprietary sheep concentrate for 30 min outside the arena. On day 2 they were introduced to the arena as a group, with all 100 bowls containing 25g concentrate and allowed to forage for 1 hour. On day 3 they were introduced singly into the arena with 20 randomly allocated bowls containing 25g concentrate and allowed to forage for 30 minutes. Following this training period the sheep were introduced individually into the arena from a grass background on each of days 6-10 (trial days) and were allowed to forage for 20 minutes. The experiment was designed as a regression experiment with either 10, 20, 30 40 or 50 randomly allocated bowls filled with 25g of concentrate. This procedure was repeated over 5 consecutive days. The number and position of the full bowls was constant for each sheep over these 5 days. Each treatment was replicated 3 times with different sheep and different randomly allocated full bowl positions. Time and duration of visits to all bowls were recorded. For each sheep the total distance walked before finding 5 full bowls was recorded and expressed as a proportion of the shortest possible path. Food remaining at the end of each session was weighed and food consumed from each full bowl calculated by difference. Results were analysed by analysis of variance.

Results

Although all animals demonstrated some spatial learning over the 5 trial days they appeared to continue to sample non-food patches where these occurred *en-route* between food patches. All animals appeared to follow the grid lines when searching. With only 10 food patches sheep reduced their search path length on day 5 to 0.40 of that on the first day, whereas with 50 food patches the reduction was only to 0.64 (Table 1). There was no significant difference between path lengths to find 5 full bowls on day 5 expressed as a proportion of the shortest possible path indicating that the sheep with less food patches had made greater use of spatial memory in optimising their search path. There was no significant difference in total amount eaten in the 20-minute trial on the 5th day although animals with more than 30 full bowls appear to be satiated after eating 420-480 g concentrate.

Table 1. Search performance of sheep to find 5 food patches on 1st and 5th trial days

No. food bowls	10	20	30	40	50	s.e.d.	P
Distance walked day 1 (m)	206	100	70	32	48	22.7	< 0.001
Distance walked day 5 (m)	82.8	61.2	41.2	38.8	30.8	15.7	0.046
Distance walked day 5 as proportion of shortest possible path	1.58	1.45	1.25	1.56	1.32	0.421	0.92
Total food eaten in 20 min (g)	211	331	420	478	419	131.8	0.353

Conclusions

Sheep appear to make more use of spatial memory when food resource is less densely distributed and, despite *en-route* sampling, to be able to reduce the distance walked between food patches to only 1.5 times the shortest possible path within 5 days.

Acknowledgements

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Octylphenol, an environmental oestrogen, affects oocyte maturation in cattle

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Introduction 4-tert-octylphenol (OP) is an alkylphenolic compound formed as metabolite of some nonionic surfactants that are widely used in industrial detergents, as plastic additives, dispersant for insecticides, etc. (Naylor et al., 1992). OP accumulates in adipose tissue. Micromolar concentrations of these compounds may constitute health hazards to animal cells. Furthermore, it has previously been shown to exert oestrogenic activity in vivo and in vitro (White et al., 1994). A growing concern about "endocrine disruptors" and their impact on oestrogen-dependent phenomena led us investigate the effects of OP on oocyte maturation. For variuos reasons bovine oocytes were chosen as the model system. We examined the effects of OP exposure on oocyte nuclear maturation in vitro and on the expression of oestrogen receptors in cumulus cells.

Materials and methods Cumulus-oocytes complexes (COCs) were isolated from ovaries by slicing and matured in vitro for 24 h at 39 °C in a humid atmosphere of 5% CO_2 in air. Various concentrations (ranging from 4.8 to 0.0048 µM) of OP were added to the maturation medium (TCM 199 supplemented with foetal calf serum, FSH and LH). After the maturation period the oocytes were mechanically stripped from the cumulus cells and nuclear maturation was assessed by lacmoid staining. Cumulus cells from groups of 40 oocytes were snap-frozen in liquid nitrogen and stored at -80 °C. Total RNA was extracted using Trizol Reagent (Gibco BRL Life Technologies) and subsequently reverse transcribed in the presence of 1 pg of rabbit alpha-globin mRNA as external standard. Oestrogen receptor (ER) alpha and beta transcripts were amplified using specific primers and the fragments were visualised on 1.8 % agarose gels. The relative transcripts amounts were analysed using a BioCapt quantification software (LTF). All experiments were replicated at least three times.

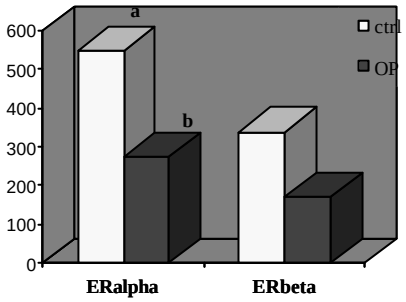
Results Effects of OP exposure on oocyte nuclear maturation are given in Table 1 (GV/GVBD: germinal vesicle/ GV breakdown, MI, II: metaphase I, II). In the highest doses employed (4.8 µM to 0.48 µM) the rate of degenerated (DEG) and immature oocytes (GV/GVBD, MI) was dramatically increased ($P < 0.05$) compared to the control and the lower doses. Semi-quantitative RT-PCR on cumulus cells showed that oestrogen receptor alpha mRNA is down-regulated by 24 h OP exposure, while oestrogen receptor beta levels were not significantly affected (Figure 1).

Table 1. Effect of OP on *in vitro* maturation of bovine oocytes

4-OP µM	GV/GVBD (%)	MI (%)	MII (%)	DEG (%)
4.8	9.3[a]	2.8[a]	0.0[a]	87.9[c]
0.48	40.3[b]	23.0[b]	2.8[a]	33.9[b]
0.048	1.9[a]	32.4[b]	57.4[b]	8.3[a]
0.0048	0.0[a]	17.6[ab]	78.0[c]	4.5[a]
ctrl	0.0[a]	17.3[ab]	81.6[c]	1.1[a]

[abc] different superscript denote significant differences ($P < 0.05$)

Figure 1 Effect of OP (0.048 µM) on ERs transcript levels in bovine cumulus cells



Conclusions These results clearly demonstrate that OP affects *in* vitro oocyte maturation in cattle in a nanomolar concentration range. The parallel effects on oestrogen receptors mRNA levels in the cumulus cells suggest that the observed toxic effects could be mediated, at least partially, by oestrogen receptors. The significance of these results depends on the degree of exposure of farm animals to oestrogenic alkylphenolic compounds.

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Ah receptor expression and signal transduction in bovine and rabbit reproductive tissues and embryos

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Introduction Polychlorinated biphenyls (PCB) are persistent environmental contaminants that have been reported to adversely affect reproduction in mammals. The exact mechanism of action of these chemicals on oocyte and embryo development are not known. Previous studies in our laboratory demonstrated that PCB adversely affect in vitro oocyte maturation and embryo development in two different *in vitro* systems (cattle, rabbit). The objective of present study was to investigate the possible role of the arylhydrocarbon receptor (AhR) in PCB toxicity. The AhR is a transcription factor activated by ligands such as dioxins or PCB. Its transcriptional activity depends on dimerisation with its nuclear partner ARNT. The presence of AhR and ARNT was investigated in bovine and rabbit genital tract (uterus, ovary) and embryonic tissues, employing RT-PCR and immunohistochemistry.

Materials and methods Paraffin embedded tissues were sectioned (5 µm) and subjected to immunohistochemistry using polyclonal mouse AhR and ARNT antibodies (Affinity Bioreagents). The signal was localised by incubation with a peroxidase linked goat anti-mouse antibody followed by color development with diaminobenzidine. Bovine cumulus-oocytes complexes (COC) were collected by slicing ovaries from slaughtered animals. Bovine COC and 6 day old rabbit embryos were snap-frozen in liquid nitrogen until PCR analysis. The rabbit embryos had been exposed to PCB in vitro for 4 h before (0.1 ng/ml per congener; congeners studied in mixtures: coplanar PCB 77, 126, 169; non-coplanar PCB 28, 52, 101, 118, 138, 152, 180; controls cultured in solvent DMSO without PCB). Total RNA was extracted using the Trizol reagent and subsequently reverse transcribed. AhR and ARNT transcripts were amplified using specific primers and the fragments were visualised on 1.8 % agarose gel. For whole mount immunohistochemistry, single COC were fixed in 3% paraformaldehyde, incubated overnight with anti-AhR and the signal was visualised after incubation with a FITC-conjugated secondary antibody and analysed using a fluorescence microscope.

Results (1) Bovine tissues. AhR and ARNT expression in the bovine ovary is summarised in Table 1. AhR was localised in the cytoplasm (cyt) of both the germinal and somatic compartments of the follicle from the primordial to the large antral stage. In contrast, ARNT protein appeared only in the nucleus (nucl) of the granulosa cells. Oocytes did not express ARNT at any stage of development, revealing an incomplete AhR signal transduction. The expression of AhR and ARNT mRNA and protein in isolated immature cumulus-oocytes complexes confirmed the data obtained for the protein in the whole ovary. (2) Rabbit tissues. In the rabbit ovary, AhR expression was strong in the endocrine interstitial cells and weaker in the follicle. In the uterus, the luminal epithelial cells showed a specific AhR expression pattern during the preimplantation period, changing from a distinct apical cytoplasmic to a more diffuse and nuclear localisation at later stages. Preimplantation embryos transcribed AhR and ARNT. Treatment with coplanar PCB for 4 h, however, did not result in the expression of genes from the AhR gene battery.

Table 1. Immunolocalization of AhR and ARNT protein in the bovine ovary

	AhR			ARNT		
	cyt	**nucl**	**mRNA**	**cyt**	**nucl**	**mRNA**
oocyte	+	-	+	-	-	-
granulosa cells	+	-	+	-	+	+

Conclusion Our results show that AhR and its nuclear partner ARNT are expressed in reproductive tissues and oocytes and early embryos in the cattle and rabbit. The expression patterns in the uterus indicate an endocrine control and an involvement in endometrial proliferation. Therefore a possible site of action related to PCB toxicity was identified in both, the mother and the embryos, in these species. The lack of ARNT in the oocyte implies an incomplete signal transduction, suggesting a pivotal role of cumulus-granulosa cells in mediating AhR activity in the ovary.

A wax diet for administration of octylphenol to laboratory rodents as a tool for the investigation of oestrogenic activity

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Introduction The use of controlled dietary intake for administration of environmental oestrogens to laboratory animals is essential in assessing the real threat of natural exposure to these compounds. Although human and animal exposure to environmental chemicals is mostly through diet or water, administration of these chemicals to laboratory animals is usually via injection. In this study, in order to overcome the problem of unpalatability of high doses of chemicals, a wax and a powdered diet were designed. These diets were used as vehicles for administration of the environmental oestrogen octylphenol (OP) to non-pregnant, pregnant and lactating laboratory rats and the dose of dietary octylphenol required to induce oestrogenic effects in the reproductive tract of female animals was determined.

Materials and Methods The wax diet consisted of various doses of OP dissolved in paraffin wax and then mixed with powdered rodent diet. The powdered diet contained the same amounts of OP without the wax. Intact Wistar rats (approx. 200g) were given increasing doses of OP in wax or powder diets over 2-day periods up to a dose that was unpalatable in either form. Whilst on the highest palatable dose, the females were mated and the diets were continued during pregnancy and lactation. The oestrogenic effects of dietary OP were subsequently investigated in an additional group of ovariectomised rats that were given the wax diet over 72 hours. Oestrogenic activity was determined by an increase in uterine wet weight and increases in cell proliferation in the uterine and vaginal luminal epithelium as measured by the number of cells in mitosis.

Results The average amounts of OP and food consumed daily are summarised in Table 1. The amount of OP consumed daily varied little between those on the wax or the powder diets. The average consumption of OP by non-pregnant animals in the highest dose groups was 90 – 100 mg/kg/day. However, the powder diet was often tipped out of the feeding receptacle and so estimation of the amount consumed was less accurate than that of the wax food. Five out of six females mated successfully. During pregnancy, the amount of food consumed increased slightly in both groups. Litters of 10 – 11 pups were born approximately 21 days after vaginal plugs had been found. During lactation, the amount of food consumed more than doubled in both groups.

	Food consumed (g/animal/day)		OP consumed (mg OP/kg BW/day)	
OP dose (µg/g wax)	Powder Food	Wax food	Powder Food	Wax Food
0	19 (±0.5)	31 (±0.8)	-	-
25	18 (±0.4)	27 (±1.3)	0.96 (±0.04)	0.91 (±0.01)
75	17 (±0.7)	26 (±1.6)	2.68 (±0.1)	2.69 (±0.2)
750	16 (±0.7)	26 (±1.2)	26.05 (±0.2)	26.60 (±1.4)
3000	13 (±0.6)	25 (±1.5)	96.80 (±9.3)	99.4 (±8.6)

Table 1 The average amounts of OP and food consumed daily by rats fed a wax or powder diet containing different doses of OP. Data are expressed as mean ± sem (n = 3 for each diet).

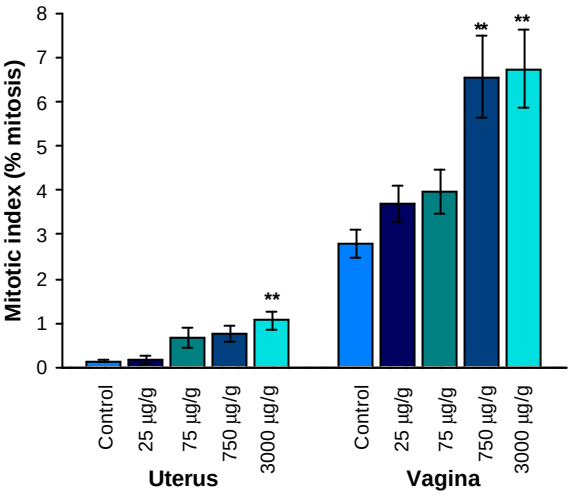

Figure 1 The effect of OP in a wax diet on mitosis in the uterine and vaginal luminal epithelium of rats after 72 hours. Data are expressed as mean ± sem (n = 3-5). **P<0.01 compared to controls.

The mitotic indices of both the uterine and vaginal luminal epithelia of the animals on the highest dose of OP were significantly higher than those of the control animals (P<0.01) (Figure 1). In a lower dose group (750 µg/g OP), the mitotic index of the vaginal luminal epithelium was also significantly higher (P<0.01) compared to that of control animals. There were no significant differences in uterine weight compared to the control, although the mean uterine weight in the highest OP group was 125 mg compared to 85 mg in the control group.

Conclusions Although both diets have been shown to be efficient vehicles for OP administration to both pregnant and lactating rats, the wax diet is preferable because of the greater accuracy of measurement of the amount of OP consumed. This study has shown that dietary OP up to 100 mg/kg/day does not disrupt mating, embryo implantation, which is a critical time for pregnancy failure, or litter size. However, dietary doses of OP above 20 mg/kg/day may induce significant oestrogenic effects in the vaginal luminal epithelium and doses over 60 mg OP/kg/day may also significantly affect uterine growth.

Bioaccummulation of the endocrine disrupting compound, dioctyl phthalate, in sheep grazing pasture treated with sewage sludge or inorganic fertiliser

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Introduction Endocrine disrupting compounds (EDC) are chemically diverse and ubiquitous compounds which are released into the environment as a result of industrial, domestic and agricultural processes. They are generally readily absorbed from ingested food and water, can accumulate in animal tissue and can have adverse effects on reproductive and immune systems at very low concentrations. They are present in high concentrations in sewage sludge which is likely to be used increasingly as a fertiliser on pasture and arable land and so there is potential for bioaccumulation in animal tissues and associated, adverse biological effects. The aim of the study was to determine the concentrations of one EDC, dioctyl phthalate (DOP), in the livers of ewes and lambs maintained on control and sludge-treated pastures.

Materials and methods Commencing in mid-summer, groups of ewes were maintained for a period of 19 months, until slaughter, on control pastures treated with inorganic fertiliser applied at a rate of 225kg nitrogen (N)/ha/year or on pasture treated with liquid sludge containing equivalent amounts of N. From two months of age until slaughter at 6 months of age (year 1), or throughout life (including foetal life) until slaughter at 6 months of age (year 2), randomly selected lambs born to ewes of each treatment were maintained on these pastures. Liver tissue was recovered from ewes and lambs at slaughter and DOP content determined following extraction, using gas chromatography linked to mass spectrometry. Treatment groups were compared, for ewes and lambs separately, by analysis of variance.

Results Mean concentrations (ng/gDM) of dioctyl phthalate (DOP) were higher in the liver of ewes from treated than control pastures. Mean concentrations were similar in lambs of treated and control groups in each year (Table 1).

Table 1 Mean log concentrations (ng/gDM) of dioctyl phthalate in liver of ewes (n = 15/group) and lambs (n =18/group). Back-transformed values are given in brackets).

	Control	Treated	s.e.d.	Significance
Ewes	3.15 (1413)	3.58 (3802)	0.160	**
Lambs				
Year 1	3.95 (8913)	4.16 (14454)	0.145	NS
Year 2	3.55 (3548)	3.52 (3311)	0. 249	NS

Conclusions The results indicate that DOP accumulated in the liver of grazing sheep in concentrations that are likely to be biologically effective. Use of sewage sludge as a fertiliser was associated with higher concentrations in ewe liver compared with control animals but levels were equally high in liver from both sludge-exposed and control lambs. The long-term consequences of such bioaccumulation for both animal and human health and reproductive function remain to be determined.

Low Tetrachlorodibenzo-p-dioxin (TCDD) concentrations affect gene expression patterns in cultured bovine fibroblasts before any cytotoxicity appears at the cellular level

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Introduction Hormonally active compounds of different origins such as polychlorinated biphenyls (PCBs) and their derivatives are widely dispersed in the environment by various industrial processes. They represent a threat to human health since their toxic effects can result from biological accumulation of low doses of active compound during extended periods of exposure. Critical doses for a cytotoxic effect have been determined using cellular models such as cultured hepatocytes but it is still not known if lower doses can affect in vitro cell functions before any toxic effect can be detected. To address this issue we have used a functional genomic approach to characterize changes in the profile of genes expressed by cultured bovine fibroblasts exposed during only 26 hours to concentrations as low as 10^{-10} to 10^{-12} molar of TCDD

Materials and methods Bovine fibroblasts were cultured in DMEM-10% foetal calf serum (FCS) - 2mM L-glutamine - 1 mM sodium pyruvate - 100 U/ml penicillin - 100 µm/ml streptomycin. Cells were plated on tissue culture dishes and cultured at 38.5°C with 5% CO_2**.** Total RNA was extracted from bovine fibroblasts using RNAzol ™ (Appligene, France) and polyA+ RNA was further purified using oligodT-coated beads (Dynal, France). Single-stranded cDNA complex probes were generated for each culture condition (DMEM, DMSO 0.1%, TCDD 10^{-10} and 10^{-12}M) according to Piétu et al., 1996 (*Genome Res.*, **6**, 492-503). PolyA+ mRNA (500ng) was reverse-transcribed using Superscript II reverse transcriptase (RNAse H^-) as described in the manufacturer's protocol (GIBCO-BRL, France), using random hexamers for priming. Labelling was performed simultaneously by incorporation of α^{33}P dATP (3000 Ci/mmole, Amersham, France). Quadruplicate filters of our bovine macroarray were then hybridized to each complex probe and hybridization signals were captured by exposure to Phosphor screens, followed by scanning with the Phosphorimager (Molecular Dynamics). Identification of the spots was performed using the Xdotsreader software (Cose, France), in collaboration with Dr C. Auffray (Genexpress, CNRS, 94801, Villejuif, France). TCDD preparation: 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) was a kind gift from Dr. S. Safe (Texas A&M University, College Station, TX, USA). 2,3,7,8-TCDD was dissolved in toluene after which an aliquot was transferred into DMSO. Toluene was evaporated after which the 2,3,7,8-TCDD concentration in DMSO was analysed using HRGCMS. Dilutions of 2,3,7,8-TCDD were prepared in DMSO immediately prior to experiments.

Results Bovine fibroblasts were exposed to two concentrations of 2,3,7,8-TCDD in DMSO 0.1%, to DMSO 0.1% alone and to usual DMEM-FCS, during 26 hours. They all looked healthy after culture. After total RNA extraction, purification of polyA+ RNA, generation of complex probes and hybridization to a bovine macroarray (Hue et al., unpublished), it appeared that the hybridizing patterns generated by the cells exposed to DMEM and DMSO 0.1% looked very similar (Fig. 1). The two TCDD concentrations looked similar when compared to each other, but compared to their closest control (DMSO 0.1%) new spots clearly appeared.

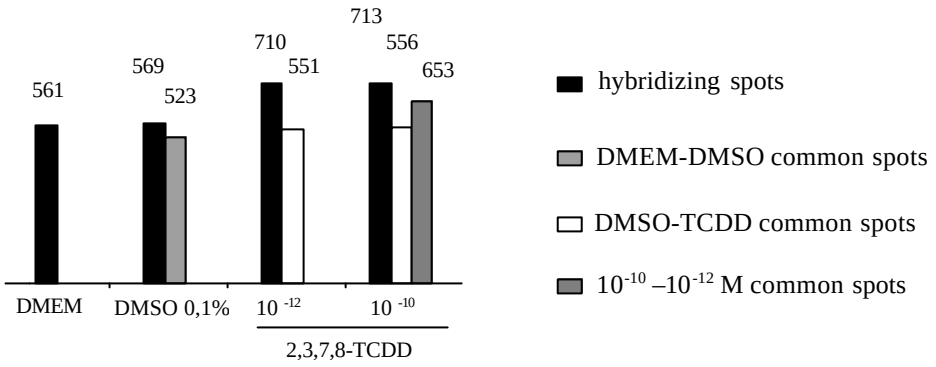

Figure 1 **Analysis of the hybridizing patterns of the four complex probes on the bovine macroarray**

Conclusion Present results first illustrate that DMSO alone, at the concentration used, does not disturb the cultured bovine fibroblasts *per se* and that early TCDD effects on gene expression in these cells can be detected by the employed experimental approach. Increasing the concentration of TCDD in culture from 10^{-12} to 10^{-10} M does not disturb the development of bovine fibroblasts strongly. TCDD, however, seems to induce the expression of new transcripts. It is therefore of great interest to confirm this preliminary experiment, evidencing for the first time that low TCDD concentrations may already affect gene expression patterns before any cytotoxic effect is visible at the cellular level.

Changes in carcass composition with age in 16-26 month old Red Deer

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Introduction Modern retailing of venison through supermarkets requires that the product be on the shelf for an extended period compared with the traditional short season in autumn/early winter. However, deer are highly seasonal animals and a range of strategies is needed to increase marketing opportunities for venison. One strategy utilizes extended winter daylength and high levels of nutrition to advance the slaughter season (Davies, 1995; Fisher *et al*.,1995). Another strategy is to adopt more natural, low input systems with reduced production costs, in which deer reach slaughter condition during their third summer at 22-26 months of age, but this needs full investigation. This study involved sequential slaughtering of deer to ascertain how rapidly carcass composition and meat quality changes over this period.

Materials and Methods This study used 32 red deer stags and 32 red deer hinds of 16 months of age, which had spent their second summer grazing a perennial ryegrass/white clover sward maintained at 8-10 cm surface height. The stags weighed 93.4 kg (s.e.m. 2.91kg) and the hinds 77.2 kg (s.e.m. 2.13kg) at housing on 12 October 1998. Deer were allocated randomly to eight slaughter date treatments from each quartile of the live weight dataset. Two initial groups of 4 stags and 4 hinds were slaughtered on 20 October at 16.3 months of age (end of second summer; 'baseline' groups). The remaining deer were fed a grass silage-based diet designed to maintain live weight over winter, and groups of 4 stags and 4 hinds were slaughtered at approximately 3-week intervals commencing at turnout on 6 April ('serial' groups). At each slaughter point, two animals of each sex were randomly selected for a half carcass dissection into lean, fat and bone. The toughness (maximum yield force) of the *longissimus lumborum* muscle from each animal in the serial groups, aged for 7 days at 2^0C and cooked to an internal temperature of 78^0C, was measured using a Stevens CR Analyser.

Fig. 1 Stags: tissue weights on age

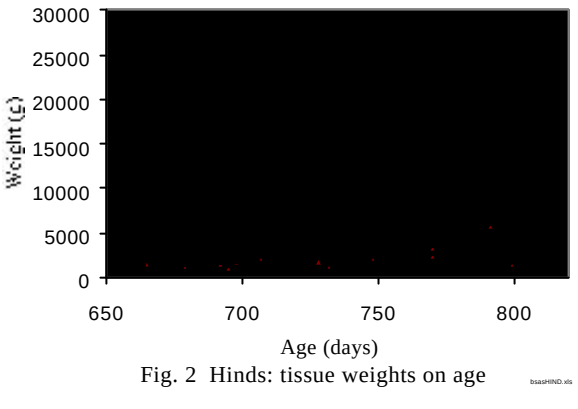

Fig. 2 Hinds: tissue weights on age

Results Growth rate of deer over winter was +12 and -17 $g.d^{-1}$ for stags and hinds, respectively. Regressions of live and carcass weights on age for the serial groups gave mean growth rates ($g.d^{-1}$) of 310 and 160 respectively for stags and 170 and 100 for hinds during their third summer at pasture. Regressions of tissue weights on age (Figs 1 and 2) showed that growth rates of all three tissues were greater in stags than hinds and were in the order lean>fat>bone. In terms of tissue proportions, fatness ($g(fat).kg^{-1}$ (total tissue)) only exceeded that in the baseline groups (stags 70, hinds 86) after 2 months following turnout and, apart from one hind in the oldest group (197 $g.kg^{-1}$),all animals had an acceptably low fat content. There was no change in meat texture with age, but stags were significantly tougher than hinds (yield force = 3.38 and 2.60 kg, respectively ($p<0.001$)).

Conclusions The results demonstrate that provided deer are fed a 'store' diet through their second winter, a substantially heavier carcass, which is not overfat, can be produced for up to 18 weeks after turnout. This increases the range of options available to extend the marketing season for venison, and is particularly valuable for hinds whose carcass weights are commonly too light at 15-18 months of age. Although the yield force measurements were low overall, indicating tender meat, differentiation of product quality may be made on the basis of gender.

Acknowledgements The financial support from MAFF for this work is gratefully acknowledged.

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Productive and carcass classification traits on chemical and instrumental meat quality characteristics of ten local cattle breeds of the Southwest of Europe

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Introduction The EU is the second largest world producer of beef meat, but production is fragmented, consisting of highly variable local systems, compared with homogenous, highly intensive feedlot systems. The current EU agricultural policy requires a reorientation of beef market to take advantage of this diversity by creating meat quality labels relating to geographical areas and with specific genotype and production systems, symbols of guaranteed quality. The aim of this experiment was to analyse the relationships between productive and carcass parameters with some meat quality traits, within breed-production systems in different European countries.

Materials and methods Seventy-five animals of ten local EU beef breeds, seven from Spain: Asturiana de los Valles (AV), Asturiana de la Montaña (AM), Avileña (A-NI), Bruna dels Pirineus (BP), Morucha (MO), Pirenaica (PI) and Retinta (RE) and three from France: Aubrac (AU), Gasconne (GA) and Salers (SA) were studied. All animals were reared under local production systems. Slaughter weight ranged between 450-550 kg for Spanish breeds and 615-750 kg for French breeds. Carcass conformation (CS) and fatness score (FS) were evaluated using the EUROP scale. *Longissimus dorsi* (LD) was used to determinate haematin content (HC), intramuscular fat (IF) and total collagen (TC). Texture of cooked meat was determined as maximum load (WB) using a Warner Bratzler device. Stress in raw meat at 20% of maximum compression (C20) was measured with an INSTRON. Linear regressions were assessed, within each breed-production system, between carcass and meat quality traits and daily gain (DG), slaughter weight (SW), carcass conformation (CS) and fatness scores (FS) as independent variables.

Results Significant regressions are shown in Table 1. DG showed negative significant slopes in some breeds related to HC, as DG increased HC fell resulting in lighter muscle, but DG had no significant effect on IF or TC. SW was weakly correlated with IF or texture parameters, although age influences insoluble cross-link formation and this is highly related to meat toughness (Monin, 1991). Slopes of CS related to IF, TC and HC were negative in several breeds, showing that higher muscle development would represent leaner and lighter meat, with lower haematin and total collagen composition. As FS increased, positive slopes appeared with IF, showing an important relation between visual and intramuscular fatness. TC and HC also increased with FS in some breeds, directly related to increments in age within the same production system although no marked relation with texture parameters was found.

Table 1. Significant slopes for each breed-system [(1)] in the linear regressions relating some meat quality traits (intramuscular fat (IF), total collagen (TC), haematin content (HC), maximum load Warner-Braztler (WB) and stress at 20% compression (C20) with DG, SW, CS and FS as independent variables.

	IF	*TC*	*HC*	*WB*	*C20*	*IF*	*TC*	*HC*	*WB*	*C20*
	Daily gain (DG)					Slaughter weight (SW)				
AV					-1.7*				0.02*	
AM			-87.8**							0.01*
PI					3.0**			0.18**		0.01*
A-NI			-49.4**					-0.51*		
GA			-93.2**	-1.5**						
SA			-31.2**						-0.01**	
	Conformation score (CS)					Fatness score (FS)				
AV	-0.16**	-0.09**				0.17**	0.09**	6.16**		
PI							0.11**		0.18*	
BP	-0.27*		-6.06*	0.44**		0.18**				
RE	-0.39*	-0.12*				0.58**				
AU	-0.09*		-5.59**							
GA	-0.19**		-4.63*			0.21**				

[(1)] Only breeds with significant slopes are showed. * = P<0.05; ** = P<0.01

Conclusion Every breed- production system had different effects on meat quality characteristics evaluated in relation to SW, DG, CS and FS. Considering commercial products, the increase of daily gain, slaughter weight or conformation and fatness scores implies important meat quality changes in terms of total pigments or intramuscular fat, taking into account the Mediterranean European market requirements for a pale and lean meat.

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Time course of incorporation of n-3 PUFA from linseed in pigs and effects on D9-desaturase activity and pork odours

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Introduction The n-3 polyunsaturated fatty acids (PUFA) are healthy nutrients which can be increased in pork by feeding sources such as linseed to the growing animal. The levels achieved depend on many factors such as the concentrations of lipid classes in tissues (eg phospholipids containing high PUFA levels are more abundant in muscle than adipose tissue) competition for incorporation with n-6 PUFA and possible inhibitory effects of PUFA on synthesis of saturated and monounsaturated fatty acids. This study examined the time course of the incorporation of n-3 PUFA into tissue lipids and the effects on the major synthetic enzyme Δ9-desaturase. The effects on pork odour were also studied.

Materials and methods Forty eight Duroc x Landrace gilts were used. From 40kg body weight, 1 group was fed a control diet C (24g/kg fat of which 0.06 18:3) and the other a diet containing 60g/kg whole linseed, L (40g/kg fat, of which 0.29 18:3) at Cotswold's Wye College Experimental Unit. Pigs were fed *ad libitum* and 8 slaughtered after 20, 60 or 100 days. They were then transported to Langford for slaughter. Pieces of subcutaneous fat were immediately removed and assayed for Δ9-desaturase activity. After overnight chilling of the carcass the hindloin was conditioned for 10 days at 1°C prior to sensory analysis by the trained taste panel. Steaks were grilled to an internal temperature of 80°C and scored on a 1-8 scales (60 and 100 d groups only). Fatty acids were measured in muscle and subcutaneous fat from the loin using procedures described by Whittington *et al* (1986). Analysis of variance compared the 2 diets and 3 time periods in a factorial design and for the sensory tests, diets alone were compared.

Results After only 20 days the proportions of 18:3 and 20:5 were close to their peaks in backfat and muscle, with higher levels of 18:3 in backfat and 20:5 in muscle (Table 1). The activity of Δ9-desaturase tended to be lower in diet L, especially at 60 days where 18:3 and 20:5 were highest. In the combined 60 and 100 day groups pork odour was low in L and abnormal odour higher (Table 2).

Table 1. Effects of diet (C and L) and days of feeding on fatty acid proportions in muscle and subcutaneous fat (g/100g fatty acids) and Δ9-desaturase in fat (nmol oleic acid/mg protein/hr)

	20d		60d		100d		s.e.d.
	C	L	C	L	C	L	
Fat:							
18:3	1.98^{b}	4.66^{c}	1.47^{a}	6.51^{d}	1.13^{a}	5.04^{c}	0.240
20:5	0.05^{a}	0.10^{b}	0.03^{a}	0.07^{b}	0.02^{a}	0.05^{a}	0.010
Δ9-desaturase	12.2^{a}	9.3^{a}	29.8^{c}	17.9^{b}	18.9^{b}	17.4^{b}	1.35
Muscle:							
18:3	0.97^{b}	2.77^{d}	0.65^{a}	3.00^{d}	0.48^{a}	2.19^{c}	0.170
20:5	0.30^{a}	0.68^{c}	0.26^{a}	0.77^{c}	0.17^{a}	0.44^{b}	0.060

Means in a line with different superscripts are significantly different ($P<0.05$)

Table 2. Effects of diet on pork odours in combined 60d and 100d groups (1-8 scale)

		C	L	s.e.d.	
Fat:	pork odour	3.5	3.2	0.12	*
	abnormal odour	2.9	3.2	0.13	*
Muscle:	pork odour	3.4	3.4	0.11	NS
	abnormal odour	3.3	3.4	0.13	NS

Conclusions 18:3 n-3 and its product 20:5 were already very high after 20 days of feeding the linseed diet. This was associated with a lower activity of Δ9-desaturase, especially after 60 days when incorporation of n-3 PUFA was greatest. There was a tendency for abnormal odours in L presumably linked to the high level of n-3 PUFA.

Acknowledgement We are grateful to MAFF for funding this work.

Reference Whittington, F.M., Prescott, N.J., Wood, J.D. and Enser, M. 1986. The effect of dietary linoleic acid on the firmness of backfat in pigs of 85kg live weight. Journal of the Science of Food and Agriculture 37, 753-761.

Effects of substitution of a carbohydrate source by highly polyunsaturated or partially saturated oil on the fatty acid composition of backfat tissues, marbling fat and vitamin E content of meat in growing-fattening pigs

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Introduction The fatty acid and vitamin E concentration in the diets of growing pigs are known to affect backfat and meat quality. The aim of this study was to determine the effects of substitution of a carbohydrate dietary source (maize) by a series of oils on the fatty acid (FA) composition of backfat and marbling fat at slaughter. Vitamin E concentration in muscle (*longissimus dorsi*) was also determined.

Materials and methods Four treatments were used in a completely randomised design (**see accompanying summary; Ocampo and Lean, 2001** and table 1). Four backfat and meat samples per treatment taken from the region of the last rib of crossbred gilts were analysed. Standard techniques were used for FA analysis of all components, together with vitamin E content of meat. Data were analysed using ANOVA with treatment as a factor.

Table 1 Fatty acid composition of treatments

	Saturated	MUFA	PUFA	U:S ratio
Treatments (mg/100 g total fatty acids)				
Maize	21	30	49	2.4
Palm oil	48	39	13	0.3
Maize oil	14	28	58	4.2
Soybean oil	16	26	58	3.5

MUFA= monounsaturated FA; PUFA= polyunsaturated FA and U:S= Polyunsaturated:Saturated ratio.

Results Means of the results are given in Table 2. In general, Fatty acid composition was similar between maize and palm oil treatments (A), and between maize oil and soybean oil treatments (B). Between A and B fatty acid composition for the majority of the FA was statistically different (P≤.05). Vitamin E was higher in the oil treatments (P≤.001) than in the maize treatment. Palm oil had the highest vitamin E concentration.

Table 2 Fatty acid composition of backfat tissues, marbling fat and Vitamin E content of meat

	Backfat tissues (mg/100 g total fatty acids)					Marbling fat (mg/100 g tissue)				
	Maize	PO	MO	SO	SED[1]	Maize	PO	MO	SO	SED
14:0	12.1^{a}	9.5^{a}	2.4^{b}	2.4^{b}	0.006	11.2^{a}	9.1^{a}	3.3^{b}	2.8^{b}	1.4
16:0	228.3^{a}	263.9^{a}	124.4^{b}	112.2^{b}	0.024	290.0^{ab}	350.1^{b}	192.2^{a}	161.5^{a}	67.7
16:0 ald						26.6^{a}	24.3^{a}	14.2^{b}	13.4^{b}	3.5
16:1	19.4^{a}	18.5^{a}	5.9^{b}	4.4^{b}	0.008	26.7^{a}	24.7^{a}	10.8^{b}	6.8^{b}	5.5
18:0	99.8^{a}	72.0^{b}	46.6^{c}	56.0^{bc}	0.019	107.9^{a}	99.3^{ab}	86.5^{b}	88.2^{b}	6.0
18:1	427.1^{a}	452.4^{a}	307.4^{b}	284.2^{b}	0.017					
18:1 ald						12.4^{a}	9.9^{b}	9.1^{b}	10.5^{b}	0.8
18:1 trans						5.3^{a}	6.9^{a}	24.0^{b}	19.5^{b}	3.8
18:1 cis 9						330.0^{ab}	404.4^{a}	238.8^{ab}	206.9^{b}	77.1
18:1 cis 11						34.2^{a}	30.5^{ab}	20.4^{c}	26.3^{bc}	3.2
18:2	202.4^{a}	175.4^{a}	501.9^{b}	499.0^{b}	0.029	207.9^{a}	219.9^{a}	479.9^{b}	435.4^{b}	30.1
18:3	8.5^{a}	6.8^{a}	11.3^{b}	41.6^{c}	0.009	3.7^{a}	3.7^{a}	6.8^{a}	23.6^{b}	1.5
CLA						0.7	0.6	0.5	0.8	0.2
20:4						53.8^{a}	55.9^{a}	47.5^{ab}	40.5^{b}	4.6
22:4						6.5^{a}	4.8^{b}	3.1^{c}	2.0^{d}	0.5
22:5						5.2^{ab}	6.1^{b}	4.3^{a}	7.8^{c}	0.5
U:S ratio	0.6	0.5	2.9	3.2		0.6	0.7	1.8	1.9	
Total fatty acids (marbling fat)						1,169	1,295	1,189	1,097	187.8
Vitamin E a**-tocopherol** m**g/g tissue**						1.9^{a}	4.0^{c}	2.8^{b}	3.6^{c}	0.3

[1] *Standard errors of differences of means (Arcsin transformation was used for anova analysis for backfat tissues).*
FA data <5 mg/100 g total FA or <5 mg/100 g tissue are not included.
Means within rows with unlike superscript letters were statistically different (P £.05).

Conclusions The fatty acid composition of backfat tissue and marbling fat in the *Longissimus dorsi* was similar in animals fed a maize based diet compared to a palm oil based diet. The palm oil diet resulted in a higher vitamin E content in the meat. Maize and soybean oil diets resulted in tissues containing high PUFA concentrations which may affect backfat consistency and pigmeat quality.

Acknowledgements Support from Colciencias (Colombia), Cotswold Pig Development Company Ltd and University of Bristol Division of Food Animal Science (meat analysis) is gratefully acknowledged.

Reference Ocampo and Lean (2001). Effects of a high-fat diet based on palm oil, soybean or maize oil on growth performance and carcass characteristics in growing-fattening pigs. BSAS annual meeting (No.152), University of York.

Evidence for calpastatin cleavage by calpain in postmortem porcine longissimus dorsi

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Introduction Calpastatin is the endogenous inhibitor of the calcium-dependent protease family known as calpains and is thought to be an important determinant of postmortem tenderisation rates in beef, lamb and pork (Koohmaraie, 1996; Parr *et al*, 1999). Regulation of calpastatin is therefore of great importance if efforts to overcome unpredictable incidences of pork toughness are to succeed. Recent evidence in ovine tissue has shown that calpastatin is susceptible to fragmentation by calpains (Doumit and Koohmaraie, 1999). The ability of calpain to fragment calpastatin in porcine tissue was investigated and the consequences for pork quality are considered.

Materials and methods Nineteen pigs of Large White and Duroc origin were brought together into a common finishing environment 5 weeks before slaughter and finished to a target weight of 90 kg. Samples (5 g) of longissimus dorsi (LD) were taken from the region of the last rib and snap frozen in liquid N_2 within 2 h of commercial slaughter and stored at –70°C for subsequent analysis. Calpastatin and calpain were extracted from the LD for activity and immunoreactivity measurements as described previously (Sensky *et al*, 1999). Activity was measured using a fluorescent substrate cleaved by calpains and immunoreactive bands of extractable calpain and calpastatin were identified by probing western blots with specific porcine anti-calpain or anti-calpastatin antibodies and visualising using enhanced chemi-luminescence. Immunoreactivity was quantified using Optimas 5.2 image analysis software (Optimas Corporation, Seattle, USA). Shear force (SF) was measured using Volodkevitch-type jaws on cooked samples taken from loin chops that had been excised from each carcass 24 h after slaughter and conditioned for 1 week at 2°C. Linear regression analysis was used to investigate the relationships between calpain, calpastatin and SF.

Results Calpastatin activity did not correlate with calpain activity or immunoreactivity. The anti-calpastatin antibodies identified several bands of calpastatin immunoreactivity of which bands at 135 kDa and 80 kDa were quantified. The intensity of the 135 kDa calpastatin band did not correlate with any calpain parameters, whilst the 80 kDa band was found to correlate significantly to the activities of μ-calpain ($r = 0.687$, $p < 0.01$), m-calpain ($r = 0.512$, $p < 0.05$) and total calpain ($r = 0.800$, $p < 0.001$; Figure 1). The total calpain activity, as well as that of m-calpain also correlated with total calpastatin immunoreactivity ($r = 0.473$ and 0.503, respectively, $p < 0.05$). The intensity of the 80 kDa calpastatin band correlated negatively with 8 d SF values ($r = -0.561$, $p < 0.05$; Figure 2).

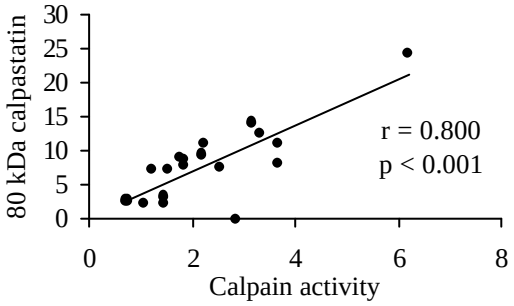

Figure 1 Correlation between calpain activity ($x10^7$ fluorescence units/g tissue) and intensity of 80 kDa calpastatin in porcine LD

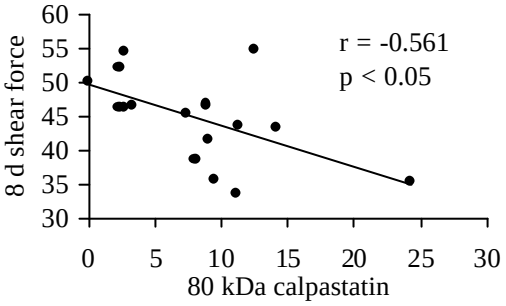

Figure 2 Correlation between intensity of 80 kDa calpastatin and 8 d shear force (N/m^2) in porcine LD

Conclusions The data indicate that, as in ovine skeletal muscle, calpastatin in porcine LD may be susceptible to fragmentation by calpain, with an increase in 80 kDa calpastatin immunoreactivity correlating to increased calpain activity. The fragmentation of calpastatin does not appear to have any effect on the activity of calpastatin, but the observation that the intensity of the 80 kDa band correlates negatively to 8 d SF suggests that cleavage of calpastatin permits calpain mediated tenderisation of skeletal muscle to proceed more effectively.

Acknowledgements This work was funded by the BBSRC and the Meat & Livestock Commission.

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The role of P450IIE1 protein and mRNA expression in determining adipose tissue skatole level

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Introduction High concentrations of skatole in adipose tissue are a major factor in boar taint - the offensive odor and taste in cooked pork from some intact male pigs. Skatole is produced by bacterial fermentation of tryptophan in the large intestine, absorbed into the blood and transported into the liver, where it can be metabolised via the cytochrome P450 system. One reason for high skatole levels in pig adipose tissues could be low expression of hepatic cytochrome P450IIE1 in liver and hence a reduced rate of skatole clearance, but no correlation was found between the rate of skatole metabolism and the P450IIE1 content of isolated liver microsomes. The present study re-investigates the relationship between backfat skatole, liver P450IIE1 expression and the rate of microsomal skatole metabolism in two breeds of pigs.

Materials and Methods 85 intact male pigs of two breed types provided by the Cotswold Pig Development Company were used. These were Large White x Landrace (LW) and Meishan x Landrace (M). The pigs were fed a standard pelleted diet and slaughtered at the same age to provide carcasses of 63-74 kg. Liver samples were frozen in solid CO_2 and subsequently stored at –80°C. Skatole in backfat was measured by high resolution gas chromatography. Microsomes were isolated by differential centrifugation. Mitochondrial skatole metabolism was measured by separating skatole from its products by thin layer chromatography in hexane/ether. P450IIE1 levels in microsomes were measured by Western blotting with a commercial antibody. mRNA levels were measured by Northern blotting with a cDNA probe specific for P450IIE1.

Results The majority of the LW pigs had skatole levels in the range from 0 to 0.1 µg/g which is below the level characteristic of boar taint. Only about 5% of LW pigs had backfat skatole levels which were higher than this. In contrast, the M breed had very high backfat skatole levels (from 0.2 to 1 µg/g) most of which were above the boar taint threshold level (0.2µg/g). When microsomal metabolism was measured under appropriate conditions the rate of metabolism was always proportional to the microsomal P450IIE1 content. confirming that metabolism via P450IIE1 was the major route of skatole breakdown in liver. Fig 1 shows the correlation between backfat skatole and microsomal P450IIE1 protein. For the LW there was a good inverse correlation for all the pigs tested. All the M pigs expressed low levels of P450IIE1. Backfat skatole levels in M pigs were always high but varied over a ten-fold range. Fig.2 shows that there was an inverse correlation between backfat skatole and P450IIE1 mRNA expression in LW. In the M breed P450IIE1 mRNA levels were always low and backfat skatole levels were relatively high but there was no clear correlation between mRNA levels and backfat skatole.

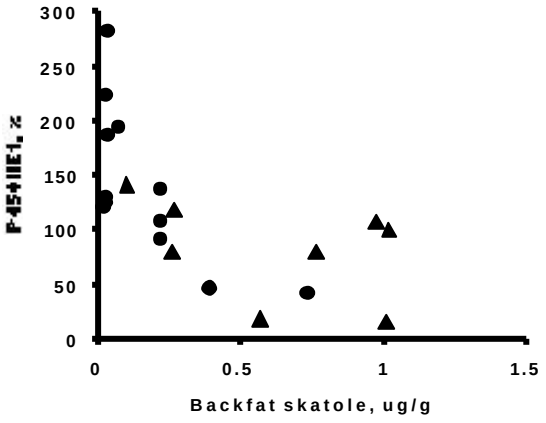

Fig 1. Microsomal P450IIE1 in LW (●) and M (▲) pigs

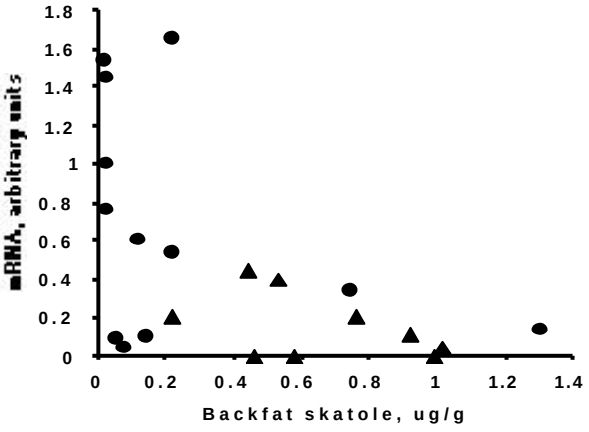

Fig 2. mRNA level in liver of LW (●) and M(▲)

Conclusions These results confirm the importanace of hepatic cytochrome P450IIE1 protein and mRNA expression in determining adipose tissue skatole levels. In the LW pigs high backfat skatole is clearly correlated with abnormally low cytochrome P450IIE1 expression. In M pigs some additional factor increases the skatole level above that determined by the low level of cytochrome P450IIE1 expression. An understanding of the molecular basis of boar taint will require elucidation of the factors controlling the expression of cytochrome P450IIE1 in pig liver.

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Effect of red and white clover on beef meat quality

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Introduction Legumes, such as red and white clover, are potentially important constituents of low input, environmentally friendly beef production systems. However, the effects of grazing clover on the quality of beef have not been assessed fully. Important aspects of meat quality are shelf-life (colour and lipid stability), flavour and human nutritional value, all of which are affected by components of the animals diet such as antioxidants and fatty acids. Tissues from lamb finished on swards containing white clover were reported to contain more linoleic and α-linolenic acid and less eicosapentaenoic acid (EPA, 20:5 n-3) (Vipond *et al*., 1993) compared to animals grazing grass. The objective of this study was to determine the quality of meat from two beef breeds raised on grass or grass plus white or red clover.

Materials and methods Forty eight steers, 24 purebred Hereford and 24 Welsh Blacks (average initial liveweight 230 kg) were randomly allocated within breed to one of 3 forage systems: 1) grass only, 2) grass and white clover or 3) grass and red clover. Swards were grazed from May to October in the first year and from May in the second year and received silage prepared from the same forage during the intervening winter. Vitamin E (HPLC) and fatty acids (GLC) were analysed on samples of *m.longissimus* removed 48h post-mortem and colour (L*a*b*) and lipid oxidative shelf-life and eating quality were assessed on meat conditioned for 10 days at +1°C and packed in a modified atmosphere (0.75 O_2, 0.25 CO_2) for simulated shelf-life display.

Results At slaughter, carcasses from Welsh Blacks were heavier but leaner and had a better conformation than the Herefords (Table 1). Diet did not affect hot carcass weight or conformation but steers on white clover were fatter although intramuscular fat contents were not different. The meat from animals off grass/red clover was oxidatively less stable despite similar levels of vitamin E to the other two treatments. Colour tended to be poorer but a* and saturation were not significantly different. Clovers increased 18:2 and 20:4 in muscle phospholipid and 18:2 and 18:3 in neutral lipids. C22:6 n-3 was not affected by feed but was significantly higher in Hereford steers. Muscle contents of trans 18:1 and conjugated linoleic acid (CLA) did not differ significantly between diet or breed with overall means (mg/100g muscle ± SEM) of 44.6 ± 6.4 and 16.8 ± 1.2 respectively. Beef flavour was unaffected by feed or breed. There were no breed x feed interactions in any measurements.

Table 1 *Effect of forage and breed on carcass and meat composition and quality.*

		Forage					Breed			
		Grass	Grass/ red clover	Grass/ white clover	SED	P	Hereford	Welsh Black	SED	P
Carcass weight hot (kg)		317.0	312.7	333.7	8.8	NS	311.7	330.6	7.17	*
	Conformation (1-15)	7.7	8.1	8.3	0.73	NS	7.6	8.5	0.42	*
	fatness (1-15)	8.1	8.0	9.2	0.56	*	9.2	7.6	0.46	***
Muscle	Lipid oxidation†	0.70	1.73	0.83	0.30	**	1.12	1.06	0.24	NS
	vitamin E (mg/kg)	3.42	2.95	3.13	0.18	NS	3.01	3.32	0.22	NS
Colour	a*	16.3	13.8	15.3	1.02	NS	15.5	14.7	0.83	NS
	saturation	18.4	16.2	17.4	0.94	NS	17.7	17.0	0.79	NS
Fatty acids (mg/100g muscle)										
Phospholipid	18:2 n-6	38	51	45	1.6	***	45	44	1.3	NS
	18:3 n-3	28	28	30	1.5	NS	28	29	1.2	NS
	20:4 n-6	20	23	21	0.8	***	23	20	0.6	***
	22:6 n-3	3.0	2.9	2.6	0.26	NS	3.2	2.5	0.21	**
Neutral lipid	18:2 n-6	17	24	22	24	*	22	20	1.9	NS
	18:3 n-3	15	21	20	2.2	*	19	19	1.8	NS
Beef flavour (1 low-100 high)		31.4	31.4	30.2	1.90	NS	31.2	30.8	1.54	NS

†mg malonaldehyde/kg meat

Conclusions The increased PUFA in beef from animals produced on clover is highly desirable in terms of human nutrition but some mechanism, such as vitamin E supplementation, is necessary to prevent the oxidative instability produced by feeding red clover.

Acknowledgement We are grateful to MAFF for funding this work.

References

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Investigating the accuracy and usefulness of ultrasonic scanning and muscle scoring in predicting carcass conformation, fat and composition in cattle

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Introduction Increasingly the market demand is for carcasses of better conformation which is reflected in carcass prices. Therefore the breeding policy for suckler herds must reflect market requirements. This study examined the usefulness of ultrasonic scanning and muscle scoring in predicting carcass conformation, fatness and meat yield.

Materials and Methods Forty-two ¾ and $^{7}/_{8}$ Charolais, Limousin and Simmental bulls were used. They were spring born, singled suckled, weaned on October 21, 1999, and received high quality silage plus an average of 4.3kg of concentrates per head daily, until slaughter on June 6, 2000, at 15 months of age. Ultrasonic measurements of eye muscle and back fat were taken at the 10th-11th and 12th-13th ribs at slaughter using an Aloka 500V scanner. Eye muscle measurements taken were area, maximum depth (single depth at the deepest point) and average depth (average of four depths). Back fat measurements taken were area and depth (average of three depths). Scanning was also carried out using a Dynamic Imaging scanner at the 13th rib and 3rd lumbar vertebra. Depth of eye muscle was obtained at the 3rd lumbar and depth of back fat was obtained at the 3rd lumbar and 13th rib. A muscle score was assigned by three scorers using the Signet muscle scoring procedure. Carcass data collected included, standard EU carcass conformation and fatness scores, eye muscle area and depth and back fat depth measurements between the 12th-13th ribs and meat fat and bone percentage from pistola dissection of one side of the carcass. Relationships between live animal assessment and carcass data were examined using simple correlation procedures.

Results Comparisons of ultrasonic scanning of eye muscle with corresponding carcass measurements at the 12-13th rib showed that the most accurate measurement was area (r = 0.82), followed by average depth (r = 0.71) with maximum depth being least accurate (r = 0.54). Unadjusted eye muscle measurements (Table 1) had r values ranging from 0.17 to 0.60 and 0.40 to 0.66 with conformation and percentage meat, respectively. When adjusted for live weight nearly all coefficients decreased in value. No single scan measurement or site emerged as the best, however some scanned measurements had as good a relationship with carcass traits as did carcass eye muscle measurements. Correlations between ultrasound measurements of back fat with corresponding carcass measurements (Table 2) showed the Dynamic Imaging scanner (r = 0.45 for depth of fat at 13th rib) to be more accurate then the Aloka (r = 0.27 for depth of fat at 12-13th rib). Adjustment for live weight did not affect r values. Correlations between scanned measurements of back fat with carcass fatness traits were better using the Dynamic Imaging scanner (e.g. r = 0.67 between 13th rib fat depth and fat score) than the Aloka scanner (e.g. r = 0.40 between 13th rib fat depth and fat score). Muscle score had a strong positive correlation with conformation (r = 0.75 to 0.81) and percentage meat (r = 0.63 to 0.67) between scorers.

Table 1. Correlation coefficients between scanned and carcass eye muscle measurements and carcass conformation and meat percentage.

Scanned eye muscle	Conformation	Meat %
Area 10-11th rib [1]	0.45	0.56
Max. depth 10-11th rib [1]	0.58	0.65
Average depth 10-11th rib [1]	0.53	0.62
Area 12-13th rib [1]	0.48	0.61
Max. depth 12-13th rib [1]	0.17	0.40
Average depth 12-13th rib [1]	0.35	0.52
3rd lumbar muscle depth [2]	0.60	0.66
Carcass eye area 12-13th rib	0.60	0.69
Carcass max. depth 12-13th rib	0.54	0.65

Table 2. Correlations between scanned fat measurements and carcass fat traits

Scanned back fat	Fat score	Fat %	Carcass Back fat
Area 10-11th rib [1]	0.04	-0.17	-0.16
Depth 10-11th rib [1]	0.01	-0.26	-0.15
Area 12-13th rib [1]	0.40	0.39	0.19
Depth 12-13th rib [1]	0.41	0.30	0.27
3rd lumbar depth [2]	0.73	0.69	0.48
13th rib depth [2]	0.67	0.61	0.45
Carcass back fat depth	0.42	0.55	1.00

[1] Aloka Scanner, [2] Dynamic Imaging Scanner

Conclusions Area was the eye muscle trait most accurately measured when scanning between the 12-13th rib. Correlation coefficients for scanned eye muscle measurements at the 10-11th ribs and 3rd lumbar with conformation and percentage meat were close to corresponding r values obtained with carcass eye muscle measurements. Measurements of carcass back fat depth were more accurate with the Dynamic Imaging scanner. Scanned measurements using the Dynamic Imaging scanner were highly correlated with both carcass fat score and percentage fat. Muscle score showed a high ositive relationship with conformation and percentage meat.

Effect of breed and nutritional management on fatty acid composition of lambs of dairy Greek breeds of sheep

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Introduction Carcass fat of meat producing animals has long been identified as one of the most important characteristics of overall meat quality. In this respect, consumer choices of particular food reflect their awareness of the link between health and consumption of fats particularly saturated. Lamb meat is considered to be excessively fat and this results in substantial loss in its marketability. This study was carried out to assess the effect of breed, sex, post-weaning nutrition, live weight at slaughter and their interactions on the fatty acid (FA) composition in carcass fat of lambs of three indigenous dairy Greek breeds of sheep.

Materials and methods A total of 336 lamb carcasses were used. They were obtained from lambs of three indigenous dairy Greek breeds of sheep, the Boutsko (B), Serres (S) and Karagouniko (K). After weaning (at about 42 days) lambs were reared under different conditions of housing and nutritional management in three (3) consecutive experiments (Exp). In Exp 1, lambs (60 intact males and 60 females) were individually penned and fed *ad libitum* on a concentrate ration (11.3 MJ Metabolisable Energy (ME) and 192 g crude protein (CP) per kg DM) together with 100 g/day of Lucerne hay (8,3 MJ ME and 182 g CP per kg DM). In Exp 2, lambs (108 intact males only) were also individually penned but were fed on three (3) different levels of concentrate: High (H), Medium (M) and Low (L) and *ad libitum* on Lucerne hay. In Exp 3, lambs (108 intact males only) were initially group fed indoors for 63 days on 3 different levels of concentrate (also denoted as H, M and L) together with *ad libitum* Lucerne hay, and thereafter they finished on irrigated sown pasture (*Lolium perrene + Trifolium repens*). Lambs were assigned to be slaughtered at one of 5 standard proportions of mature weight (PMW) for each breed in Exp 1 (i.e. 0.20, 0.30, 0.45, 0.60 and 0.90); at three fixed live weights (TSLW; i.e. 23, 28 and 33 kg), common for all breeds in Exp 2 and at two fixed PMW in Exp 3 (PMW; i.e. 0.48 and 0.54). The right hand side of each lamb carcass was minced and one random sample, of approximately 200 g, was taken. Samples were freeze-dried and then grounded in a small mill, to pass through a 1-mm mesh screen. From the ground samples, a quantity of 2gr was taken for lipid extraction. Samples were assayed by gas-liquid chromatography to identify and quantify the proportions of various FA. Results were analysed separately for each experiment using the general linear model procedure of analysis of variance.

Results Fatty acid composition of carcass fat was significantly affected by breed, sex and degree of maturity (e.g. live weight at slaughter) of lambs (P<0.05 - P<0.001).. Generally, carcass fat of the lambs studied had a high proportion of palmitic (22.7%), stearic (15.8%) and oleic fatty acid (40.9%) and a low proportion of palmitoleic (3.7%), linoleic (4.9%) and linolenic fatty acid (1.8%). In Exp 1 and Exp 2, saturated FA of carcass fat were higher in lambs of K than those of S and B breeds. However, in Exp 3, saturated FA of carcass fat were higher in lambs of the B than S breed.

Table 1. Weight percentage proportion of individual fatty acids (FA) in the total composition of FA of the carcass fat of the Boutsko (B), Serres (S) and Karagouniko (K) breed.

	Experiment 1					Experiment 2					Experiment 3				
FA	B	S	K	SEM	P	B	S	K	SEM	P	B	S	K	SEM	P
10:00	0.31	0.25	0.29	0.021	NS	0.26	0.33	0.26	0.018	<0.01	0.27	0.29	0.29	0.020	NS
12:00	0.43	0.37	0.41	0.028	NS	0.28	0.39	0.46	0.022	<0.001	0.63	0.46	0.44	0.025	<0.001
14:00	5.36	4.59	5.05	0.138	<0.001	3.88	4.20	4.84	0.110	<0.001	5.91	4.67	4.61	0.140	<0.001
15:00	0.64	0.78	0.81	0.035	<0.01	0.63	0.69	0.68	0.016	<0.05	0.83	0.74	0.66	0.018	<0.001
16:00	22.27	22.91	24.56	0.237	<0.001	22.54	21.94	23.71	0.024	<0.001	23.01	21.40	21.89	0.217	<0.001
16:1? 7	3.56	3.52	3.53	0.055	NS	3.56	3.70	3.43	0.045	<0.001	3.89	3.74	3.46	0.036	<0.001
17:00	1.69	2.15	2.17	0.049	<0.001	1.87	2.11	1.91	0.038	<0.001	1.67	1.75	1.59	0.023	<0.001
18:00	13.55	14.02	13.85	0.263	NS	17.95	17.36	18.08	0.302	NS	16.08	16.45	16.36	0.219	NS
18:1?9	42.43	42.59	41.30	0.331	<0.05	40.64	40.33	38.92	0.335	<0.01	38.57	41.08	41.46	0.356	<0.001
18:2?6	5.81	5.52	4.83	0.110	<0.001	4.58	4.98	4.27	0.106	<0.001	4.43	4.59	4.58	0.055	NS
18:3? 3	1.89	1.14	1.00	0.037	<0.001	1.75	1.57	1.47	0.050	<0.001	2.35	2.48	2.47	0.05	NS
20:00	0.21	0.25	0.24	0.013	NS	0.19	0.24	0.18	0.021	NS	0.06	0.07	0.06	0.008	NS
Others	1.85	1.88	1.94	0.062	NS	1.84	2.13	1.75	0.062	<0.001	2.32	2.27	2.13	0.068	NS

Conclusions The results of the present study suggest that there are possibilities of modifying FA composition, especially reducing the saturated FA in carcass fat, by manipulation of post-weaning nutrition of lambs whilst slaughtering them at a wide range of live weights that are much heavier than those traditionally produced in Greece.

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Plasma and meat hormones and metabolites of lambs grazing high-formononetin red clover

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Introduction There is increasing interest in the use of red clover (*Trifolium pratense*) as a high protein forage crop to finish growing lambs. Red clover contains the isoflavanoid compound formononetin which is converted to the non-steroidal oestrogen analogue equol by rumen micro-organisms. Equol is absorbed by the animal, and can have significant effects, such as suppressing reproductive cycling in ewes. Very few commercial red clover varieties have been bred with a low formononetin content to prevent this problem. Although human health benefits have been linked to the consumption of oestrogenically active compounds in foods such as soya (Kurzer and Xu, 1997), there is a need to investigate the presence of oestrogenically active compounds in animal products. The objective of this study was to investigate plasma metabolite and hormone concentrations, and the residual levels of equol in meat, of lambs grazing two varieties of red clover differing in their formononetin contents, compared to control animals grazing grass.

Material and methods Sixty Suffolk × Mule lambs, 30 wethers (30.3 kg, s.e. 0.29) and 30 ewe lambs (29.4 kg, s.e. 0.18) were used for the experiment. Five male and five female lambs were weaned onto duplicated plots of each of three different forages: HF, a high formononetin red clover sward (predominantly cv. Astra); LF, a low formononetin red clover sward (cv. Formica); and Control, a perennial ryegrass (cv. AberSilo). The lambs were rotationally grazed on subplots of these swards until selected for slaughter at fat-class 3L. The mean number of days to finish were 46, 43 and 43 for treatments HF, LF and Control respectively (s.e.d. 4.8; NS). Animals were slaughtered using conventional techniques approximately 48 h after removal from the plots, following starvation and transportation to the abattoir. Blood samples were taken from all lambs immediately after they were removed from the experimental plots following selection for slaughter. Teat length was measured as an indication of the biological activity of red clover formononetin content, and meat samples (*Longissimus dorsi*), bulked for each sex on each duplicate forage plot, were analysed for concentrations of equol and oestradiol-17β. Data were analysed as a split plot analysis of variance, blocking on a dummy factor created to separate sexes within forage plots, and with a treatment structure of replicate + forage × sex.

Results Formononetin contents of the forages were 2.2, 1.6 and 0.0 g/kg dry matter (DM) for HF, LF and Control forages respectively. The DM contents of the forages were 131, 146 and 163 g/kg, and the crude protein (CP) contents were 255, 223 and 193 g/kg DM. Main effects of forage on plasma and meat metabolite and hormone concentrations are presented in Table 1. There was a significant effect of forage type on concentrations of plasma urea, probably due to the increased CP contents of the clovers. Red clover-fed animals also had significantly increased plasma concentrations of growth hormone and insulin-like growth factor (IGF)-I. While grazing the three different forages, male lambs had significantly increased mean plasma concentrations of insulin (6.24 vs 5.29 μIU/ml for male vs female; s.e.d. 0.222; $P<0.01$) and IGF-I (181.6 vs 151.8 ng/ml; s.e.d. 5.23; $P<0.01$). There was a significant effect of forage on mean teat length indicating a biological effect of the clover formononetin. There was a significant effect of forage on meat, but not plasma, oestradiol-17β concentrations (highest in the LF-fed lambs of both sexes: 20.6 pg/g for males, 22.2 pg/g for females), but no equol was detectable in meat samples.

Table 1. Mean effects of forage on plasma and meat metabolites and hormones (with interaction s.e.d.). See text for mean effects of lamb sex and interaction effects. HF and LF are high- and low-formononetin red clovers.

	Forage				Sig[†]		
Plasma	Control	HF	LF	s.e.d.	F	S	F×S
Total protein, g/l	67.0	68.9	67.3	1.46			
Albumin, g/l	33.6	35.5	34.7	0.88			
Globulins, g/l	33.4	33.4	32.6	0.84			*
Urea, mM	8.8	13.1	13.2	0.52	***		
Glucose, mM	3.71	3.84	3.82	0.145			
Growth hormone (GH), ng/ml	7.0	10.8	9.3	1.29	*		
Insulin, μIU/ml	5.6	5.9	5.8	0.39		**	
GH/Insulin, ng/μIU	1.3	1.9	1.6	0.18	*	**	
IGF-I, ng/ml	136	186	178	9.1	**	**	*
Oestradiol, pg/ml	4.8	5.6	5.8	0.89			
Meat							
Oestradiol, pg/g	7.6	8.1	21.4	4.26	*		
Equol	ND[‡]	ND	ND	-			
Teat length, mm	16.0	24.7	26.7	1.81	***		*

[†] Significance of effect: *, $P<0.05$; **, $P<0.01$; **, $P<0.001$; F = Forage, S = Sex.
[‡] Not detectable, < 5 ng/g fresh tissue.

Conclusion Despite several effects of forage type on plasma metabolite and hormone concentrations, and an effect of clover formononetin on teat growth in lambs of both sexes, there was no carry-over of equol into the meat of lambs finished on red clover with either a high or low formononetin content. Equol is rapidly excreted from the body, and was probably reduced to the undetectable levels during the time that animals were starved and transported for slaughter.

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Postmortem proteolysis in pork does not depend on fibre type distribution

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Introduction Proteolytic degradation is known to be faster in white muscles than in red muscles (Whipple & Koohmaraie, 1992). Variation in eating quality between muscles has often been correlated to their metabolic properties, as determined by the fibre type distribution. Correlations between fibre type distribution and postmortem proteolysis could result from two possible effects: (1) Due to their inherent differences in metabolic potential, composition and content of proteolytic enzymes, fibres of some types may degrade more than others. (2) The balance of fibre types controls postmortem (p.m.) metabolic characteristics of the muscle as a whole, with all fibre types within it being equally affected. An experiment was conducted to compare the rate of postmortem proteolysis in five porcine muscles differing in fibre type distribution and to compare the rate of proteolysis in type II fibres isolated from these muscles.

Materials and methods Three pigs (all the same crossbreed of Duroc, Landrace and Yorkshire) were used in this study. At 1 hour p.m. Semitendinosus (ST), Semimembranosus (SM), Longissimus dorsi (LD), Soleus (S) and Vastus intermedius (VI) were removed from the left side of the carcasses. Muscle samples (~ 10 g) were cut and frozen in liquid nitrogen. At 24 hours p.m. the five muscles from the right side of the carcass were removed and muscle samples were either frozen immediately or stored for 2 and 7 days, respectively, at 2^{o}C. Fibre types were identified by staining for myofibrillar ATPase activity (Brooke & Kaiser, 1970). Muscle homogenates and type II fibres were dissolved in urea buffer (Fritz et al., 1989). Desmin and its degradation products were resolved by SDS-PAGE on 10% separating gels (Novex, USA). Proteins were electrophoretically transferred to polyvinylidene fluoride membranes and incubated with primary mouse anti-desmin (DE-R-11, 1:5.000, Dako, UK) antibody. Antibody binding was visualised by exposure to BCIP/NBT. Densitometric scans of membranes were performed using a CREAM software program (Kem-En-Tek, Denmark).

Results The rate of desmin degradation in the five muscles is shown in figure 1. Desmin degraded faster in LD and SM than in ST, S and VI. The rate of desmin degradation was expected to be similar for ST, LD and SM because the fibre type distribution of these muscles is similar (data not shown). However, ST exhibited the same rate of degradation as VI and S, even though VI and S have more type I and IIa fibres than ST. The inter-muscle differences can therefore not be explained solely by the fibre type distribution but may also be influenced by other muscle-specific traits (e.g., proteolytic potential). Desmin degradation in type II fibres is illustrated in figure 2. The relative change in the band intensity of native desmin from day 1 to 8 p.m. was calculated and used as an estimate of the rate of degradation because degradation products could not be detected. The highest relative change of desmin occurred in type II fibres isolated from LD, while desmin did not change from day 1 to 8 p.m. in ST. These results indicate that type II fibres show different patterns of proteolysis depending on the muscle in which they are located.

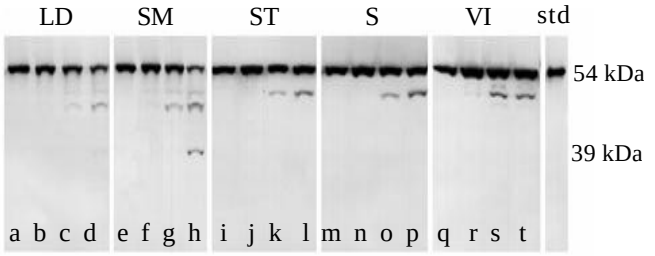

Figure 1. Western blot of muscle homogenates at 0 (lane a, e, i, m, q), 1 (lane b, f, j, n, r), 3 (lane c, g, k, o, s) and 8 (lane d, h, l, p, t) days postmortem. Purified desmin appears in the single track on the right. Each muscle was analyzed from three different animals.

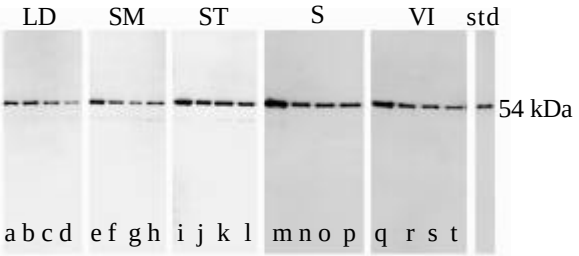

Figure 2. Western blot of type II fibres at 0 (lane a, e, i, m, q), 1 (lane b, f, j, n, r), 3 (lane c, g, k, o, s) and 8 (lane d, h, l, p, t) days postmortem. Purified desmin appears in the single track on the right. Each muscle was analyzed from one animal.

Conclusions Differences between muscles in the rate of degradation do not seem to be a direct result of the fibre type distribution. The rate of degradation probably depends more on the local environment (i.e., pH and proteolytic potential) within the muscle rather than on variations between individual fibres of given types.

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Conjugated linoleic acid in cows milk: independent effects of dietary linoleic and linolenic fatty acids

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Introduction It is desirable to increase the level of conjugated linoleic acid (CLA) in milk as a health benefit in human nutrition. CLA has been shown to affect carcinogenesis, atherosclerosis, diabetes, the immune system, bone mineralization, body fat accretion and nutrient partitioning. The predominant CLA isomer present in foods from ruminants is *cis*-9, *trans*-11 CLA. It is formed in the rumen by anaerobic bacteria as an intermediate in the hydrogenation of linoleic acid. Recent evidence has shown that CLA can also be produced in the mammary gland by desaturation of *trans*-11 $C_{18:1}$. Previous researchers have used various oils or oil seeds to try and elevate CLA levels in milk. A problem with this approach is that most oils contain mixtures of fatty acids so responses cannot be attributed to individual acids. Up to now there has been no report looking at how individual fatty acids affect CLA production. The objective of this work was to separate the effects of linoleic and linolenic acids on CLA production in dairy cows.

Materials and methods

Eight Holstein-Friesian cows were used in this study. Four diets were designed that differed in their linoleic and linolenic acid contents. All cows received a basal diet of 30 kg/day of good quality grass silage. The concentrate (7kg/day) consisted of barley (583g/kg), soya (315), oil blend (77) and minerals/vitamins (25). Four oil blends with the desired amounts of linoleic and linolenic acids were produced from olive, linseed, rape, soya and sunflower oils (Table 1). Cows were fed on each diet for ten days, with samples collected on the last three days of each period, in a 4x4 Latin Square design. Data were analysed using analysis of variance with cows as blocks to avoid problems of repeated measures.

Table 1 Fatty acid profile of the oil blends (g/100g FAME)

	Fatty Acid				
Treatment	**C16:0**	**C18:0**	**C18:1**	**C18:2**	**C18:3**
Low linoleic/low linolenic acid (**LL**)	12	3	59	13	13
Low linoleic/high linolenic acid (**LH**)	9	3	41	14	32
High linoleic/low linolenic acid (**HL**)	6	3	43	33	12
High linoleic/high linolenic acid (**HH**)	6	4	21	34	34

Results There were significant treatment effects on milk yield and milk fat content, but not milk protein content. High linoleic, with or without high linolenic acid, significantly decreased the palmitic acid content of milk fat. No difference was observed in the stearic or oleic acid content of milk fat despite the large variation in precursors. *Trans* $C_{18:1}$ fatty acids were increased with the HH diet indicating incomplete hydrogenation in the rumen. Significant differences in linoleic and linolenic acids in milk fat were observed that reflected the dietary supply of these fatty acids. Increasing the linoleic and/or linolenic acids in the diet increased the concentration of CLA in milk fat. High levels of both had an additive effect increasing the CLA content in milk by 27% from the LL treatment.

Table 2 The effects of treatment on production parameters and the fatty acid composition of milk fat (g/100g FAME)

	LL	LH	HL	HH	SED
Milk yield (kg/d)	17.1^{a}	18.0^{b}	17.9^{ab}	17.4^{ab}	0.39
Milk fat content (g/kg)	44.7^{a}	48.5^{b}	45.4^{ab}	44.7^{a}	1.63
Milk protein content (g/kg)	32.4	32.7	32.4	32.3	0.35
Milk fat composition (g/100g FAME)					
$<C_{14:0}$	20.3^{a}	20.8^{ab}	21.9^{c}	21.5^{bc}	0.42
Palmitic acid ($C_{16:0}$)	26.1^{a}	25.5^{ab}	25.0^{b}	24.2^{c}	0.34
Stearic acid ($C_{18:0}$)	14.8	14.8	14.8	15.1	0.39
Trans $C_{18:1}$	2.9^{a}	2.9^{a}	2.8^{a}	3.3^{b}	0.16
Oleic acid (*cis*-9 $C_{18:1}$)	26.9	26.6	26.1	26.0	0.57
Linoleic acid ($C_{18:2}$)	1.2^{a}	1.3^{a}	1.5^{b}	1.6^{b}	0.03
Linolenic acid ($C_{18:3}$)	0.4^{a}	0.5^{b}	0.4^{a}	0.5^{b}	0.01
cis-9, *trans*-11 CLA	0.8^{a}	0.9^{b}	0.9^{b}	1.1^{c}	0.04

[a,b,c] Means in the same row with different superscripts differ significantly (P<0.05)

Conclusions The results demonstrate that the CLA content of cows milk can be increased by increasing linoleic and/or linolenic acids in the diet. Different oil blends affected the extent of hydrogenation in the rumen and consequently the fatty acid profile of milk. By obtaining high CLA levels on both the LH and HL diets we have confirmed recent proposals that CLA can be produced from *tran*-11 $C_{18:1}$ in the mammary gland by the Δ^9-desaturase enzyme as well as in the rumen through incomplete hydrogenation.

Acknowledgement A.L. Lock would like to thank MAFF for the provision of a Ph.D. Studentship.

Altering carcass composition during a winter store period does not affect the final carcass composition following zero-grazing at the end of an 18-month finishing system

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Introduction The ability to produce high quality beef carcasses cost-effectively in an 18-month finishing system is partly determined by the feeding regime during winter and the subsequent response to grass during the finishing period. Animals fed on grass silage throughout winter have been shown to develop fatter carcasses (Baker *et al.*, 1985). The aim of this study was to investigate the effects of altered carcass composition at the end of a winter store period on composition at the end of a summer grazing period.

Method Thirty-eight spring born Limousin cross steers weaned in autumn, were fed on grass silage and a concentrate diet for two weeks. Steers were randomly divided into three groups, blocked by weight, one group (Autumn 1) consisted of ten steers and the remaining two groups (Spring and Autumn 2) each consisted of fourteen steers. Autumn 1 steers were scanned using velocity of sound (VOS) at the 10th rib, last rib and 3rd lumbar vertebra, slaughtered, and the protein and fat content of the carcass was determined. The remaining 28 animals were trained to use Calan-Broadbent gates. Fourteen steers (7 from Spring and 7 from Autumn 2) were fed on a diet of barley (58%) and straw (36%) supplemented with rumen protected soya (6%). The remaining steers were fed a diet of silage (86%) and barley (13%) supplemented with rumen bypass fat (1%). The diets were adjusted to achieve an overall growth rate of 0.6 kg/day over a six month winter store period for both groups. At the end of the winter store period (Spring) VOS measurements were carried out on seven animals per treatment prior to slaughter. The remaining fourteen steers were zero grazed *ad libitum* on a grass diet for eleven weeks and finished on a silage diet for the last seven weeks due to a shortage of grass. In autumn the remaining fourteen steers were VOS scanned and slaughtered (Autumn 2). Rates of carcass, protein and fat gain were determined by the sequential slaughter. The mean of all three sites scanned using VOS was used for the statistical analysis. Statistical analysis was carried out using ANOVA or regression.

Results There was no significant difference in mean animal liveweights between barley based and silage based treatments at the different slaughter points. Mean weights for the steers were 197 kg (sem 5.3), 289 kg (sem 4.6) and 382 kg (sem 4.5) for Autumn 1, Spring and Autumn 2 periods respectively. Steers slaughtered in Spring and fed on the barley based diet had a higher protein and lower fat carcass content than steers fed on the silage based diet. Steers fed on the silage based diet had a higher killing out percentage then steers fed on the barley based diet. At the end of the summer finishing period there was no significant difference in carcass characteristics between the barley and silage treatments but fat gain was significantly higher in barley fed animals. There was no significant difference in the mean of the VOS results, from the three sites, and different treatments at the slaughter points. The VOS results showed a significant correlation with the lean:fat ratio; $p<0.001$, $r^2 = 0.36$.

Table 1. *Chemical composition of half carcass ,VOS data and carcass gain for steers at different slaughter points and different treatments.*

Carcass	Autumn 1	Spring		Autumn 2	
Characteristics		Barley	Silage	Barley	Silage
Carcass weight (kg)	105 ± 3.6^{a}	144 ± 3.2^{b}	160 ± 4.3^{c}	201 ± 5.5^{d}	211 ± 5.2^{d}
Killing Out (%)	53.2 ± 0.92^{ab}	50.2 ± 0.64^{b}	55.0 ± 0.28^{a}	53.4 ± 0.99^{ab}	54.7 ± 0.96^{a}
Protein (%)	18.6 ± 0.18^{a}	20.2 ± 0.53^{bc}	18.4 ± 0.31^{ad}	19.9 ± 0.26^{acd}	20.1 ± 0.52^{d}
Fat (%)	11.6 ± 0.53^{a}	11.6 ± 0.94^{a}	17.6 ± 1.05^{b}	18.0 ± 0.46^{b}	18.4 ± 1.30^{b}
VOS	6.30 ± 0.006^{a}	6.27 ± 0.006^{a}	6.29 ± 0.011^{a}	6.30 ± 0.005^{ac}	6.34 ± 0.014^{bc}
Live wt. Gain (g/day)		621 ± 45.9^{a}	640 ± 52.9^{a}	717 ± 83.7^{a}	799 ± 88.2^{a}
Carcass gain (g/day)		271 ± 23.1^{a}	376 ± 32.4^{b}	449 ± 48.0^{ab}	431 ± 58.2^{b}
Protein gain (g/day)		66.5 ± 8.39^{a}	68.0 ± 6.35^{a}	86.7 ± 9.13^{a}	110.3 ± 17.8^{a}
Fat gain (g/day)		30 ± 10.1^{a}	107 ± 11.9^{bc}	152 ± 10.1^{b}	85 ± 13.8^{c}

Values in the same row with different superscripts are significantly different ($p<0.05$).

Conclusion Steers fed a silage based diet had higher carcass weights and gained more fat during the winter period. Despite differences in carcass weight and fat composition at the end of the winter period between silage and barley fed animals, differential responses in tissue growth resulted in similar carcass weights and composition at the end of the summer period. Although there was a correlation between the lean:fat ratio of the carcass and the VOS results, the treatment effects of carcass composition could not be detected using VOS.

Acknowledgements The funding of this work by MAFF is gratefully acknowledged

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Effects of a high-fat diet based on palm, soybean or maize oil on growth performance and carcass characteristics in growing-fattening pigs

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Introduction Diets for growing-fattening pigs are normally low in fat and based on cereals, which supply approximately two thirds of the total energy required by pigs. Restricted concentrations of oil (between 2 and 10% of total dry matter) have been used to improve growth rate and feed efficiency. Palm oil and oil palm by-products have been used as the main energy source in the growth cycle as substitutes for cereals (Ocampo and Lean 1999). The objective of this experiment was to evaluate three oils as dietary substitutes for maize grain in growing-fattening pigs, based on growth performance and carcass characteristics using ultrasonic measurements.

Materials and methods Thirty-two crossbred gilts were randomly distributed into 4 treatments (Table 1), each with 8 animals per pen but fed individually. A constant level of feed was offered for each treatment for the duration of the trial (1,840, 1,250, 1,210 and 1,210 g/day for maize, palm oil, maize oil and soybean oil treatments respectively). All animals being offered the same levels of energy and lysine. Gilts had two adjustment weeks, were fed twice daily (0800 and 1600 h) and the rejected feed was weighed daily. Animals were weighed every two weeks and ultrasonic measurements of backfat (P_2) and muscle depth were taken at 46, 65 and 89 kg live weight, using a portable linear/convex sector ultrasonic scanner (ultrasonic frequency 3.5 MHz). Data were analysed using ANOVA procedure of Genstat as a completely randomised design, using treatment as a factor. The total lipids were extracted from oil and diet using the method of Bligh and Dyer (1959).

Results Means of results obtained during 9 experimental weeks are given in Table 2. Feed intake was c. 5% lower than was expected during the first two experimental weeks. Growth rate, P_2 (backfat) and muscle depth were similar for all treatments (P>.05). Feed conversion (F.C.) was statistically different (P<.001) between the maize treatment and the oils treatments, but digestible energy (DE)/kg gain Mj were similar for all treatments (P>.05). Fat intake was c. 4.5 times greater in the oil treatments than that in the maize treatment. Fat intakes represented c. 42% of the total intake in the oil treatments.

Table 1 Ingredients and composition of treatments, as-fed basis

	Treatment[1]			
	Maize	PO	MO	SO
Ingredients (g/kg)				
Maize grain	652	--	--	--
Soybean meal[2]	239	440	455	455
Rice bran	109	160	165	165
Palm oil	--	400		
Maize oil	--	--	380	--
Soybean oil	--	--	--	380
Composition (g/kg of diet)				
Fat	62	438	406	414
Crude Protein[3]	163	196	203	203
Lysine[3]	8.6	12.4	12.8	12.8
D.E. Mj/kg[3]	14.2	21.3	22.4	22.0

[1] PO=Palm oil; MO=Maize oil; SO=soybean oil
[2] Soybean fortified with vitamins and minerals (92.4% soybean meal, 2.5% mixture of vitamins and minerals and 5.1% dicalcium phosphate)
[3] Calculated data

Table 2 Means of performance according to treatments

	Treatment				
	Maize	PO	MO	SO	sed
Live weight kg					
Initial	47.3	46.2	47.6	46.6	
Final	90.0	89.0	89.0	87.0	
Growth rate g/d	677	682	661	643	23.8
Daily intake					
Fat g	108[a]	524[b]	479[c]	486[c]	6.55
Crude protein g*	285	237	243	241	
Lysine g*	15.0	15.0	15.3	15.2	
D. E. Mj*	24.7	25.1	25.7	25.6	
Total feed kg	1.75	1.19	1.18	1.17	
F. C. (DM)	2.3[a]	1.6[b]	1.7[b]	1.7[b]	0.08
D E /kg gain Mj	37.0	36.9	39.1	40.2	1.16
Carcass, mm					
P_2 fat depth	11.8	13.2	14.1	12.9	1.0
Muscle depth	52.5	54.1	53.9	53.1	1.56

Means within rows with unlike superscript letters were statistically different (P<0.001)
* Calculated data

Conclusions The results suggest that the breakdown processes of the energy from a carbohydrate source (maize) and a fat source (oils) were equally efficient for muscle growth and the use of oils did not increase fat deposition. Diets containing high levels of fat (up to 42%) in the place of cereals may be used efficiently in some pig production systems.

Acknowledgements Support from Colciencias (Colombia) and Cotswold Pig Development Company Ltd is gratefully acknowledged.

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Implications of changing the housing conditions of pigs prior to slaughter for the eating quality of bacon

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Introduction Commercial housing conditions are known to have an effect on the eating quality of bacon. Significant differences have been shown between pigs reared on solid concrete floors or straw-bedded accommodation (Maw *et al*. In press). Also high levels of dietary Vitamin E have been shown to improve pigmeat quality (Buckley *et al*., 1995).This experiment was carried out to examine whether the effects of housing could be eliminated or reversed by altering housing conditions or reduced by dietary supplementation with Vitamin E.

Materials and methods Sixty four Large White × Landrace pigs balanced for sex were assigned on the basis of liveweight to one of 4 treatments, in 4 replicates for 4 weeks prior to slaughter. The treatments were as follows:-

CONCRETE FLOORED- Pigs kept in solid floored accommodation with a slatted dunging passage for 4 weeks prior to slaughter.

VITAMIN E – As Concrete Floored with the addition of a Vitamin E supplement of 250 mg/kg of feed.

CHANGEOVER – Pigs kept in solid floor accommodation for 3 weeks and then moved to straw-bedded accommodation for the final week before slaughter.

STRAW - Pigs kept in straw-bedded accommodation for 4 weeks prior to slaughter.

The middle backs from each pig were processed for bacon. Once mature the backs were pressed, frozen to –12 °C, sliced and stored. Sample slices from each pig were subjected to a sensory profile analysis by a trained taste panel of 10 persons. A 5-point scale, where increasing value indicates increasing strength, was used to assess 14 attributes describing appearance, texture, taste and aroma. Analysis of variance was used to test for differences between the treatments.

Results Those sensory attributes which showed significant differences ($P<0.05$) are given in the table below:-

Table 1 Treatment means of a 5-point scale for 4 sensory attributes

Attribute	**Treatment**				**s.e.d**
	Concrete	**Vit. E**	**Ch/over**	**Straw**	
Aroma					
Androstenone	1.72^{a}	1.77^{ab}	1.87^{b}	1.64^{a}	0.068
Off-aromas	1.29^{a}	1.27^{a}	1.44^{b}	1.27^{a}	0.053
Flavour					
Skatole	1.56^{a}	1.54^{a}	1.77^{b}	1.68^{ab}	0.076
Off-flavours	1.27^{a}	1.47^{b}	1.30^{a}	1.34^{a}	0.062

^{ab} means on the same row with same superscript are not significantly different

Bacon produced from pigs in the changeover treatment gave significantly higher scores ($P<0.05$) for androstenone and off- aromas compared to those from solid concrete and straw-bedded accommodation. For skatole flavour there was also a significant difference ($P<0.05$) between the changeover and concrete treatments. The intensity of off-flavours was significantly higher ($P<0.05$) in the Vitamin E treatment than the other three.

Conclusions The changeover of the housing conditions of pigs 1 week prior to slaughter has a detrimental influence on subsequent eating quality with an increase in strength of undesirable aromas and flavours. This maybe associated with a disturbance of the gut microflora, possibly due to the effect of straw consumption. Contrary to expectation, Vitamin E appeared to have a detrimental effect on the strength of off-flavours. No explanation for this can be offered.

Acknowledgements The authors are grateful to D A Halls Ltd for providing the meat samples and the staff and students at The Robert Gordon University, Aberdeen who were involved with the taste panel evaluations. Appreciation is also extended to MAFF and D A Halls Ltd for funding this work.

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The effects of straw-only feeding prior to transport and journey time on faecal pathogen excretion and hide contamination of finished cattle.

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Introduction Many cases of food-borne illness in the UK are related to the consumption of contaminated meat products. This has highlighted the importance of adopting hygienic procedures throughout the meat production chain, including the farm environment (Pennington, 2000). Many factors are known to affect the hygienic condition of finished cattle (Davies *et al*., 2000) and various husbandry practices may be used to improve cleanliness at slaughter. Feed withdrawal, for example, may be used to reduce faecal output and improve the visible cleanliness of hides. However, the extent to which this impacts upon microbiological contamination of the hide, and its effects on pathogen levels following transport to the abattoir remain to be determined. This study investigated the interactive effects of feeding a straw-only diet prior to transport and journey time on the microbiological status of cattle faeces and hides.

Methods A total of 72 Limousin cross cattle, finished on a silage-based ration, were used in a four by two factorial design experiment. Animals were housed in pens of eight, and randomly allocated to a straw-only diet for 0, 1, 2 or 3 days prior to transport to the abattoir (n = 18 per treatment). Following each straw-only feeding period, animals were transported to the abattoir in a single-deck cattle wagon, with the journey time being either 2.5 or 6 hours (n = 9 per journey time). Rectal faecal samples were taken from all animals before transport, and a postmortem faecal sample was taken at the abattoir. A brisket swab sample was taken from all animals before transport to the abattoir and following unloading. As an index of bacterial load, Total Viable Counts (TVCs) in faecal samples and swabs were measured using automated plate counting equipment, following plating of diluted inocula onto nutrient agar plates and incubation for 72 hours at 30°C. Data were transformed to $\log_{10}$ values, analysed using ANOVA and are expressed as a mean and standard error.

Results Straw only feeding for 0, 1, 2 or 3 day prior to transport significantly affected faecal (Fig 1a; $P<0.01$) and hide (Fig 1b; $P<0.05$) TVCs. Following transport to the abattoir, both faecal (Fig 2a) and hide (Fig 2b) TVCs tended to be greater after a 6 hour journey, compared with a 2.5 hour journey, although this did not reach statistical significance. There was no significant interaction between the duration of straw only feeding and journey time.

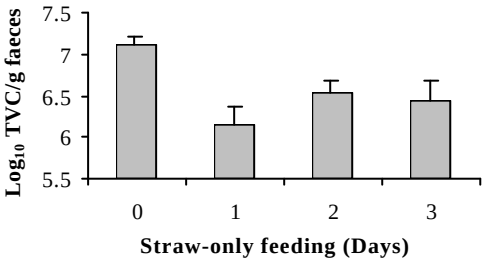

Figure 1a *Faecal TVCs($\log_{10}$ values) before transport*

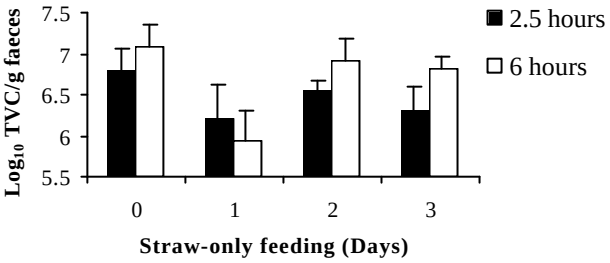

Figure 2a *Faecal TVCs ($\log_{10}$ values) after transport*

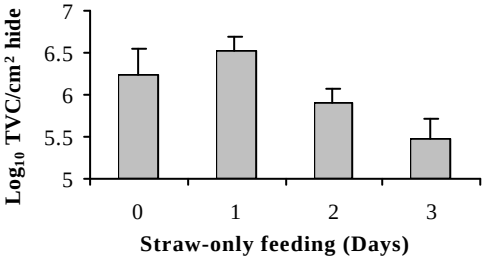

Figure 1b *Brisket TVCs($\log_{10}$ values) before transport*

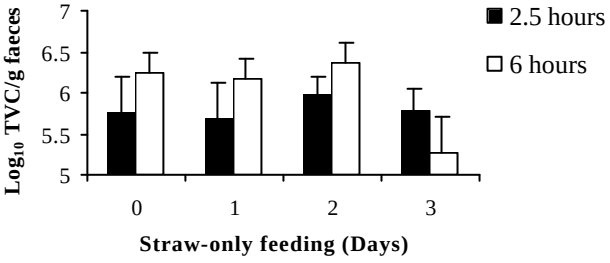

Figure 2b *Brisket TVCs ($\log_{10}$ values) after transport*

Conclusions Straw-only feeding following a silage-based finishing ration may be used to reduce the level of TVCs in the faeces of cattle, and therefore has the potential to reduce the contamination of hides with bacteria pathogenic to humans. This may be of particular importance as journey time to the abattoir increases.

Acknowledgements The funding of this work by the Food Standards Agency is gratefully acknowledged.

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The effect of the lambing distribution on the evolution of bulk tank milk composition in the Latxa dairy sheep of the Basque Country (Spain)

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Introduction According to the lambing distribution more than 90% of the Latxa flocks existing in the Spanish Basque Country can be classified into four typologies (Oregui *et al*., 1996, Ruiz *et al*., 1997). Generally speaking, flocks located in the highlands or mountainous areas (Groups 1 and 2) usually concentrate the lambing season at the end of winter. As winter conditions are less severe, lambings tend to begin before (early-winter or late-autumn) and follow a more scattered pattern (Groups 3 and 4). The objective of this analysis was to study the possible relationship between lambing distribution and solids composition of bulk tank milk, which affects cheese-making performances at the farm.

Materials and Methods Data from 1076 bulk tank milk samples recorded monthly in 70 flocks and 3 consecutive productive seasons (1995, 96 and 97) were analysed, which meant 207 flock-year levels. Fat and protein contents (g/100 ml.) determined from these samples were statistically analysed according to the following general linear model:

$$y_{ijk} = a + b \times AMY + T_i + Mm_j + T_i \times Mm_j + FY_k(G)_i + e_{ijl}$$

AMY is the average daily milk yield per ewe in the corresponding test day; T_i is the effect of the lambing typology (i=1 to 4); Mm_j is the effect of the month of milking (j=1 to 7); FY_k is the flock-year effect (k=1 to 207). As each typology used to begin the milking period in a particular month of the year, the sample was considered to be unbalanced to allow introducing the effect of the month of the year (My_j) and Mm_j simultaneously. Because of that, the analysis was repeated substituting Mm_j by My_j. Fat and protein monthly average concentrations were estimated by least square means.

Results Both models explained 76-77% of the variance, having all the factors included in the analysis a significant effect ($p<0.05$) on fat and protein concentrations. Apart from the conjugated effect of FY_k ($p<0.001$), the analysis showed the negative effect of *AMY* on solids concentration ($p<0.05$), which is well known at the animal level

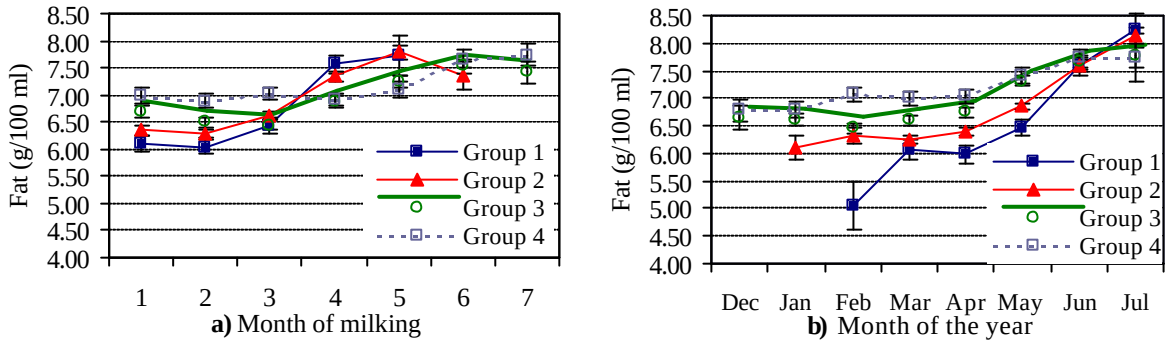

Figure 1 Evolution of fat concentration (g/100 ml) according to the lambing distribution typology.

The significant effect of $T_i x Mm_j$ ($p<0.01$) indicated differences in the evolution of fat and protein concentrations along the milking season in each typology. The increase in lambing concentration (from Group 4 to 1) involves a faster conformation of the milking flock and a more similar physiological state of every productive sheep. In consequence, the evolution of fat (Figure 1a) and protein concentrations throughout the milking season remembers the existing for just one sheep. On the contrary, the average physiological stage of the milking flock is more constant in Groups 3 and 4 due to a more dynamic milking flock, and solids concentration is more homogeneous throughout the season. Together with the higher maintenance of milk yield (Ruiz et al., 1997), this is regarded to be a positive feature for cheese making. But it was amazing to find that milk concentration in the early milking season increased as it begun earlier. This effect was more evident when My_j was included in the statistical model instead of Mm_j (Figure 1b). The causes of this effect have not yet been determined, but it can probably be related to differences in the management of the flocks, the capacity to mobilise body reserves, and the photoperiod at lambing.

As a consequence of these different tendencies in the milk concentration patterns, significant differences ($p<0.05$) were found between Groups for the fat content (g/100 ml) estimated (lsmeans) for the whole milking season: 6.5±0.14 and 6.7±0.07 in Groups 1 and 2, 7.0±0.04 in Group 3, and 7.2±0.07 in the Group 4. As for protein, only the Group 1 (5.9±0.07) differed significantly ($p<0.05$) from the others (5.7±0.05 in Group 2, and 5.7±0.04 in Groups 3 and 4).

Conclusions Lambing concentration is related to a higher increasing tendency in fat and protein concentrations of bulk tank milk throughout the milking season. But milk concentration in the early milking season decreases as it begins later.

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Increased intake responses from beef steers zero-grazed on *Lolium perenne* selected for high levels of water soluble carbohydrate

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Introduction Fresh forages may result in the loss of up to 40% of dietary nitrogen as rumen ammonia, as the microbial population is unable to utilise all the non-protein nitrogen released from rapidly degradable plant proteins. This may be due to the characteristically low levels of readily available energy released in the rumen, primarily as a result of the low levels of water soluble carbohydrate (WSC) in traditional forages. In a previous experiment Lee *et al,* (1999) found an increase in liveweight gain of pre-weaned lambs grazing *Lolium perenne* selected for high WSC concentrations. This study examined whether the enhanced performance on high WSC grass may be related to increased supply of nitrogen to the small intestine.

Materials and methods Eight Hereford x Friesian steers 430 (se 9.0) kg, prepared with rumen, duodenal and ileal cannulae were allocated at random to receive either one of two fresh grass varieties *ad libitum.* AberDove, an experimental variety bred to express high levels of WSC or AberElan, a commercially available variety used as the control. The diets were given for a 21d experimental period and over the last 7d, rumen (1d), duodenal (2d) and ileal (1d) samples were obtained. The grasses were cut daily from plots of primary growth material that had all previously been fertilised with approximately 62.5 kg N/ha, two months prior to the start of the experiment. AberElan (control) was cut at 10:00h, and AberDove (high WSC) was cut at 14:00h, to maximise diurnal WSC differences. Immediately following cutting, the fresh grass was chilled for 2h in a blast freezer, and was then moved to cold storage at 4^{o}C until being offered *ad libitum* twice daily, at approximately 16:00h and 09:00h. Following the end of the experimental period animals were slowly changed onto a standard experimental silage for a 14d period after which intakes were then determined over 5d to act as a covariate period. All samples were subject to general analysis of variance with a treatment structure of grass variety adjusted for covariate intakes (Genstat 5; Lawes Agricultural trust, 1997)

Results The WSC concentration of AberDove (236 g/kgDM) was higher (P<0.001) than AberElan (152 g/kgDM). Fibre content of AberDove was lower (P<0.001) than AberElan (ADF, 258 and 303 g/kgDM respectively) but there was no significant difference in crude protein content of the grasses (98.2 and 99.3g/kgDM respectively). Rumen parameters and nitrogen flow to and absorption from the small intestine are shown in Table 1. Animals offered AberDove had lower rumen ammonia-N concentrations (P=0.001), but higher DM and N intakes (P<0.001) and greater flows of nitrogen into and absorption of nitrogen from the small intestine (P=0.05 and P=0.004 respectively). Total volatile fatty acid (VFA) and butyrate production were not significantly different between the two grasses. However the ratio of propionate : (acetate + butyrate) was significantly greater on AberDove.

Table 1 Rumen parameters and nitrogen flow to and absorption from the small intestine (residual d.f. =7)

	AberElan	AberDove	s.e.d	Significance
DM Intake (kg/d)	6.7	9.3	0.02	0.001
N Intake (g/d)	105.9	153.9	2.86	0.001
Duodenal N (g/d)	99.6	129.9	11.8	0.05
Apparent Absorption (gN/d)	50.7	70.7	4.07	0.004
		Rumen Parameters		
Total VFA (mmol/l)	61.9	60.7	1.74	NS
Acetate (mmol/l)	40.4	37.7	1.10	0.01
Butyrate (mmol/l)	6.9	7.2	0.31	NS
Propionate (mmol/l)	12.4	13.7	0.42	0.002
Ratio (P/A+B)	0.26	0.30	0.010	0.001
Ammonia-N (mgN/l)	26.4	14.0	1.95	0.001

NS = Not significant to P<0.05

Conclusions Animals consumed approximately 40% more of the higher WSC (AberDove) forage, which may be related to the significantly lower rumen ammonia-N or the lower fibre content of the high WSC grass. Since the N content of the grasses were similar the increased DM intake on AberDove resulted in an increased flow of N to and absorption from the small intestine. This along with the greater propionate : (acetate + butyrate) ratio may contribute to an increase in animal performance.

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The effect of forage type and host animal diet on the *in situ* rumen degradation of grass silage and pea/wheat bi-crops containing different pea varieties

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Introduction Compared to grass silage, pea/wheat bi-crops produce higher dry matter (DM) yields, higher feed intakes and nitrogen (N) retention (Adesogan *et al*., 2000). The improved performance of animals fed bi-crops may be due to a postulated synchronous supply of readily fermentable energy and protein for ruminal microbial protein synthesis. This study attempted to validate this theory by measuring the rumen degradability of grass silages and pea/wheat bi-crop silages containing different pea varieties. To determine if grass silage-fed animals could be used to determine the degradability of bi-crops, the effect of host animal diet on rumen degradation was also examined.

Materials and methods Two pea/wheat bi-crops that were established from spring wheat (var. Axona) and either a tall (var. Magnus; MW) or short straw (var. Setchey; SW) spring pea variety were evaluated. A first cut, perennial ryegrass silage (GS) was also evaluated as a control. The silages were incubated in three dry, ruminally cannulated Friesian cows in a 3x3 change over design with three week periods. The cows were adapted to the diets in the first 2 weeks of each period and the last week was used for the rumen degradation studies. The bi-crops and GS silages were fed at the maintenance level of intake (with no concentrates) in two equal portions twice daily at 09.00 and 16.00 h. Rumen degradation was measured using the nylon bag technique. Each forage was incubated in quadruplicate during each period for each of the following incubation times: 4, 8, 16, 24, 48, 72, and 96 h. In order to study the effect of host animal diet, four replicate samples of each forage were also incubated for 48 h in the rumen of cows fed on the other forages in each period. The kinetic degradation parameters were described with the model of McDonald (1981; ($D = A+B(1-e^{c(t-t_L)})$) where A = the zero time washing loss, B = the potentially degradable insoluble fraction, c = the fractional rate of degradation and t_L = lag time). The effective degradability (ED) was calculated using a fractional outflow rate (k) of 0.05/h. Statistical analysis was carried out using the general analysis of variance

Results The DM washing loss was significantly higher in MW than in SW or GS but the other DM degradation parameters were not significantly affected by forage type (Table 1). Nitrogen loss after 24 or 48 h was also similar across the forages. However, starch and NDF losses after 48 h were lower (P<0.001) in SW than in MW or GS. MW had the highest starch loss after 48 h while GS had the highest NDF loss after 48h. SW had the lowest NDF and starch losses after 48 h. The host diet did not affect the ruminal degradation of DM, N and starch in the bi-crops (Table 2). In contrast, incubating GS in the rumen of cows fed SW significantly reduced the DM, N, starch and NDF degradation of GS.

Table 1. DM degradation characteristics (g/kg) and 48 h N (g/kg N), starch (g/kg starch) and NDF (g/kg NDF) losses of bi-crops and GS

	MW	SW	GS	SED
A	481[b]	451[a]	449[a]	6.00
B	372	412	416	24.6
A+B	853	862	864	22.3
ED (k=0.05/h)	614	629	629	8.40
c	0.040	0.049	0.049	0.01
Lag time (h)	2.72	2.33	2.36	0.44
24 h N loss	905	912	908	5.88
48 h N loss	937	933	933	3.77
24 h starch loss	634[a]	731[b]	722[b]	19.7
48 h starch loss	821[b]	771[a]	805[b]	9.89
24 h NDF loss	368[a]	358[a]	539[b]	30.7
48 h NDF loss	558[b]	468[a]	661[c]	19.1

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

Table 2 The effect of forage consumed on 48 h rumen degradation of DM (g/kg), N (g/kg N) and starch (g/kg starch)

Test forage	Forage fed	DM	N	Starch
SW	SW	724	933	771
	MW	746	938	791
	GS	762	942	801
	SED	23.7	5.83	19.1
MW	MW	766	937	821
	SW	744	932	804
	GS	781	942	832
	SED	12.9	3.33	9.1
GS	GS	802[b]	933[b]	805[b]
	SW	706[a]	900[a]	711[a]
	MW	782[b]	926[b]	785[b]
	SED	24.3	8.17	24.6

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

Conclusions This study confirms that the starch and N components of bi-crops are rapidly degraded, thus supporting the theory that such forages can supply energy and protein synchronously to the rumen. Since host diet did not affect the rumen degradation of the bi-crops, grass silage fed animals can be used to generate accurate degradability data on bi-crops. In contrast, the degradation of GS may be underestimated if it is measured the rumen of cows fed some bi-crops.

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Influence of wilting time on silage compositional quality and microbiology

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Introduction Wilting grass before ensiling has become a firmly established practice, since it provides economic advantages due to the increment of the dry matter content of the forage before ensiling. Most forage crops contain less than 20 per cent of dry matter when they are cut thus reduction of the weight of crop to be transported provides advantages (Gordon *et al.*, 1999). Also, research has shown that such practices yield improved and reduced effluent loss from the silo and higher feeding value of the silage. Wilting of grass prior to ensiling has been widely adopted as a means of reducing effluents produced and improving the fermentation quality of silage. The aim of this research was to assess the effect of differing wilting periods and silage moisture levels of silage quality and microbial growth levels.

Materials and methods Silage was prepared from second-cut grass/white clover mixture harvested at the 4 week growth stage. The crops were cut using a Haldrop mechanical harvester on 7 June 1999 and were field wilted to 30-60% DM. The grass-clover mixture was then precision-chopped and ensiled in laboratory silos following cutting after 24, 48, 52 and 60 hours, with 3 replicate silos of each time period. The silage was allowed to ferment at ambient temperature for 2 months and sub-sampled. Three composite samples of each treatment were analysed for the variables in Table 1 by NIR analysis. Microbial analysis after fermentation included total aerobic count (plate count agar, 1.5% glucose, 35ºC/24 h), lactic acid bacteria (MRS agar, 4.5% CO_2, 30ºC/48 h) and yeast and moulds (malt extract agar, 30ºC/24 h). The data (normally distributed) was statistically analysed using ANOVA in Minitab.

Results The composition of the silage in g/kg DM unless otherwise stated is presented in Table 1.

Table 1 Silage quality in grass and clover (25 % clover) wilted for 0, 24, 48, 52, 60 hours pre ensiling

	Wilting time	0	24	48	52	60	SEM
Chemical composition	Toluene dry matter, DM (%)	20.3 ^{d}	34 ^{c}	37 ^{c}	51 ^{b}	63 ^{a}	36.3
	Crude protein, CP (g/kg)	202	208	211	195	192	157.1
	DCP (g/kg)	141.7	146.6	149.6	136	133.1	109.3
	D value (%)	77.2 ^{a}	77.9 ^{a}	76.6 ^{a}	71.1 ^{b}	69.3 ^{c}	58.7
	Metabolisable energy, ME (MJ/kg)	12.3 ^{a}	12.4 ^{a}	12.2 ^{a}	11.4 ^{b}	11.1 ^{c}	9.4
	pH	3.8 ^{c}	4.3 ^{c}	4.4 ^{c}	4.9 ^{b}	5.3 ^{a}	3.7
	NH_3-N (g/kg)	47 ^{a}	41 ^{b}	40 ^{b}	15 ^{c}	15 ^{c}	2.0
	Ash (g/kg)	96 ^{b}	94 ^{b}	95 ^{ab}	105 ^{ab}	107 ^{a}	76.3
	Neutral detergent fibre, NDF (g/kg)	510 ^{a}	458 ^{b}	442 ^{b}	378 ^{c}	268 ^{d}	302.2
	Lactic acid (g/kg)	137.7 ^{a}	83.1 ^{b}	63.9 ^{c}	17.8 ^{d}	8.7 ^{e}	33.4
	Sugars (g/kg)	29 ^{c}	49 ^{b}	55 ^{ab}	26 ^{c}	47 ^{b}	33.5
	Volatile fatty acids, VFA's (g/kg)	34.4 ^{b}	16.5 ^{c}	18.6 ^{c}	8.4 ^{c}	52.4 ^{a}	14.8
Metabolisable protein	FME (MJ/kg)	7.7 ^{b}	9.1 ^{a}	9.3 ^{a}	8.9 ^{a}	8.1 ^{ab}	7
	ERDP (g/kg)	142.9 ^{a}	140.5 ^{a}	140.1 ^{a}	119.0 ^{b}	109.3 ^{b}	97.3
	DUP (g/kg)	15 ^{c}	24.6 ^{b}	28.8 ^{b}	39.1 ^{ab}	49.4 ^{a}	26.7
Acids (FM)	Lactic acid (g/kg FM)	8.6 ^{a}	5.4 ^{b}	4.8 ^{c}	1.9 ^{c}	1.3 ^{a}	2.6
	Ethanol (g/kg FM)	1.1 ^{b}	1.7 ^{a}	0.55 ^{a}	0.46 ^{c}	0.2 ^{a}	0.4
	Acetic acid (g/kg FM)	4.2 ^{a}	2.4 ^{b}	3.1 ^{b}	1.2 ^{c}	0.5 ^{a}	1.2
	Total VFA (g/kg FM)	4.3 ^{a}	2.4 ^{bc}	3.1 ^{ab}	1.2 ^{c}	0.5 ^{c}	1.2
Microbial analysis	Total aerobic count (Log_{10} cfu/g)	8.1 ^{a}	6.8 ^{b}	7.9 ^{a}			0.20
	Lactic acid bacteria (Log_{10} cfu/g)	8.0 ^{a}	5.9 ^{c}	7.2 ^{b}			0.31
	Yeast and moulds (Log_{10} cfu/g)	4.1 ^{b}	7.0 ^{a}	6.8 ^{a}			0.47

Means on the same line followed with the same superscript or no superscript, do not differ significantly ($p<0.05$).

Wilting silage up to 37% DM produces greater levels of ME, ERDP and DUP. However above 37% DM resulted in lower levels of ME, ERDP and DUP. Wilting samples to 34-37% DM maintains higher sugar levels, resulting in lower lactic acid and VFA's levels compared with un-wilted silage. The total VFA's were lower in the wilted samples indicating a different fermentation pattern, which was also reflected in the higher counts of yeasts and moulds and lower MRS counts.

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Influence of maturity stage on *in situ* dry matter degradability of six maize varieties in fistulated sheep

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Introduction The fresh weight, dry matter (DM) contents and nutritional quality in maize vary considerably with variation in varieties, stages at which harvested, climatic conditions and agronomic factors. Recently, agronomists, nutritionists, and dairy producers have placed increased emphasis on factors affecting the nutritive value of maize. However, very little information is available on quantitative variability of the feed value of maize fodder as affected by such factors. This study was, therefore, carried out to assess the effect of harvesting of six different maize varieties at two stages (dates) of grain maturity on quality of both the stover and cobs.

Materials and methods Six varieties of maize viz., Aura (FAO 190), Bezemara (FAO 190), Pirat (FAO 220), Diva (FAO 230), Boss (FAO 250) and Carlos (FAO 260) were grown under identical agronomic conditions. Sown in early spring with standard NPK applications. These varieties were harvested at two-grain maturity stages during previous year (2^{nd} and 16^{th} September) and are designated as early and late stage. At harvest, the plants were separated into cobs (which includes grain) and stover (rest of the plant). The *in situ* DM degradability of the samples was determined using three rumen fistulated male Blackhead sheep. The sheep were fed 500 g DM of maize silage and 60 g soybean meal twice daily. Samples were incubated for 2, 4, 8, 12, 24, 36, 72 and 96 h. The DM degradation data were fitted to the exponential equation $p= a + b (l – e^{-ct})$ using the Neway Excel Programme where p is DM degradation (%) at time 't', and index value was calculated (Ørskov, 2000). The overall *in situ* degradability of maize stover and cobs was calculated form the data of six varieties. Statistical significance of DMD of different varieties and two stage of maturity mean differences was independently tested using the least significance difference (LSD) based on residual variability between the samples following two-way analysis of variance.

Results The overall degradability of maize stover was higher in early stage of maturity than late stage (Fig. 1). The DMD of stover of all varieties harvested at later stage was lower (48.1%) as compared to those in early stage (58.4%). The stover of Carlos and Bezemara harvested in early stage had higher ($P< 0.05$) 48-h DMD as compared to others. Based on index value the six varieties of maize stover showed the following ranking order: Carlos > Pirat > Bezemara > Diva > Aura > Boss in early stage and Bezemara > Diva > Carlos > Pirat > Aura > Boss in later stage. There was no significant difference ($P>0.05$) between maturity stages on the overall DMD of maize cobs (72.8 and 72.1%, Fig. 2). Carlos (12 h: 73.6%) and Boss (24 h: 84.7%) showed the highest DMD in cobs harvested in early stage.

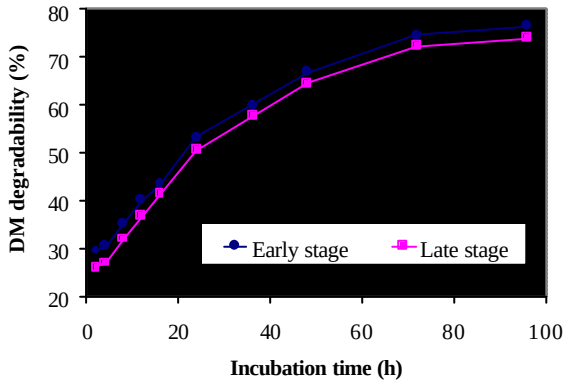

Figure 1 Overall *in situ* DMD (%) of maize stover

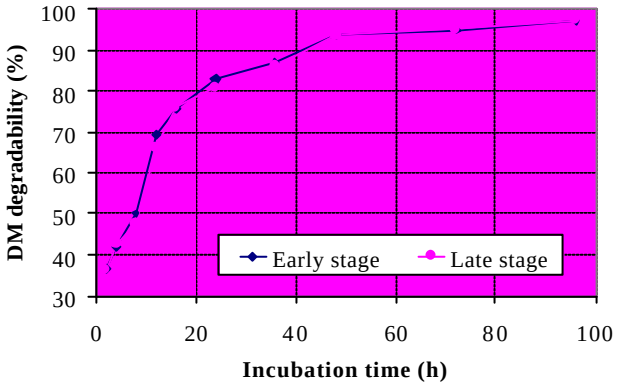

Figure 2 Overall *in situ* DMD (%) of maize cobs

Conclusions The overall results showed evidence of varietal as well as maturity stage differences in quality of cobs and stover, and indicated the superiority of maize varieties Carlos and Bezemara having higher desirable nutritional characteristics.

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Voluntary intake and apparent digestibility in ponies offered alfalfa based forages *ad libitum*

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Introduction There is increased interest in using forages other than grass hay as the basal diet for equines in the UK. Whilst a range of short-chopped dehydrated alfalfa based forages may be used as alternatives to grass hay in equine diets, there is very little information available on their likely intake characteristics, apparent digestibilities or their nutritive values. The objectives of the current experiment were:- 1) to determine the voluntary feed intake (VFI) characteristics of three alfalfa based forages when offered *ad libitum* to ponies, 2) to determine the *in vivo* apparent digestibility, digestible energy (DE) and digestible crude protein (DCP) contents of these forages and 3) to compare the actual energy and protein intakes with theoretical energy and protein requirements.

Materials and methods Six mature Welsh-cross pony geldings were individually housed and offered one of three alfalfa based forages *ad libitum* (approximately 30% excess) according to a replicated 3x3 latin square changeover design experiment with 3 periods of 21 days. The 3 forages under study were short-chopped, dehydrated and molassed alfalfa (AA), a blend of AA and oat straw (AA-OS) and AA with added oil and a probiotic yeast culture (AA-OIL). Each 21 day period consisted of a 16 day adaptation phase and a 5 day collection phase when VFI, *in vivo* apparent digestibilities of dry matter (DMD), organic matter (OMD), crude protein (CPD), ether extract (EED), acid hydrolysis EE (AHEED), acid detergent fibre (ADFD), neutral detergent fibre (NDFD) and gross energy (GED) along with the energy and protein intake parameters were recorded. Forage DE and DCP contents were also calculated whilst theoretical energy (DE REQ) and protein (DCP REQ) requirements were predicted according to equations published in NRC (1989) for stalled equines and compared with actual DE and DCP intakes (DEI and DCPI respectively).

Results There were only small differences between the CP and NDF composition of forages AA and AA-OIL as offered to the ponies and the CP and NDF composition of the forage refusals indicating only a minimal degree of dietary selection by the ponies. In contrast however, the CP and NDF contents (g/kg DM) of the feed refusals from the AA-OS diet were markedly lower (98 *cf* 75) and higher (602 *cf* 675) respectively. This suggests that despite the thoroughly mixed nature of the forage on offer, ponies actively selected from amongst the dietary constituents in forage AA-OS in favour of the alfalfa components and against the straw components. Pony liveweight (LW), voluntary dry matter intakes (DMI), *in vivo* apparent digestibilities, DE and DCP contents along with nutrient intake parameters are shown in Table 1. Although DMI was slightly lower when forage AA-OS was offered, no significant differences in VFI were seen between the forages. However, whilst not always reaching statistical significance, apparent digestibilities were generally lower when AA-OS was fed. Similarly, inclusion of oat straw significantly reduced ($P<0.001$) both the DE and DCP contents of the AA-OS forage compared with the AA forage alone. In contrast, inclusion of oil and probiotic significantly increased ($P<0.001$) the DE but not the DCP content of the AA-OIL forage compared with the AA forage alone. Both the energy and protein intake / requirement ratios were significantly reduced ($P<0.01$) by the AA-OS forage compared with the AA and AA-OIL forages.

Table 1. Voluntary intake, apparent digestibilities (g/kg) and nutrient intakes in ponies offered alfalfa based forages

	AA	AA-OS	AA-OIL	sed	Sig		AA	AA-OS	AA-OIL	sed	Sig
LW (kg)	315^{a}	319^{ab}	322^{b}	2.58	*	DE	9.4^{a}	7.9^{b}	10.6^{c}	0.19	***
DMI						(MJ/kg DM)					
(kg/d)	5.95	5.52	5.90	0.294	NS	DCP	93^{a}	62^{b}	96^{a}	2.86	***
(g/kg LW)	18.9	17.4	18.4	0.880	NS	(g/kg DM)					
(g/kg LW $^{0.75}$)	79.5	73.3	77.7	3.760	NS	DEI	56.2^{a}	43.4^{b}	62.5^{a}	3.33	**
						(MJ/d)					
DMD	526^{a}	476^{b}	548^{a}	16.5	*	DE REQ	31.8^{a}	32.1^{ab}	32.4^{b}	0.23	*
OMD	506^{a}	462^{b}	529^{a}	15.4	*	(MJ/d)					
CPD	642^{a}	565^{b}	650^{a}	17.8	**	DCPI	558^{a}	338^{b}	565^{a}	39.6	**
EED	-1150^{a}	-280^{b}	-144^{b}	319.5	*	(g/d)					
AHEED	404	396	442	115.7	NS	DCP REQ	189^{a}	192^{ab}	193^{b}	1.55	*
ADFD	352	366	338	24.0	NS	(g/d)					
NDFD	275^{ab}	349^{a}	228^{b}	32.6	*	DEI/DE REQ	1.76^{a}	1.36^{b}	1.93^{a}	0.095	**
GED	487^{a}	423^{b}	527^{c}	9.2	**	DCPI/DCP REQ	2.95^{a}	1.77^{b}	2.93^{a}	0.201	**

Conclusions The results indicate that in practice, equines are likely to consume a wide range of alfalfa based forages in similar amounts and that suitable equine forage mixes with varying nutritional characteristics can be manufactured by blending additional dietary ingredients with short-shopped dehydrated alfalfa.

Acknowledgement This work was funded by Dengie Crops Ltd.

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The effect of freezing and thawing fresh grass silage on near infrared reflectance spectroscopy (NIRS) predictions

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Introduction The accurate analysis of forage is paramount if the nutritive value to the ruminant animal is to be estimated. NIRS has the potential to accurately and rapidly evaluate the chemical and biological parameters of fresh silage. In many laboratory situations the pressure of proximate and specialized chemical analyses means that routinely, fresh forages have to be stored frozen (-20°C) before analysis. Research by MacRae *et al.*, (1975) has shown that although the physico-chemical composition of high quality forages changed upon freezing and subsequent thawing there were no differences in the apparent digestibilities and total nitrogen remained relatively constant. The objective of this study was to examine the effect of freezing and thawing fresh silage on NIRS predictions.

Materials and methods Nineteen fresh grass silages with a wide range in alcohol toluene corrected dry matter (ATDM, g/kg) were selected. One kilogram of each silage was chopped to approximately 3cm lengths and thoroughly mixed to ensure homogeneity. Three separate packings of each silage were prepared in non-PVC cling film and the samples scanned on a Foss 6500 NIRS spectrometer, using the coarse transport cell. Spectral data were recorded as log 1/Reflectance. These packages were then placed flat into a snap sealed polythene bag, identified with the silage number and stored flat in a freezer at -20C. The remainder of the chopped silage sample was split in two, sealed in labelled polythene bags and stored in the same freezer. After 1 week the 3 packages plus one of the loose bags of sample for each silage were removed from the freezer and left to thaw for 48 h to attain room temperature. The loose samples were mixed, packed in triplicate and scanned. The 3 original packages were also scanned and then returned to the freezer. This methodology was repeated at three months for the 3 packages and the remaining bag of loose sample. Spectral scans for each silage were meaned for the three treatments and potential intake ($MJ/kg^{0.75}$), ATDM, (g/kg) and D Value (g/kg) were predicted using the models of Park *et al.*, (1999). Treatments were denoted as fresh (P1), 1 week frozen packages, (P2), 3 month frozen packages (P3), 1 week frozen loose (L2) and 3 months frozen loose (L3). The data were analysed by analysis of variance using a randomised block model with silages as blocks. The biases of P2, P3, L2 and L3 relative to P1 were calculated and t-tests used to test for significant differences from zero. Regression analyses based on the 19 silage means were carried out.

Results Comparisons of predictions of fresh, 1 week and three month frozen silage are shown in Table 1. Using the same packages (a, b & c) throughout the study should reflect differences due to freezing and thawing. The errors for the loose samples combine the effects of freezing, sub-sampling and packing. It would be expected that freezing causes cell wall disruption and therefore the loss of cell nutrients. This would account for the overall lower predictions after freezing. However the standard error of difference (SED) for intake and D value are well within the cross validation error of the calibration (SECV 7.23 and 23.9 resp). ATDM however gives a larger SED than SECV (8.34).

Table 1 Comparison of NIRS predictions for each treatment in relation to the reference fresh silage, P1

Parameters	treats	Mean	Range	S.d.	SED	Bias	SED (C)	Slope	R^2
Intake	P1	76.4	57.0 – 95.8	10.05					
($MJ/kg^{0.75}$)	P2	75.6	54.0 – 96.8	10.63	2.94	0.79^{NS}	2.91	0.91	0.93
	P3	73.1	49.9 – 98.5	10.69	4.92	3.36^{***}	3.69	0.88	0.88
	L2	76.7	53.9 – 94.7	10.04	1.63	-0.30^{NS}	1.65	0.99	0.97
	L3	74.7	53.1 – 92.7	10.22	2.61	1.75^{**}	1.99	0.96	0.96
ATDM	P1	252.0	185.7 – 376.5	45.49					
(g/kg)	P2	243.2	175.6 – 383.4	47.07	12.24	8.85^{***}	8.68	0.95	0.97
	P3	238.8	170.4 – 363.9	45.01	16.21	13.29^{***}	9.55	0.99	0.96
	L2	237.4	170.1 – 329.1	40.10	19.45	14.61^{***}	13.19	1.09	0.92
	L3	239.5	171.5 – 330.0	39.63	17.85	12.58^{***}	13.01	1.11	0.93
D Value	P1	659	560 – 755	50.4					
(g/kg)	P2	656	586 – 742	48.0	12.3	4.1^{NS}	11.9	1.02	0.94
	P3	648	581 – 727	44.7	22.4	11.9^{**}	19.5	1.04	0.85
	L2	647	550 – 737	50.7	17.7	12.3^{**}	13.2	0.96	0.93
	L3	646	550 – 726	49.2	19.8	14.0^{***}	14.4	0.98	0.92

Conclusion The freezing and thawing process in general lowers NIRS prediction values of fresh silage. However these differences with reference to the fresh silage predictions are within acceptable calibration errors for intake and D value but not for ATDM.

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The prediction of digestible and metabolisable energy concentrations in grass-based diets of producing cattle using data tested with sheep trials at maintenance feeding level

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Introduction In the UK metabolisable energy (ME) system (AFRC, 1993) it is recommended that dietary ME concentration is reduced by proportionately 0.018 per unit increase in feeding level (FL) above maintenance. This factor was reported by Van Es (1975) two decades ago. At this Institute a total of 33 silages have been offered to sheep as a sole diet at maintenance for measurements of energy intake and outputs in faeces and urine. These silages were also fed to cattle, together with concentrates, at production level for determination of energy metabolism data in calorimetric chambers. The objective of the present study was to use these data to evaluate the combined effects of animal species and level of feeding on ME and digestible energy (DE) concentrations in diets.

Material and methods The cattle data were derived from 75 beef cattle and 242 lactating dairy cows. Twelve beef cattle (from a single trial) were offered the silage as a sole diet, but otherwise all animals were offered the mixed diets (mean concentrate proportion, 0.529, s.d. 0.139). Milk yield for dairy cows ranged from 3.2 to 49.1 kg/d. The mean FL (AFRC, 1993) was calculated to be 3.27 (s.d. 1.047) for both dairy and beef cattle. For sheep DE and ME concentrations of mixed diets at maintenance level were estimated using the measured silage data, predicted silage methane energy output and tabulated energy values of concentrates. The overall mean ME and DE concentrations for sheep fed at maintenance (ME_{maint} and DE_{maint}) were respectively 12.40 (s.d. 0.642) and 14.83 (s.d. 0.801) MJ/kg DM. The corresponding data for cattle fed at production level (ME_{prod} and DE_{prod}) were 11.95 (s.d. 0.671) and 13.96 (s.d. 0.665) MJ/kg DM. The treatment mean data (n=59) for both sheep and cattle were used to evaluate the relationship between ME_{prod} (DE_{prod}) and ME_{maint} (DE_{maint}) in a number of linear and multiple regression equations.

Results Regression eqs. are presented in Table 1. Relating FL above maintenance (FL-1) to the rate of reduction of ME or DE ((production-maintenance)/maintenance, $ME_{(P-M)/M}$ or $DE_{(P-M)/M}$) revealed that the constant had no significant effect on the relationship (eqs. (1) or (2)), indicating that there was little difference in dietary ME or DE concentration between sheep and cattle when fed at maintenance. These 2 eqs. also indicated that each unit increase in FL above maintenance would reduce dietary ME and DE concentrations by 0.016 and 0.025 respectively when the constant was omitted. The same results were also obtained from eqs. (3) and (4). The reduction rate of 0.016 for dietary ME concentration obtained in the present study is marginally lower than that (0.018) reported by Van Es (1975).

ME_{prod} and DE_{prod} were significantly related to ME_{maint} and DE_{maint} ($p<0.001$) respectively (eqs. (5) and (6)). These 2 relationships were significantly influenced by FL ($p<0.001$) and the eq. (6) was further affected by silage DM proportion in total diets (S/T) ($p<0.001$). The eqs. (7), (8) and (9) were therefore developed to predict ME_{prod} and DE_{prod} using respectively ME_{maint} and DE_{maint}, FL and S/T (for DE_{prod} only).

Table 1. Regression equations (the values in brackets are s.e. data)

Regression equations	R^2	
$ME_{(P-M)/M} = -0.016_{(0.003)} * (FL-1) + 0.002_{(0.008)}$	0.57	(1)
$DE_{(P-M)/M} = -0.022_{(0.003)} * (FL-1) - 0.008_{(0.007)}$	0.58	(2)
$ME_{prod} = [1 - 0.016_{(0.001)} * (FL-1)] * ME_{maint}$	0.51	(3)
$DE_{prod} = [1 - 0.025_{(0.001)} * (FL-1)] * DE_{maint}$	0.53	(4)
$ME_{prod} = 0.891_{(0.077)} ME_{maint} + 0.910_{(0.952)}$	0.87	(5)
$DE_{prod} = 0.681_{(0.066)} DE_{maint} + 3.855_{(0.987)}$	0.84	(6)
$ME_{prod} = [1.068_{(0.072)} - 0.019_{(0.004)} * (FL-1)] * ME_{maint} - 0.755_{(0.851)}$	0.88	(7)
$DE_{prod} = [0.847_{(0.055)} - 0.019_{(0.003)} * (FL-1)] * DE_{maint} + 2.042_{(0.780)}$	0.84	(8)
$DE_{prod} = [0.891_{(0.063)} + (-0.027_{(0.006)} + 0.018_{(0.013)} * S/T) * (FL-1)] * DE_{maint} + 1.355_{(0.919)}$	0.86	(9)

Conclusions The eqs. (7) and (9) are recommended to calculate ME_{prod} and DE_{prod} for cattle respectively. Alternatively, it is suggested that dietary ME or DE concentration is reduced by proportionately 0.016 (eq. (3)) or 0.025 (eq. (4)) respectively with each unit increase in FL above maintenance.

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Prediction of silage dry matter digestibility from digestible organic matter digestibility

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Introduction Grass silage forms the basal forage for the majority of dairy and beef cattle during the winter indoor feeding period. However its feeding value, as determined by intake potential and digestibility can differ dramatically at farm level as indicated by the Hillsborough Feeding Information System (HFIS). For example, for 7000 silages which were offered to dairy and beef cattle during the 1999/2000 indoor feeding period in Ireland and analysed through the HFIS, dry matter digestibility (DMD) varied from 540 to 830 g/kg DM (Keady, 2000). Many models used to predict feed intake by dairy cattle include a digestibility component (Keady and Mayne, 2000). However some models use DMD whereas others use digestible organic matter digestibility (DOMD). Furthermore commercial laboratories in Ireland measure silage digestibility as DMD while in the UK it is measured as DOMD. To facilitate the use of different models to predict food intake by dairy cattle, often it is necessary to be able to predict DMD from DOMD or vice versa. The present study was undertaken to develop a relationship between DMD and DOMD to facilitate the use of different models for the prediction of food intake when digestibility is available only either as DMD or as DOMD.

Materials and Methods *In vivo* DMD and DOMD data were available for 61 different silages from 15 experiments undertaken at the Agricultural Research Institute of Northern Ireland between 1984 and 2000. The digestibility coefficients of 29 and 32 silages were determined through sheep and growing cattle respectively. The silages were offered once daily for 6 days as the sole diet *ad libitum* to the young growing cattle and as the sole diet at maintenance to the sheep. The sheep and cattle were housed in digestibility crates designed to facilitate the separate collection of urine and faeces. Collection of urine and faeces commenced 48 hours after the first feed-in and continued for 6 days. Urine and faeces were weighed and sampled daily. On completion of the digestibility studies the daily sub-samples were bulked together, mixed and sub-sampled for chemical analysis. Feed and faecal samples were analysed for dry matter, ash, crude protein and gross energy. Urine samples were analysed for gross energy and protein.

Results Silage DOMD ranged from 546 to 740 g/kg DM, DMD 580 to 783 g/kg DM and ash concentration 67 to 129. Regression analysis using treatment means was undertaken to develop a relationship between DOMD and DMD. The dataset was randomly divided into two sub-groups of 41 and 20. The sub-group of 41 was used to investigate the relationship between potential DOMD and DMD.

This produced a linear relationship, with no significant differences between the species, of the form:

DMD = 49.1 (se 24.30) + 0.988 (se 0.0356) DOMD $R^2 = 0.95$ ***

where DMD = dry matter digestibility (g/kg DM) and DOMD = digestible organic matter digestibility (g/kg DM).

This relationship was validated using the remaining data from the sub-group of 20. Linear regression analysis of the actual DMD values and their respective predicted values from the model based on the sub-group of 41 was performed. This identified a linear relationship described by the following equation:

PDMD = 8.2 (se 59.1) + 0.9919 (se 0.0814) ADMD $R^2 = 0.89$ ***

where PDMD = predicted DMD (g/kg DM) and ADMD = actual DMD (g/kg DM).

As the constant was not-significantly different from zero ($P>0.05$), a further regression was undertaken which forced the line through the origin. This line had a slope of 1.00321, with a standard error of 0.00309 and R^2 of 0.89, clearly indicating that DMD of the validation dataset was determined with a high degree of precision.

Conclusions It is concluded that the DMD of silage can be predicted from a highly significant linear relationship with DOMD, enabling the use of many feed intake prediction models requiring digestibility measured either as DMD or DOMD.

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Sheep avoidance of faeces creates a foraging trade-off between nutrient and parasite intake

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Introduction Gastrointestinal (GI) parasites, acquired by sheep through the action of foraging, are the most pervasive challenge to their survival and reproduction. The eggs of many GI parasite species are deposited on pasture in faeces where they develop into infective stage larvae that contaminate surrounding swards. We test the hypotheses that (1) faeces and hence parasite avoidance behaviour of sheep creates a grazing trade-off between nutrition and parasitism and (2) the relative costs and benefits of the trade-off in relation to animal state of infection (parasitized, non-parasitized, immune) determines their subsequent grazing behaviour.

Materials and methods 30 female parasite naïve Scottish Blackface sheep were equally divided into three treatment groups balanced for live weight (five months old; 27.6±0.57 kg LW at the start of the experiment). A parasitized and an immune state treatment were created by daily dosing two groups of 10 sheep with 2,500 infective larvae of *Ostertagia circumcincta*, for three weeks and four months prior to the start of the experiment, respectively. The remaining 10 sheep were maintained as non-parasitized controls. Each of the three treatment groups was divided into two replicates (n=5), each of which was placed in a 0.25 ha experimental field plot. The six groups of sheep were rotated daily around the field plots for two weeks. Each experimental plot was composed of a chequer board of 100 (5m x 5m) patches with alternate patches contaminated with 240g/m^2 of sheep faeces. The herbage intake of the animals was determined using the n-alkane method. Activity time and grazing behaviour (bite rate and step rate) were measured using direct observations and vibracorders. Faecal egg counts and blood pepsinogen concentrations were used to monitor the development of immunity in immune animals and the development of sub-clinical parasitism in parasitized animals before and during the experiment. All sheep wore faeces collection bags to prevent contamination of clean patches.

Results Sward **N**itrogen, **O**rganic **M**atter **D**igestibility and **D**ry **M**atter were not affected by faeces contamination. Sward DM and OMD increased in week 2. Initially, all animals strongly selected to graze the non-contaminated patches (Figure 1) resulting in disproportionate sward depletion. The strong avoidance of the faeces contaminated plots by parasitized and non-parasitized sheep remained throughout the experiment, by which time the contaminated swards were 15.1±0.37 cm and the non-contaminated swards 7.1±0.37 cm. Immune animals reduced their avoidance of faeces contaminated swards during the experiment resulting in a non-selective grazing strategy (i.e. 0.5 bites from clean patches: t-test, P>0.05) by the end of the experiment (day 13). Compared to non-parasitized control sheep, immune sheep had increased rates of herbage intake and movement, and *vice versa* for parasitized sheep which had lower bite rates compared to the other animal treatments (Table 1).

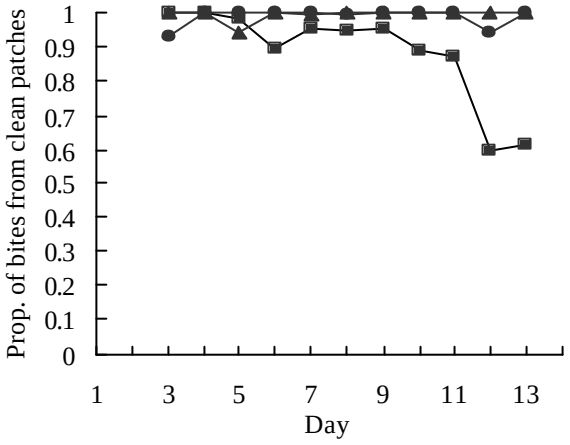

Figure 1. Patch selection (arcsine back-transformed prop.). Squares denote immune, circles denote non-parasitized and triangles denote parasitized sheep; Coefficients of variation = 8.2%, 6.9% and 6.8% respectively

Table 1. Effect of state of infection on sheep grazing behaviour. Values given are means over two weeks. Effects based on REML, Wald statistics: N.S.=P>0.05, *=P<0.05, **=P<0.01, ***=P<0.001.

	Treatment				Effects		
	Immune	**Parasitized**	**Non-parasitized**	**s.e.d.**	**D**ay	**T**reatment	**D**x**T**
Bite rate (bite/sec)	0.84	0.80	0.90	0.045	N.S.	*	***
Step rate (step/sec)	0.21	0.15	0.18	0.015	N.S.	**	N.S.
Time active (min/day)	667	737	655	44.4	N.S.	N.S.	N.S.
Intake (kg DM/day)	0.947	0.736	0.851	0.0251	*	***	N.S.

Conclusions Selective grazing in relation to the distribution of faeces and hence parasites, creates a highly heterogeneous sward structure and a trade-off between the benefits of increased forage intake rate (through grazing relatively tall swards) and risks associated with parasite ingestion (through grazing faeces contaminated swards). Faced with this trade-off, herbivores face a dilemma in that their behavioural strategies to maximize nutrient intake (select tall swards) conflict with their strategies to avoid parasitism (avoid faeces). The timing of reduced faeces avoidance may be determined by the animal's ability to deal with parasites (i.e. their parasite/immune state). Knowledge of the state of parasite infection of domestic herbivores may then be used in farm and disease management strategies to reduce the production losses through parasitism.

A decision support system for controlling bovine viral diarrhoea (BVD) in beef suckler herds

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Introduction The success of any farm business relies on allocating resources optimally across all farming activities. This is particularly important for disease prevention activities because of their potential impact on farm business viability, the temptation to reduce such activity when funds are scarce and the possible implications for animal welfare and food safety. The objective of the current study was therefore to explore the relative impact of alternative disease prevention strategies under a range of different circumstances. This was done by example, using the case of BVD in a typical Scottish beef herd.

Materials and methods A state-transition (Markov-Chain) model incorporating a Monte-Carlo process was developed using a computer spreadsheet (Microsoft Excel) to simulate the effect of a variety of control measures on BVD at the farm level. A fixed, closed herd management strategy with 100 breeding cows was used. Each year these cows produced 50 female and 50 male calves, of which 30 females were kept on with the aim of using about 15 as replacements. Default input values for the model were derived from the literature (see Bennett et al., 1999), expert knowledge and the SAC Farm Management Handbook 1999/2000. The output included the total costs of BVD, i.e. output losses and control expenditure over 10 years. The control option examined here was vaccination (2 doses/animal/year @ £3.50/dose). The model was run assuming 89 of the cows were susceptible to BVD, 10 immune and 1 persistently infected (PI, the source of the disease). Three control options were investigated. No control, vaccinating cows and heifers before the introduction of a PI animal ('early') or the year after ('late'). Each scenario was run assuming various levels of efficacy of the vaccine. All runs were repeated 30 times to calculate standard deviations.

Results Figure 1 shows that most costs occur in the first 5 years. The average cost over 10 years for the disease without a vaccination program was £32/cow/year (sd 2.9). Using a 100% effective vaccine after infection ('late') this cost could be reduced to £30 (sd 3.2). However, the extent of this benefit was dependent on the efficacy of the vaccine. When the vaccination program was used before the introduction of a PI animal into the herd ('early') the cost of an outbreak of the disease was much reduced and less dependent on the efficacy of the vaccine (Figure 1). The average cost over 10 years for this scenario was £14/cow/year (sd 3.0).

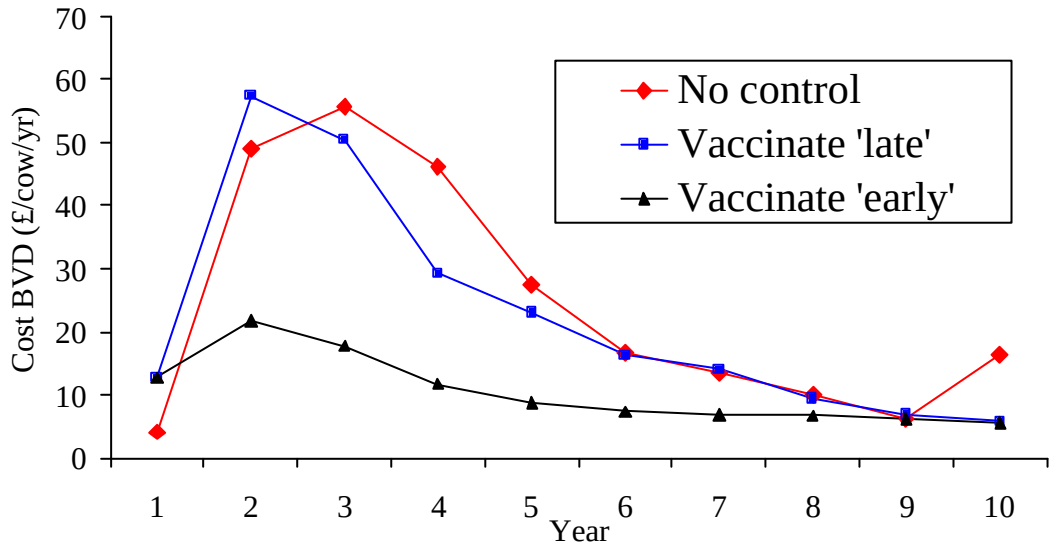

Figure 1: Total cost (including vaccine) of BVD over time at present day values. Vaccines assumed 100% effective.

Conclusion The model demonstrated the significant economic impact of a BVD outbreak on a beef suckler herd. This effect was alleviated to a great extent through a vaccination program applied in advance of the outbreak, but much less benefit was gained from vaccinating an already infected herd. This suggests that the approach adopted here may have value as part of a decision support system.

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Sample test-day heritability estimates for somatic cell score for Hungarian Holstein- Friesian crossbreds

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Introduction There have been many studies of genetic variance in somatic cell score (SCS). Reported estimates of heritability (h^2) range from 0.02 to 0.16, the lowest h^2 obtained in the 1st parity and increased in the later lactations (Mrode and Swanson 1996). The objective of the present study was to examine the effect of introducing Holstein-Friesian genes in the Hungarian native breed on the inheritance of sample test-days SCS.

Material and methods Sample test-day observations for six genetic groups are shown in Table (1). Data included records of Hungarian Native Breed (HNB), Holstein-Friesian (HF) and their crossbreds. Hungarian Holstein-Friesian crossbreds were <25%HF, ≥25-<50%HF, ≥50-<75%HF, and ≥75%HF.The total number of lactation records were 348108 of 130335 cows, daughters of 2124 sires in the first three parities. Records included in the present study had at least 220 days of production with at least five subsequent monthly sample observations. Data were of the first three parities of cows calving from June 1990 to December 1994. Records with missing two consecutive sample test day observations were also ignored. SCC was transformed to somatic cell score (SCS) as $\log_2[(SCC/100)+3]$. The general model was $Y_{ijklmn} = u + G_i + S_{Ji} + C_{kji} + P_l + STG_{ml} + e_{ijklmn}$. Where:- Y_{ijklmn}: is the SCS, μ: population mean, G_i: fixed effect of ith genetic groups, S_{ji} is the effect of jth sire nested within ith genetic group, C_{kji}: random effect of kth cow nested within jth sire and within ith genetic group, P_l: fixed effect of lth order of lactation, STG_{ml}: is the effect of mth lactation stage within lth parity, ε_{ijklmn}: is the random. Co-variance components were computed applying multi-traits derivative free restricted maximum likelihood procedure (MT-DF-REML), Boldman (1997).

Results Estimates of h^2, sire additive and residual variance components for all traits are shown in Table (1). h^2 of SCS increased with HF genes in different crosses except in ≥75%HF. HF had the lowest h^2 of SCS and is less than NHB by 33%. In general, residual variance increased with advancing percentage of HF inheritance. Crossbreds of ≥75%HF genes showed a greater residual variance and a smaller sire variance than the other crossbreds. h^2 for SCS in the first three parities are shown in Figures 1. h^2's of SCS in the 1st parity are within a narrow rage among different genetic groups. Replacing half or little more of NHB genes by HF genes causes increasing h^2 for SCS. Estimates of h^2 for SCS overall parities (Table 1) and by parities (Figure 1) for crossbreds were higher than for purebreds. In general differences between h^2's of SCS across parities were not small. This is may be due to change amount of residual variance across parities. These results are in agreement with that reported by Coffey et al. (1986). They also concluded that SCS early and late in life could genetically constitute different traits. Differences in h^2's for SCS among genetic groups indicate there is a real difference in the inheritance of resistance to mastitis not only across parities but also among different stages of lactation.

Table 1 Estimates of sire (σ^2_S), residual (σ^2_e) variances, heritability (h^2) and standard error (SE) for SCS.

Genetic groups	Somatic cell score (SCS)		
	σ^2_S	σ^2_e	$h^2 \pm$SE
NHB	9871	197890	.21±.19
<25% HF	12314	182162	.29±.20
≥25-<50% HF	17189	203016	.37±.11
≥50-<75% HF	14921	201433	.32±.12
≥75% HF	8814	216202	.17±.19
HF	10470	309612	.14±.07

NHB: native Hungarian Breed, HF: Holstein-Friesian.

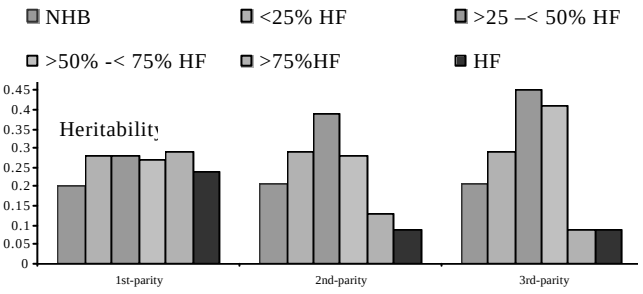

Figure 1 Heritability for somatic cell score per parity.

Conclusions STD-may reduces the amount of environmental variance as opposed to lactational measurements as well as total phenotypic variance. The highest h^2 of SCC was obtained for genetic groups of <25% and ≥25-<50%HF inheritance Genetic groups with high h^2 estimates and wide range of additive variance for SCS are more appropriate in selection programs to reduce SCS and to improve mastitis resistance. Crossbreds with medium HF inheritance may be more appropriate genetic group for selection against SCS and consequent improves udder health. Selection against SCS needs accurate study to consider the relationship between milk composition and SCS.

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Mrode, R.A., G.J.T. Swanson 1996. Genetic and statistical properties of somatic cell count and its suitability as an indirect means of reducing the incidence of mastitis in dairy cattle *Animal Breeding abstract* **64**: 847-857.

Variations among six genetic groups in relationship between sample test-day daily milk and somatic cell scores of Hungarian Holstein-Friesian

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Introduction The genetic and phenotypic relationship between somatic cell score (SCS) and milk production has been reported as both negative, positive, whereas phenotypic correlations have been mainly negative (Mrode and Swanson 1996). The aim of the present study is to investigate the variation in associations of test-day SCS with daily milk yield (DY) in six genetic groups of Holstein- Friesian (HF) and Native Hungarian Breed (NHB).

Materials and methods Crossbred groups involved in the present study are shown in Table (1). Genetic and phenotypic correlations among the various SCS means and DY were estimated using an animal model of MTDFREML package (Boldman, 1997) that includes both animals with records and genetically related animals with no records, $y=XB+Zu+e$. Where y is an $n*1$ vector of observations on the trait of interest; X is an $n * p$ incidence matrix; Z is a $t * t$ matrix equal to an $n * n$ identity matrix relating observations to the animals that made them and augmented by null rows and vectors for animals that are to be evaluated but have no records; B is a $p * 1$ vector of known fixed effects; u is a $t * 1$ vector of random breeding values, which can be partitioned into u_1, and $n * 1$ vector representing animals having records and u_2, a $(t\text{-}n) * 1$ vector for related animals with no records; and e is an $n * 1$ vector of random errors. Thus

$$E\begin{bmatrix} y \\ u \\ e \end{bmatrix} = \begin{bmatrix} XB \\ 0 \\ 0 \end{bmatrix}, \& \; V\begin{bmatrix} y \\ u \\ e \end{bmatrix} = \begin{bmatrix} V..... As^2_G..I_n s^2_e \\ As^2_G..As^2_G...0 \\ I_n s^2_e...0....I_n s^2_e \end{bmatrix}$$

where $V=A^{-1}s^2_G+I_n s^2 e$, A = additive genetic relationship matrix, s^2_G= additive genetic variance and $s^2 e$ = residual variance. Somatic cell count (SCC) has been transformed to SCS with the base 2 log scale as $SCS=\log_2[3+(SCC/100)]$.

Results Phenotypic (R_p) and genetic (R_g) correlations between SCS and DY are represented in Table (1). R_g estimates were generally in low to medium values. Overall parities across genetic groups, the highest R_g and R_p were -.21 and -.19 for NHB and HF, respectively. R_p`s and $R_g$`s were negative either across parities or across crossbreeds. The highest R_g estimates were –.25 and -.22 in the 4th parity for HF and HNB. R_g estimates were negative in all genetic groups within parity except in the 1st parity. R_g`s in the 1st parity corresponded to negative $R_p$`s for each genetic group. The highest R_p >-0.20 was obtained for HF, <25%HF, and NHB in the 4th, (2nd &3rd), and 1st parity, respectively. Crossbreeds showed moderate estimates of correlations in different parities. Estimates of R_p decreased with HF inheritance. Small differences among R_g's within the 1st parity with advancing percentage of HF inheritance were observed compared with those in other parities.

Table 1 Correlations estimates between somatic cell score and daily milk yield within parity.

	Genetic correlations R_g							Phenotypic correlations R_p						
Parity	R_g @	HNB	<25%HF	≥25-<50%HF	≥50-<75%HF	≥75%HF	HF	R_p @	HNB	<25%HF	≥25-<50%HF	≥50-<75%HF	≥75%HF	HF
1	**.10**	.13	.14	.14	.14	.11	.08	**-.14**	-.22	-.20	-.20	-.15	-.14	-.11
	.11	*.08*	*.10*	*.12*	*.09*	*.04*	*.01*	***.07***	*.07*	*.09*	*.11*	*.11*	*.18*	*.01*
2	**-.12**	-.13	-.13	-.17	.18	-.14	-.15	**-.11**	-.20	-.21	-.19	-.17	-.14	-.13
	.07	*.07*	*.07*	.18	*.04*	*.02*	*.11*	***.08***	*.10*	*.07*	*.04*	*.02*	*.07*	*.11*
3	**-.14**	-.09	-.11	-.11	-.08	-.08	-.14	**-.12**	-.20	-.24	-.18	-.11	-.15	-.17
	.01	*.07*	*.01*	.04	*.03*	*.07*	*.17*	***.01***	*.12*	*.09*	*.09*	*.01*	*.03*	*.11*
4	**-.16**	-.22	-.17	-.10	-.12	-.18	-.25	**-.20**	-.18	-.14	-.13	-.13	-.16	-.24
	.07	*11*	*.07*	02	*.06*	*11*	*10*	***.11***	*09*	*08*	*.07*	*11*	*.07*	*11*
@	**-.13**	**-.21**	**-.17**	**-.15**	**.10**	**-.10**	**-.09**	**-.15**	**-.08**	**-.08**	**-.11**	**-.13**	**-.18**	**-.19**
	.07	***.09***	***.07***	**11**	***.11***	***.04***	***.04***	***.07***	***.02***	***.07***	***.07***	***.06***	***.07***	***10***

HNB: Hungarian Native Breed, HF: Holstein Friesian, @: overall estimate of correlation, Cows no.=172065, Lactation No. = 458348, and Sires = 873, Standard errors are value in italic form

Conclusion R_g's of SCS with milk traits were generally in low to medium negative values. These results indicate to, increase SCS is moderately accompanied with decreases DY. Crossbreeds with high HF% showed markedly negative correlations for reducing DY with increasing SCS. The current results may suggest that, SCS in the early and late parities may be genetically considered different traits, implying that selection in the early lactations could be more effective to reduce SCS and increase mastitis resistance. Differences in R_g's , R_p's among different genetic groups may reflect to presence true variation among crossbreeds. Also the current results suggest that, breeding crossbreeds is better than purebreds in the production farms.

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Boldman, K. G., L. A. Kriese, L. D. Van Vleck, C. P. Van Tassell and S. D. Kachman, 1997. A Manual for Use of MTDFREML. A set of programs to obtain estimates of variances and covariances. U.S. Agric. Depart, Agric. Res. Ser.
Mrode, R.A., G.J.T. Swanson 1996. Genetic and statistical properties of somatic cell count and its suitability as an indirect means of reducing the incidence of mastitis in dairy cattle *Animal Breeding abstract* **64**: 847-857.

Total Enterobacteriaceae counts as an indicator of animal feedingstuffs hygiene.

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Introduction Bacteria belonging to the family Enterobacteriaceae enter the animal feed chain as normal contaminants of raw materials used in the manufacture of animal feeds. The family Enterobacteriaceae encompasses 30 established genera, including Salmonella spp, Escherichia spp, Shigella spp and Yersinia spp. Many of the genera exhibit pathogenicity towards man, animals, insects and plants and many of the pathogenic forms produce toxins. A number of the genera in this family occur regularly in association with animals; they are found as indigenous members of the gut microflora where they may either produce no harmful effects, or are capable of causing disease in both endothermic and ectothermic animals. There is a recognised association between the risk of isolation of salmonella and degree of Enterobacteriaceae contamination (Veldman *et al*. 1995). This has led to the consideration of recording Enterobacteriaceae contamination levels in feed stuffs as an indicator of feed hygiene and potential limits to the degree of contamination being set by the major retailers. This paper sets out data gathered from the routine analysis of feed raw materials examined for Enterobacteriaceae contamination.

Materials and Methods. Feed samples were analysed for their Enterobacteriaceae contamination using a Malthus System V impedance analyser. Test cells containing growth medium supplied, are inoculated with a suspension of the feed sample, these cells are then placed in the analyser and left for 24 hours. The analyser monitors changes in impedance in the cell due to microbial growth and records this data as a graph. Using calibrated software the point of change from lag phase to log phase growth rates are determined. This is referred to as the detection time. A calibration curve is generated using this detection time data and manual colony counts on Violet Red Bile Glucose Agar (VRBGA). The calibration curve can then be used for routine analysis of samples using the system to generate an estimate of the number of colony forming units present per gram of sample. A total of 396 samples of wheat and 939 samples of soya were analysed. Mean colony forming units (CFU:log10) were compared using the Student t-test.

Results. Raw materials can be classed as either unprocessed (whole cereals) or processed (oil seed meals and animal proteins). The mean CFU (log10) of wheat (n=396) which is a material that is not routinely processed were 5.046 log10 (range 10^1 –10^7CFU/g)(Figure 1). Soy bean meal including full fat, hy-pro and soya bean meal (n=939) which is a product that is processed showed a bimodal distribution with a high number of samples with very low Enterobacteriaceae counts (Figure 2). This is probably due to the processing environment which has reduced Enterobacteriaceae counts. A second population of samples with a peak at 4.986 log10 CFU/g indicates the high degree of recontamination which has occurred. When the samples with <10^1CFU/g were removed from the soya data set there was no significant difference between the mean CFU/g of the wheat or processed soya (P>0.05: SE 0.7612 log10).

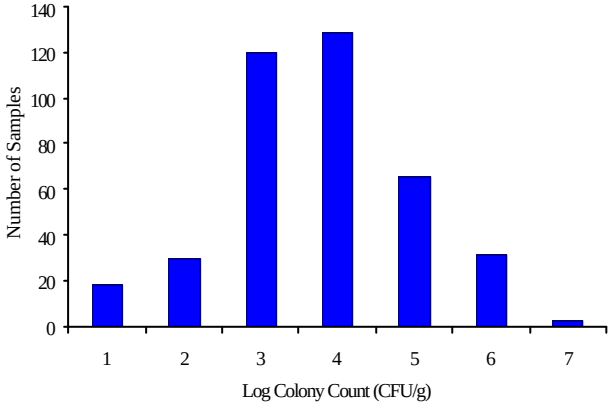

Figure 1. Enterobacteriaceae contamination of wheat

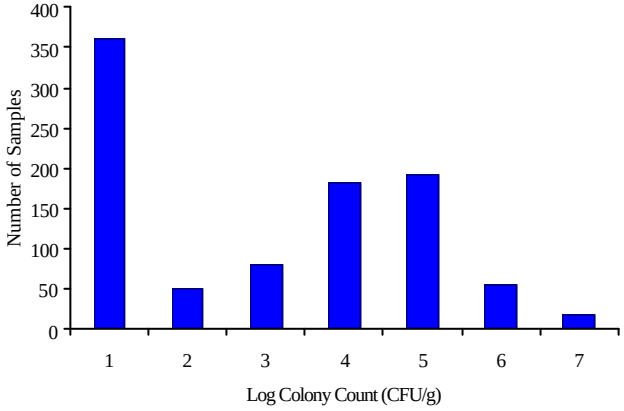

Figure 2. Enterobacteriaceae contamination of processed soya

Conclusion The data indicates that a large proportion of the raw materials used for animal feed manufacture are contaminated with significant levels of Enterobacteriaceae (>10^4 CFU/g). In the present climate every effort must be taken to eliminate contamination of food and feed with pathogenic bacteria. Processing the raw material has the potential to reduce this degree of contamination. The level of natural contamination found in the raw materials is sufficient to indicate the requirement for further processing either via heat or chemical treatment. This contamination of raw materials should be viewed as a critical control point for the entry of pathogenic bacteria into the feed and food chains.

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Total Enterobacteriaceae counts as an indicator of the internal hygiene of feed mills.

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Introduction. Raw materials used in feed manufacture are contaminated with high (>10^4 cfu/g) levels of enterobacteriaceae indicating the potential for contamination with pathogenic bacteria such as salmonella (Wood *et al*. 2001). There is urgent need to reduce the contamination of animal feed with zoonoses such as salmonella and campylobacter. During manufacture of feedingstuffs, heat and moisture are used to process and sterilise feed but this can also provide conditions for microbial growth. High temperature treatment used to sterilise feed will not protect feedingstuffs from recontamination, if residual microbial contamination remains in the feed mill. It is essential to understand the influence of feed processing and the feed mill environment on the microbial contamination of feed.

Materials and Methods. Two methods were used to quantify the microbial contamination present in the internal mill environment. A sterile 10cm^2 template was placed on the area to be evaluated and a sterile sponge used to swab the entire surface within the template. Alternatively debris was sampled from the boots of elevators, or dust adhering to surfaces and caked material in presses. At each sampling point triplicate samples were taken for microbiological analysis. Samples were analysed for their Enterobacteriaceae content using a calibrated Malthus System V impedance analyser using methods previously reported (Wood *et al.* 2001). Mean colony forming units (CFU) for each sampling point were compared by analysis of variance and differences between means compared using the Bonferroni correction.

Results. No two feed mills have identical processing systems. Results from key reference points in two mills are presented (Figures 1 & 2) which indicate similar pattern of microbial contamination. Overall microbial counts from the two mills (CFU) were not significantly different. Raw materials introduce high levels of bacteria into the system (intake). Grinding where heat is generated tended to reduce CFU. Pelleting where high volumes of steam and pressure are used significantly ($P<0.01$) reduced microbial contamination compared with all other sampling points. However in the cooler where the feed is cooled and dried there is the potential for recontamination from debris material which adheres to the surfaces of the machinery. At outload even after the feed has been processed using steam and heat, there were significant levels of enterobacteriaceae still present in the feed although the levels tended to be lower than samples taken from the intake ($P<0.095$).

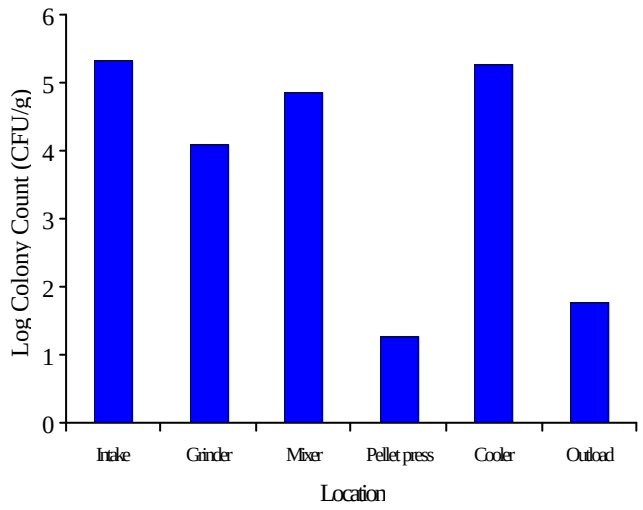

Figure 1. Enterobacteriaceae contamination in mill 1.

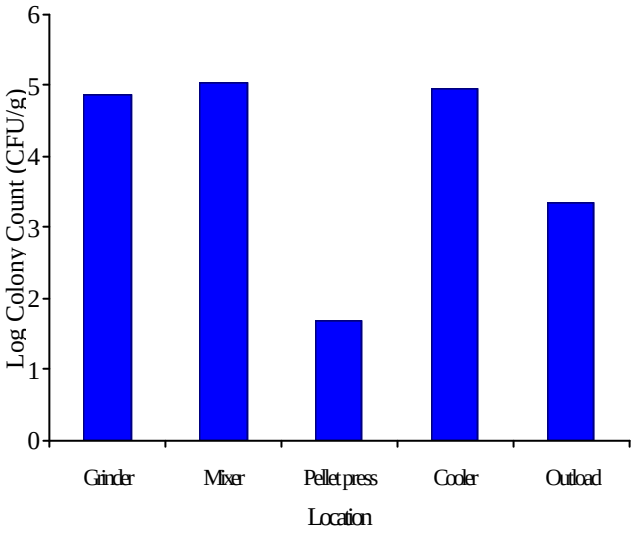

Figure 2. Enterobacteriaceae contamination in mill 2.

Conclusion. Microbiological control in feedingstuffs has been identified as an important factor in controlling the entry of zoonoses into the food chain. The results indicate that even when the feed is processed using heat and steam during pelleting the internal surfaces are still contaminated with high levels of Enterobacteriaceae which have the potential to recontaminate the feed with bacteria. The results identify coolers that are areas of heat and moisture exchange, as being high risk areas for recontamination. The level of recontamination which occurs in the mill indicates the need for additional treatment to prevent microbial recontamination.

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The influence of oxygen on the efficacy of porcine lactobacillus probiotic cultures.

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Introduction *Lactobacillus* probiotics have consistently shown *in vitro* properties of key importance in the prevention of diarrhoea at weaning. However, these probiotics have shown variable results in pigs *in vivo*, and it is important to determine the reasons for this variability if the efficacy of the preparations is to be improved. Hillman *et al*. (1993) reported that there are significant oxygen levels along the piglet intestine. As *Lactobacillus* spp. are primarily anaerobic bacteria, and are isolated and examined *in vitro* under anaerobic conditions, it is possible that the presence of oxygen along the piglet intestine could be exerting a detrimental action on their probiotic effectiveness. This experiment was set up to examine the influence of oxygen on two porcine *Lactobacillus* spp which have been previously demonstrated to inhibit the growth of *Escherichia coli* K88 *in vitro* (Hillman and Fox, 1994).

Materials and methods A porcine enterotoxigenic *E. coli* (O149:K88:K91 ; Central Veterinary Laboratory, Weybridge, UK) was maintained in nutrient broth (Oxoid, Basingstoke, UK). The two probiotic *Lactobacillus* strains (*L. acidophilus* PF32 and *L. brevis* PF42) were maintained within the collection of the Microbiology unit. Specific growth rate of *E. coli* was evaluated in the presence of each *Lactobacillus* in 100 ml of M9 medium (Hillman and Fox, 1994) in conical baffle flasks (aerobic) as well as in sealed Hungate tubes under a 50:50 mixture of N_2/CO_2 (anaerobic). Three different oxygen concentrations were produced by constant shaking (125, 75, 25 rpm; corresponding to 225, 185 and 130 µmoles l^{-1} dissolved oxygen respectively as determined using a polarographic electrode) in a water bath at 39°C. Final pH, lactic acid and volatile fatty acid production (HPLC) were also evaluated. Data were compared using one-way ANOVA and LSD tests.

Results Under highly aerobic conditions (225 µmoles l^{-1}) both probiotic species have shown an evident decrease of inhibitory effect (ie. The growth rate of *E. coli* in the coculture has increased). The data show that the specific growth rate of *Escherichia coli* was decreased significantly by both PF 42 and PF 32 at oxygen concentrations up to 185 µmoles l^{-1}. No pH differences were found between the control incubations and those with the *Lactobacillus* strains.

Table 1: Effect of porcine Lactobacillus probiotics on the specific growth rate of *E. coli* K88.

	Oxygen, µmoles l^{-1}			
	225	185	130	Anaerobic
Control	0.89 ± 0.10^{a}	0.87 ± 0.05^{a}	0.82 ± 0.05^{a}	0.77 ± 0.09^{a}
PF42	0.75 ± 0.09^{a}	0.44 ± 0.01^{b}	0.39 ± 0.04^{b}	0.39 ± 0.04^{b}
PF32	0.75 ± 0.12^{a}	0.53 ± 0.02^{b}	0.45 ± 0.02^{b}	0.41 ± 0.06^{b}

Results shown are the specific growth rate (h^{-1}) of *E. coli* in pure culture (control) and in the presence of either *L. brevis* (PF42) or *L. acidophilus* (PF32), ± SEM. For all data, n=3. Values bearing different superscript letters differ significantly ($P<0.05$).

Conclusion The results show that high aeration rates provoked a decrease in the inhibitory effect of *L. brevis* PF 42 and *L. acidophilus* PF 32, although these probiotics were still capable of significant ($P<0.05$) inhibition of the pathogen up to 185 µmoles l^{-1} dissolved oxygen. The decrease in the inhibitory activity was correlated with a decrease in the fermentative metabolism, and was probably caused by oxidative stress. These findings indicate that precautions should be considered to prevent toxic effects of oxygen when *Lactobacillus* are processed, manufactured and stored in farm situations. There was no significant effect between anaerobic and 130 µmoles l^{-1}, suggesting that the activity of these probiotics is unlikely to be affected at intestinal oxygen levels (approx 60-110 µmoles l^{-1} in weaned piglets; Hillman *et al*, 1993). Therefore, it appears that this component of variability is likely to be located in the conditions of storage and use of the product. Other data suggested that the observed inhibition did not result from acidification or from the production of organic acids during the period of the experiment.

Acknowledgements This work was funded by SERAD. JP was an ESF postgraduate student during this study.

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Distribution of potentially probiotic *Lactobacillus* spp. in pig farms.

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Introduction Isolates of *Lactobacillus* spp were obtained from colon samples from a total of 26 randomly selected Scottish piggeries. These were screened for probiotic potential, assessed by their ability to inhibit the growth of *Escherichia coli* K88. It emerged that the distribution of potentially probiotic isolates was not uniform: certain piggeries seemed to harbour populations of Lactobacillus spp. with a high proportion of potential probiotics, while other piggeries yielded few or no active isolates. We postulate that this distribution may influence the perceived variation in efficacy of commercial probiotic preparations.

Materials and Methods Samples of colon contents were obtained from three pigs on each farm, diluted and plated on MRS medium (Oxoid, Basingstoke, UK). After 48 h anaerobic incubation at 39°C, 10 colonies (per farm) from the highest dilutions showing growth were picked off and transferred to fresh growth medium. This ensured that the isolates obtained represent the most numerous groups of *Lactobacillus* spp and minimises the risk of contamination from nearby colonies, thus reducing the steps needed to obtain pure cultures. The full quota of 10 was not always obtained: farms where 5 or fewer isolates were obtained have not been included in this study (7 farms excluded). A total of 54 isolates were obtained from SAC Tillycorthie piggery since this piggery represented a convenient source of material. Pure cultures were assessed for their ability to inhibit the growth of *E. coli* K88 using the technique described by Hillman and Fox (1994). Inhibition was compared with controls using a one-way ANOVA and LSD test, and results were grouped by the degree of significance of the inhibition.

Results A total of 19 farms produced more than 5 pure isolates each. These are numbered 1-19: farm 19 is SAC Tillycorthie piggery (Table 1). Overall, of 210 isolates, 72 (34%) were capable of significantly reducing the growth of *E. coli* K88 *in vitro*. Tillycorthie (farm 19) with the largest number of isolates, produced 28% of isolates showing significant inhibition so that we could assume that approx 30% of *Lactobacillus* spp would be expected to be capable of inhibiting *E. coli* K88. This proportion is not evenly distributed throughout farms: 20-40% of isolates from farms 1, 3, 6, 8, 18 and 19 showed inhibition, 40-50% of isolates from farms 4, 7, and 12 were inhibitory. Farms 2, 10, 11 and 17 yielded over 60% of isolates as inhibitory while farm 5 yielded only 14% of inhibitory isolates. No inhibitory *Lactobacillus* spp were recovered from farms 9, 13, 14, 15 or 16. All 7 isolates from farm 11 were capable of inhibiting the growth of *E. coli* K88 with a significance of P<0.01or greater.

Table 1. Number of isolates of *Lactobacillus* spp from each farm, grouped by significance of inhibition of *E. coli* K88.

	Farm no.																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
ns	6	4	6	4	7	6	3	8	6	3		4	8	10	7	10	3	4	39
P<0.05			3	2	1		2	1		1							1	4	4
P<0.01	4	2		2		2	4	1		3	2	1					4		9
P<0.001		4								3	5	2					1	2	2
Total	10	10	9	8	8	8	9	10	6	10	7	7	8	10	7	10	9	10	54

ns, no. of isolates showing no significant inhibitory activity. *P* values, no. of isolates showing significant inhibitory activity at the levels indicated. Total, total no. of isolates per farm.

Conclusions Indigenous probiotic activity is not evenly distributed between farms. The sample obtained contained examples of both zero and 100% probiotic activity within the colonic *Lactobacillus* population of pigs, with a range of activities detected within the samples from most farms. Although only a small part of the total lactobacilli within the intestine could be sampled in this study, those isolated represent the predominant groups within the colonic population of these pigs. The implication is that this distribution could affect perceived probiotic efficacy, as an added probiotic may have a greater effect in pigs which have a low indigenous probiotic population.

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Environmentally and economically sustainable systems of sheep and goat meat production

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Introduction Recent studies on the production and marketability of sheep meat in Greece have shown that response of the small ruminant sector to changing consumer demands has been to produce consistently larger and leaner lambs all year round. Moreover, purchases of such meat are likely to favour less exploitative systems of production (*Zygoyiannis et al. 1999*). The idea is that such systems should constitute more environmentally and economically sustainable options. The objective of the current study was to investigate the potential of developing sustainable systems of sheep and goat meat production that are less depended on pharmaceutical control of gastrointestinal parasitism without comprising the quality of produced meat.

Material and methods Two consecutive experiments were performed using sixty female lambs and kids of indigenous dairy Greek breeds, respectively. Lambs of the Karagouniko breed (weaned approximately at 52 days) were initially group fed indoors for 30 days with 250 g/day/head of a concentrate ration (11.3 MJ ME/kg DM) and 500 g of Lucerne hay. Subsequently, they were finished on irrigated sown pasture (*Lolium perenne*). At turnout to pasture (day 0), all lambs were given orally an anthelmentic treatment against gastrointestinal parasites. The pasture was contaminated with 3rd stage larvae of gastrointestinal nematodes of various dominating genera from local sheep. The contamination of the pasture was monitored by means of herbage larvae counts on day 0 and thereafter every 21 days. Lambs were equally assigned into three groups ((A = controls, B = treated every 21 days with 300mg Albendazole/lamb and C = fed daily 20g barley + 80g digestible undegradable protein (DUP); Super soya 44%)) balanced for live weight (mean ± SD: 19.1 ± 2.12 kg). At 21-day intervals all lambs were weighed and condition scored and in particular those of group B were again given the anthelmentic treatment whereas those of group A and C were given a placebo. All lambs were slaughtered after grazing for 126 days. Their gastrointestinal tract was removed to assess the level of parasitism whilst the quality of their carcasses was assessed according to the European Association of Animal Production standardised methods and by chemical analysis. An identical experimental design was adopted to perform an experiment with kids (9-10 weeks old). The kids were kept indoors for 28 days post-weaning and were slaughtered after 85 days grazing in the contaminated pasture. Data were analysed by analysis of variance.

Results Table 1 shows the LW of lambs and kids at turnout to pasture and at the end of grazing period together with their carcass weight and some quality measurements of these carcasses such as conformation, fatness, PHu and chemical analysis. There were significant differences ($P<0.05$) in growth rate of both lambs and kids between the three groups. However, there were not significant differences in carcass quality measurements between controls and those animals fed either the DUP supplement or treated with anthelminthincs.

Table 1 Growth and carcass measurements of lambs and kids (means ± SD)

	Experiment 1 (lambs)			Experiment 2 (kids)		
	Group A	Group B	Group C	Group A	Group B	Group C
Weight (kg)	n=20	n=20	n=20	n=20	n=20	n=20
LW at start	19 ± 2.0	19 ± 2.0	19 ± 2.2	16 ± 1.6	16 ± 1.4	16 ± 1.5
LW at slaughter	36 ± 2.3	36 ± 3.9	37 ± 2.2	22 ± 1.8[a]	24 ± 2.2[b]	24 ± 1.6[b]
Carcass weight	17 ± 1.5	17 ± 2.4	18 ± 1.4	9 ± 1.1	10 ± 1.0	10 ± 0.7
Conformation	2.4 ± 0.49	2.5 ± 0.61	2.8 ± 0.41	1.8 ± 0.44[a]	2.0 ± 0.01[b]	2.0 ± 0.01[b]
Fatness	2.4 ± 0.37	2.2 ± 0.59	2.3 ± 0.47	1.9 ± 0.67[a]	2.4 ± 0.49[b]	2.2 ± 0.41[b]
PHu	5.5 ± 0.05	5.6 ± 0.05	5.5 ± 0.07	5.6 ± 0.15	5.6 ± 0.18	5.6 ± 0.11
Chemical analysis						
Protein (%)	19.8 ± 0.68	20.0 ± 0.97	19.8 ± 0.58	20.5 ± 0.29	19.5 ± 0.97	19.7 ± 0.69
Total fat (%)	6.2 ± 0.85	6.3 ± 0.15	6.5 ± 0.82	4.3 ± 0.47	5.3 ± 1.00	4.9 ± 0.87
Moisture (%)	72.1 ± 0.35	71.9 ± 0.44	72.8 ± 0.75	74.7 ± 0.85	74.1 ± 0.18	74.3 ± 0.38
Ash (%)	1.0 ± 0.01	1.0 ± 0.03	1.0 ± 0.01	1.1 ± 0.01	1.0 ± 0.03	1.0 ± 0.03

Conclusions The results suggest that carcasses heavier than the traditional in Greece, with high quality characteristics, could be produced by the inclusion of DUP in the feed of lambs and/or kids finished on irrigated sown pasture contaminated with larvae of gastrointestinal nematodes. It seems that such strategy could be adopted as an alternative to chemotherapeutics for the control of gastrointestinal parasitism of sheep and goats.

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Intake of lactating and dry dual-purpose cows grazing two species of *Brachiaria* pastures in Santa Cruz, Bolivia

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Introduction *Brachiaria* spp. pastures, specially *B. decumbens*, cover an area of 400 thousand ha in Santa Cruz, Bolivia. They comprise the basal diet for ruminants in dual-purpose and small-holder dairy systems of the region (Herrero *et al*. 1999). Therefore, an important portion of the animal production is conditioned by the amount and quality of these pastures and their intake. Knowledge of pasture intake is crucial for formulating bio-economically feasible feeding systems for ruminants. The objective of this work was to measure intake of *Brachiaria* spp pastures using alkanes as markers and to quantify pasture intake differences between dry and lactating cows.

Materials and methods The study was carried out in real farms in Santa Cruz, Bolivia and was divided in two trials. In the first trial, 8 lactating (501 $\pm$ 5.5 kg BW, 8.5 kg milk $cow^{-1}d^{-1}$) and 8 non-lactating (503 $\pm$ 8 kg BW) Criollo cows grazing *Brachiaria mutica* during the wet season (March – April) under an stocking rate of 33 LU ha^{-1} d^{-1} were used. Lactating cows received additionally 2 kg d^{-1} of a maize/soybean based concentrate (12 MJ ME, 180 g CP kg DM^{-1}). For the second trial we used 8 lactating (441$\pm$15 kg BW, 9 kg milk $cow^{-1}d^{-1}$) and 8 non-lactating (439$\pm$18 kg BW) Holstein/Zebu crossbred cows grazing *Brachiaria decumbens* at stocking rates of 12 LU ha^{-1} d^{-1} at the start of the dry season (June – July). Lactating cows also received 3 kg d^{-1} of the above-mentioned supplement. Pasture intakes for each cow were measured by the alkanes technique (Mayes *et al.* 1986) and analysed by gas-chromatography. Simulated grazing pasture samples were collected for chemical analysis (CP and NDF) and gas production measurements with residue collection after incubation (Jessop and Herrero, 1996) for estimation of NDF digestibility (NDFD). Green leaf allowance was also measured before animals were introduced to the paddocks.

Results Intake results and the chemical composition and herbage availability of the paddocks for both trials are presented in Tables 1 and 2, respectively. Pasture intake was less than 1.5% BW irrespective of physiological state of the animals. In general terms, lactating cows ate only 15 – 20% more pasture than non-lactating cows, which is equivalent to 0.7-0.8kg OM, thus suggesting that pasture intake only covered maintenance requirements while most of the milk produced was mainly achieved through the supplementary feeding.

Table 1 Pasture intake of lactating and non-lactating cows in the two trials.

Physiology state	N	g OM kg $BW^{0.75}$	s.e.
Trial 1 – Criollo			
Lactating cows	8	50.52 [a]	0,50
Non lactating cows	8	43.31 [b]	0,40
Trial 2 – Crossbreds	8		
Lactating cows	8	63.81 [a]	0,10
Non lactating cows		55.26 [b]	0,09

[a, b] Means with different superscripts are significantly different within trial (P<0.01)

Table 2 Chemical composition and herbage allowance of the pastures used in the two trials

Pasture species	*B. mutica*	*B. decumbens*
CP (g/kg DM)	71	67
NDF (g/kg DM)	701	713
NDFD	0.66	0.60
Instantaneous green leaf availability (t/ha)	1.9	2.0

Conclusions The results of the present study suggest that unfertilised *Brachiaria* pastures in Santa Cruz, Bolivia are of limited nutritive value, only being able to meet maintenance requirements of non-lactating dual-purpose cattle. Milk production was a function of the supplements being fed suggesting that the economic efficiency of the feeding systems can be managed through diet formulation and alternative feed ingredients.

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The nutritive value of native forage plants of Armenia

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Introduction Armenia is a typical highland country with an average altitude of 1800 m. More than half of its territory is occupied by natural pastures and hay producing areas which form an important source of feed material for animal husbandry. However, in recent years the country has been experiencing an acute shortage of feed materials and as a result the total number of livestock and animal derived products has drastically dropped. During the transition to a market economy the government has been unable to import additional forage materials, therefore there is a need to utilise local resources more rationally. Limited studies of the nutritive value of Armenia's forage plants has been carried out. Moreover, in previous studies the fundamental criteria for measuring forage quality has been largely limited to an assessment of crude protein (CP), total ash (TA), crude fat (CF), nitrogen-free extractable substances (NFS) and crude cellulose (CC) contents. Only recently have studies been undertaken to assess gross energy (GE) and in-vitro digestible organic matter (OM) in the dry matter (DOMD) contents, and OM digestibility (OMD) and digestible energy (DE) value of OM. The objective of this work was to summarize the published studies on chemical composition and to discuss the results of recent measurements of energy value and in-vitro digestibility of native forages in Armenia in order to facilitate the selection and utilisation of high quality forages by farmers.

Materials and methods A total of 363 forage samples have been analysed, representing the most common herbaceous plant species in Armenia: data on chemical composition of 297 samples were taken from published studies (Magakyan, 1963; Kazaryan et.al., 1972) and 66 mixed samples were collected by us through Armenia's Geghama mountain range. The recent study did not have an aim to analyse feeding properties of individual species, so mixed samples were taken within the groups of grasses and legumes in which the species *Agrostis alba L., Festuca pratensis Huds., Poa pratensis L., Dactylis glomerata L., Medicago sativa L., Trifolium Pratense L., Tr. repens L. Vicia variabilis Fr. et. Sint.* dominated. Samples were largely taken at flowering stage and were cut at ground level and then dried at 100 ^{0}C for eight hours and milled (1 mm screen). The CP and TA contents was measured by common methodologies that were used in the former Soviet Union (Ermakov et.al., 1972). GE content was determined using calorimetric apparatus of both the USA (Parr Inst.Comp.) and the SU (KV-08M) types. Samples (n=17) were used to determine the in-vitro DOMD content using the two-stage incubation technique of Tilley and Terry (1963), involving the incubation of the dried samples with buffered rumen fluid and acidified pepsin. The DOMD and OMD were determined in quadruplicate. Rumen fluid was collected from two mature wether sheep prior to the morning feed. The DOMD, OMD, DE and a proportion of the GE determinations were performed in the UK (ADAS, NSRU).

Results The mean values of chemical composition obtained in previous studies were (in % DM): CP, 11.1 and 17.7; TA, 7.1 and 8.1; CF, 3.0 and 3.1; NFS, 46.3 and 45.5; CC, 32.5 and 25.7 for grasses and legumes respectively. A wide variation in CP and CF contents is reported while the variation in NFS and CC is small. The CP and TA contents obtained recently (Table 1) were comparable with published literature data. A considerable difference between the two botanical groups was found in CP, DOMD content, OMD and DE value; CP content was higher in legumes but the remaining parameters were higher in grasses. The wide variation in some parameters determined may be due to different varieties and altitudes.

Table 1. The chemical composition of wild-growing forage plants in Armenia (% DM unless stated)

Fractions	Grasses			Legumes		
	mean	range	n	mean	range	n
CP	11.8	6.0-18.5	28	17.9	7.6-23.6	30
TA	8.9	5.9-14.6	28	9.7	7.2-17.8	30
DOMD	63.1	51.0-73.7	10	58.1	49.3-64.8	7
OMD (g/g OM)	0.701	0.577-0.796	10	0.642	0.543-0.723	7
GE (MJ/kg DM)	17.8	15.2-20.5	38	17.4	14.4-19.4	28
DE (MJ/kg OM)	12.4	10.2-14.1	10	11.2	9.4-12.6	7

Conclusion The findings suggest that Armenia possesses high quality wild growing forage crops. However, further investigations are required to identify more prospective species for commercial use.

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Inclusion of varying levels of urad (*Vigna mungo*) chuni in concentrate mixtures on nutrient utilization in native male buffaloes

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Introduction Pulse chunies are popular agro-industrial byproducts, obtained from processing of pulses in the preparation of dals and are available to extent of 3 million tonnes in India. These chunies comprise broken seed coat, germ and small pieces of broken cotyledons and constitute about 15-20% of total weight of pulse seeds. Very little information is available in the literature on effective levels of inclusion of chunies in complete rations for ruminants. An attempt was made to study the effect of inclusion of varying levels of urad (*Vigna mungo*) chuni in the concentrate mixtures on the nutrient utilization in native male buffaloes.

Meterials and methods Four isonitrogenous concentrate mixtures with 20% CP were prepared by incorporating urad chuni at 0 (CM-1), 20 (CM-2) 30(CM –3) and 40% (CM-4) levels. The control concentrate mixture (CM-1) consisted of maize, 270; deoiled groundnut cake, 310; deoiled ricebran, 400 and mineral mixture, 20g per Kg. The complete rations 1 to 4 comprised of 1.35 Kg of respective concentrate mixtures plus 5 Kg of rice straw and are isoenergetic to meet the nutrient requirements for maintenance as per kearl (1982). These rations were evaluated in a 4 x 4 Latin square design experiment (14 d preliminary + 7d collection period) using four fistulated native male buffaloes (288 ± 7Kg) 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 DM intake ranged between 5.44 to 5.58Kg for the four dietary treatments. Inclusion of urad chuni at 20, 30and 40% levels in the concentrate mixtures has no significant effect on DM, CP, NFE, Hemicellulose and Cellulose digestibities of the total ration in buffaloes. However, the digestibilities of NDF and ADF ($P<0.05$) linearly increased with increase of urad chuni in the concentrate mixtures indicating that fibre fraction of urad chuni was fairly digestible. All the animals were in positive balance for N, Ca and P. P retention decreased ($P<0.01$) linearly as the level of inclusion of urad chuni increased in the concentrate mixtures and might be due to lower P content of urad chuni (0.35%). The calculated DE intake increased linearly with increase of urad chuni inclusion in concentrate mixtures indicating higher digestibility of urad chuni. The NH_3-N and TVFA concentration in the rumen liquor of buffaloes increased significantly ($P<0.05$) with increase of urad chuni inclusion in the rations. Perusal of data indicate that pH, NH_3-N and TVFA concentration were optimal for cellulolytic activity, maximum microbial protein synthesis and for maximum rates of absorbption at 40% level inclusion of urad chuni. Similarly, Rao *etal* (2000) also observed higher digestibilities of cell wall constituents and increased DE intake as the level of green gram chuni increased in the concentrate mixtures of the rations fed to native male buffaloes.

Table 1 *DM intake, nutrient utilization and plane of nutrition in buffaloes fed concentrate mixtures containing urad chuni.*

	CR-1	CR-2	CR-3	CR-4	SEM	STAT. SIG
Dry matter intake (Kg/d)	5.58	5.54	5.50	5.44	0.060	NS
Dry matter digestibility (%)	56.7	56.9	57.1	57.2	1.37	NS
CP digestibility (%)	64.6	63.5	63.3	62.8	1.12	NS
NDF digestibility (%)	53.8^{a}	55.0^{ab}	55.6^{b}	56.4^{b}	0.48	*
ADF digestibility(%)	43.8^{a}	45.6^{b}	46.6^{bc}	47.5^{c}	0.50	*
Hemicellulose digestibility (%)	71.8	69.9	71.7	72.4	2.03	NS
Cellulose digestibility (%)	53.5	55.3	56.6	58.3	1.07	NS
NFE digestibility (%)	71.0	73.0	74.0	74.8	1.59	NS
Nitrogen retention (g/d)	28.8	27.1	27.5	26.3	0.65	NS
Calcium retention (g/d)	21.7	21.3	20.3	19.7	0.71	NS
Phosphorous retention (g/d)	14.2	12.5	11.3	10.4	0.54	**
DCP intake (g/d)	256	242	250	242	8.0	NS
DE intake (Mcal/d)	13.3	13.7	13.8	13.9	0.15	NS

* $P<0.05$ ** $P<0.05$

Conclusion It is concluded that urad chuni can be included at 40% level in concentrate mixtures of native male buffaloes on rice straw based rations for maintenance without any adverse effect on nutrient utilization.

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Voluntary intake of five forage trees in a cafeteria trial

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Introduction. In tropical countries is a common practice to feed cattle with variety of forage trees as supplements. In order to develop adequate strategies for management of trees, an assessment is needed of their potential use (intake) by cattle. Little research has been conducted in this area, and most effort has been focused on single forage evaluation. The objective of this experiment was to assess the preference by cattle of five forage trees. Preference was taken as the voluntary intake of a tree forage offered in a cafeteria trial.

Material and methods. Five heifers (341 ± 36 kg LW) were allocated to individual pens and fed on fresh Taiwan grass (*Pennisetum purpureum*) *ad libitum.* Simultaneously 2 kg fresh forage of each of the five following trees were offered: *Brosimun alicastrun*, *Piscidia piscipula*, *Leucaena leucocephala*, *Lysiloma latisiliquum* and *Guazuma ulmifolia* for a 10 day adaptation period. After adaptation, five additional days were employed for measuring intake in a cafeteria trial using the methodology suggested by Borman *et al.* (1991). All five forages were offered fresh, *ad libitum*, and simultaneously in separate containers for 6h. Each day the position of the tree forage was changed to avoid any conditioning and learning effects. Intake was measured hourly, and after 6h final refusals were weighed and Taiwan grass was offered as usual. Samples of the forages were taken for chemical analysis .. Data were analyzed as multiple latin squares, where: heifers=squares, position in trough = columns, days = rows, and treatments = individual trees.

Results. The chemical composition of the trees was within the range commonly reported for them (Table 1). *L. Leucocephala* had the greatest protein content but only achieved moderate intake. Polyphenol contents were highest in *L. latisiliquum* which together with *P. piscipula* gave the lowest intakes. Intake was highly correlated with lignin content. (Pearson coefficient, -0.90, P=0.039). The relationship between intake and lignin was described by:-

Intake (g/kgLW$^{0.75}$) = 90.8(±20.63) – 0.648(±0.183) Lignin (g/kgDM) (P=0.039, F=12.4, R^2=0.81, RSD 10.5)

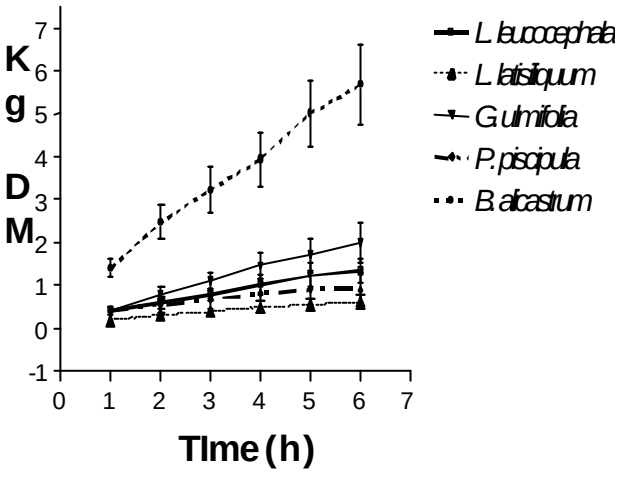
Intakes are given as the five day means of the five animals. Figure 1 shows that intake of the trees was clearly differentiated from the first hour, and the trend in the cumulative intake pattern did not show drastic changes during the six hours. Nieto *et al.* (2000) observed that eating rates of the same forages when offered alone were similar (*c.* 20gDM/min), and that time spent eating caused differences in intake. Both results suggest that cattle may detect, foliage characteristics related to feed quality before other factors are detected as post-ingestive feedback signals. A more lignified forage might be detected as being harder and 'tougher', a signal which is possibly detected during eating.

Table 1. Chemical composition (g/kg DM) and intake (gDM/kgLW$^{0.75}$) of five forage trees (n=5 animals)

	BA	PP	LL	TL	GU	Total
DM	418	385	349	471	324	
CP	169	185	267	213	155	
NDF	360	481	395	418	426	
ADF	288	289	239	212	259	
L	68	148	108	116	107	
Ash	116	126	79	79	109	
TP	17	18	24	37	14	
CT	7	7	12	12	18	
Intake	55.4a	5.4c	15.6b	5.6c	17.3b	99.3
SED #						1.63

BA= B. alicastrum, PP= P. piscipula, LL= L. leucocephala
TL= L. Latisiliquum, GU= G. ulmifolia
L= lignin, TP = total phenols, CT = Condensed tannins,
# SED = Standard error of difference between forages
Means with different letters differ at P<0.05

Figure 1. Cumulative intake of five forage trees in a cafeteria trial. (each point ± sem, n=5 animals)



Conclusion. Cattle select differentially between forage species. The rapidity of the selection suggests that the mechanism is independent of post ingestive feedback signals. This ability to select must be taken in account when designing feeding strategies which include mixtures of trees.

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Voluntary intake of grass and a forage tree when offered simultaneously

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Introduction. Voluntary intake of tropical grasses is a major constraint to animal performance in tropical countries. The use of forage trees as supplements has been associated with an improved rumen environment and hence an increased intake of forage (Umunna *et al*., 1995). Under practical conditions, grass and forage trees are commonly offered simultaneously at feeding time. However, little information is available as to the effect of this practice on intake of different forages. The objective of this experiment was to evaluate the intake of grass and a forage tree when offered simultaneously, and to assess the benefits on total DM intake with five different forage tree species.

Materials and methods. Five heifers (347 ± 33 kg LW) were allocated to individual pens and fed on fresh Taiwan grass (*Pennisetum purpureum*) *ad libitum*. Simultaneously 2 kg fresh forage of each of the five trees *Brosimun alicastrun* (BA), *Piscidia piscipula* (PP), *Leucaena leucocephala* (LL), *Lysiloma latisiliquum* (TL) and *Guazuma ulmifolia* (GU) were offered, each in a separate container for a 10 day adaptation period. After adaptation, a multiple latin square design was used to measure intake of tree forage and grass. The tree forage and grass were offered fresh, *ad libitum*, and simultaneously in separate containers for 6h. A different tree was offered to each animal. After 6h, refusals were weighed and then Taiwan grass alone was re-offered. For the next three days grass alone was offered, and then the procedure repeated with the tree offered to each animal being changed. Each animal was thus offered each tree forage with a three day interval between each measurement. The whole experimental procedure was then repeated with each animal being offered each tree forage as before, but in a different order. The intention had been to complete these repeat measurements as a within animal 5x5 latin square (ie each animal being offered each forage five times). In the event, the supply of tree forage was exhausted after three replicates, and the experiment had to be terminated. Data were analyzed for variance attributable to forage, animal, replicate and day (period).

Results. Intakes between forages differed significantly, with *B. alicastrum*, a widely used local tree forage, being the the best ($P<0.05$), including comparison with L leucocephala which is widely used throughout the tropics. This agrees with the preference ranking reported by *Nieto et al*., (2000), although intakes were lower than when the tree forages were offered alone. The intake of grass tended to be lower (ns) when *B. alicastrum* was given. However total DM intake was increased by 29% ($P<0.05$) with *B. alicastrum* compared with *L.latisquum)*, and *B. alicastrum* contributed 33% of total intake. The improvement in DM intake by including a forage tree in the diet was mainly apparent with two of the forage trees. However there was no clear relationship with any chemical compound analysed in the feed (Table 2) in explaining the higher total intake. The 24h *in situ* DM degradation has been reported as BA 84.3, LL 67.2, PP 58.1, TL 49.1 and GU 68.8% (Lizarraga, 2000). Taking degradation and condensed tannins values, the results suggest that a rapidly rumen degradable forage coupled with low condensed tannins will allow the greatest total intakes.

Table 1. Voluntary daily intake (DM) of tree forage and grass when offered simultaneously with Taiwan grass for 6h. (n=5 animals)

	Tree (kg)	Tree ($g/kgW^{0.75}$)	Grass (kg)	Grass ($g/kgW^{0.75}$)	Grass + tree ($g/kgW^{0.75}$)
L. latisiliquum	0.30^{c}	4.1^{d}	6.83	89.6	93.7^{c}
G. ulmifolia	1.04^{bc}	13.5^{cd}	6.90	86.3	99.8^{bc}
P. piscipula	1.37^{b}	17.7^{b}	6.21	79.4	97.2^{bc}
L. leucocephala	1.73^{b}	22.2^{b}	6.80	87.7	109.9^{ab}
B. alicastrum	2.98^{a}	38.6^{a}	5.90	77.4	116.0^{a}
SED	0.40	5.2	0.60	8.3	9.2

BA=B. alicastrum, PP=P. piscipula, LL=L. leucocephala, TL= L. latisiliquum, GU=G. ulmifolia.
Means with different superscripts differ ($P<0.05$)

Table 2. Chemical composition (g/kg DM) of five forage trees

	BA	PP	LL	TL	GU
DM	418	385	349	471	324
CP	169	185	267	213	155
NDF	360	481	395	418	426
ADF	288	289	239	212	259
L	68	148	108	116	107
Ash	116	126	79	79	109
TP	17	18	24	37	14
CT	7	7	12	12	18

L=Lignin, TP = total phenols, CT = Condensed tannins

Conclusion. Grass is preferred by cattle over a forage tree when the animal is given the opportunity to select. This behaviour must be taken in account of in the design of feeding strategies which include forage trees. Proper assessment of forage tree quality must be done employing complimentary techniques (as the *in situ* degradation) in addition to traditional chemical measurements.

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Rumen environment modifications in sheep fed with brewers' grain silage in Brazil

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Introduction. The roughage supplementation with some industrial by-products is an alternative on feed management. The great number of brewery in Brazil and the wet brewers' grain nutritional value are an attractive possibility for use in sheep diets, mainly for its availability during the year and its fibre quality. The inclusion of this feed in high proportions on sheep diets could modify rumen environment. The objective of this paper was to evaluate the modifications on rumen environment caused by addition of brewers' grain silage as fibre source on sheep diets.

Material and methods. The wet brewer's grain was stored under anaerobic conditions during 30 days. Three treatments were represented for three experimental diets (A - 100 % tifton hay, B - 67 % tifton hay and 33 % brewers' grain silage, C - 33 % tifton hay and 67 % brewers' grain silage). Six rumen cannuled sheep were fed with experimental diets *ad libitum*. Rumen liquor for ammonia nitrogen and pH measurements were colleted after 0, ½, 1, 1½, 2, 3, 4, 5, 6, 9½, 14 and 22 h after the first meal in the morning. Statistical design was 3x3 multiple Latin square (Mead et al., 1993) and data were analysed by SAS procedures.

Results. Ammonia nitrogen content profiles were similar for all treatments (Figure 1). The mean values at 22-h collection were 18.93, 27.03 and 29.61 mg.100 ml^{-1}, for diets A, B and C, respectively. Maximum values for ammonia nitrogen were obtained between 1½ and 3 h after meal. The greatest crude protein supply when by product was included resulted on ammonia nitrogen raise compared to diet A The low by-product intake for diet C maybe had influenced on ammonia-N values observed at some intervals (9½, 14 and 22 h). The pH variations during 22 h after meal can be observed on Figure 2. Differences between pH values were significant for treatments ($P<0.05$; T test), and the mean values for treatments A, B and C were respectively 6.26, 5.98 and 6.28 (se=0.05). Diet B showed the lowest pH values. It can be related to a great by-product intake in first hours after meal. Rumen pH variations were similar between treatment A and C at earliest intervals after the first meal, suggesting the preference of sheep for hay. The greatest amounts of non-structural carbohydrates of wet brewers' grain silage can be responsible for decrease of pH values on treatment B.

Table 1. *Chemical composition of experimental diets (A - 100% Tifton hay; B - 67% Tifton hay and 33% brewers' grain silage; C - 67% Tifton hay and 33% brewers' grain silage) and dry matter intake for sheep.*

Diets	Nutritional parameters[1]					
	DM	CP	EE	NDF	ADF	DMI
A	895.5	125.8	24.4	639.1	382.6	1.09[a]
B	687.4	160.5	63.8	684.9	366.6	1.13[a]
C	350.2	196.3	91.1	676.6	350.2	0.74[b]
std error						0.07

[1] DM-dry matter (g.kg^{-1}), CP-crude protein (g.kg DM^{-1}), EE-ether extract (g.kg DM^{-1}), NDF-neutral detergent fibre (g.kg DM^{-1}), ADF-acid detergent fibre (g.kg DM^{-1}), DMI-dry matter intake (kg.d^{1}). [a,b] different superscript letters indicate a significant difference ($P<0.05$; Tukey)

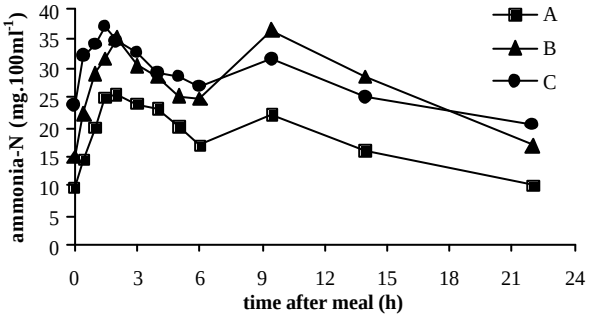

Figure 1. *Rumen ammonia-N profiles for tested diets (A – 100% Tifton hay; B – 67% Tifton hay and 33% brewers' grain silage; C – 33% Tifton hay and 67% brewers' grain silage).*

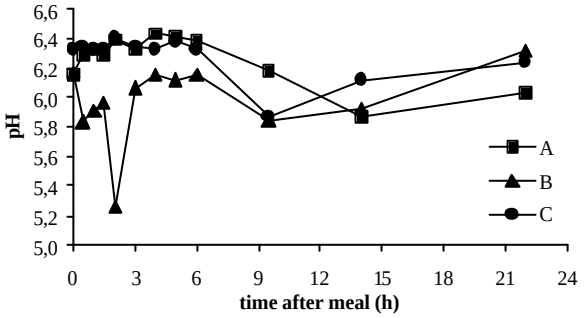

Figure 2. *Rumen pH profiles for tested diets (A – 100% Tifton hay; B – 67% Tifton hay and 33% brewers' grain silage; C – 33% Tifton hay and 67% brewers' grain silage).*

Conclusions. Under Brazilian conditions, replacement of tropical forage for wet brewers' grain silage until 67 % dry matter basis did not affect rumen environment damage, however, some characteristics of this by-product could interfere with dry matter intake.

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Evaluation of the Indonesian coffee pulp as a ruminant feed using the Reading Pressure Technique

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Introduction Indonesia is the world's third largest producers of coffee with the residual coffee pulp being included in ruminant diets. However, coffee pulp has a low palatability although studies have shown that this can be increased by ensiling. In addition, coffee pulp contains anti-nutritive factors such as caffeine and tannins. Washing the residue with hot water reduces caffein concentration by about 90 % (Kiflewahid, 1982). In this study, the effects of washing and ensilage on the degradability profiles were examined by estimating gas release using the Reading Pressure Technique.

Materials and methods Fresh coffee pulp (FCP) was obtained from coffee grown locally in Jambi, Indonesia. FCP was ensiled for two weeks (CPE). FCP was also washed in running hot water (± 90 °C) until the water drained clear. The washed FCP (WCP) was then ensiled for 2 weeks without (WCPE) or with 6% molasses (WCPEM) on a fresh weight basis. The substrates were incubated in buffered rumen fluid as described by Mauricio *et al*. (1999) at 39 ^{0}C for 96 h. Gas production was measured at 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 36, 48, 72 and 96 h post-inoculation. Rumen fluid was taken one hour before feeding from a dry cow allowed access to pasture 6 h per day plus hay/straw overnight.

Results FCP and WCPE had similar total gas production values at 96 h (Figure 1), but FCP was fermented faster than WCPE in the early stages (Figure 2) indicating that washing the substrate with the hot water removed some of the soluble carbohydrate (CHO). This negative effect increased when the washed residue was ensiled. The loss of CHO resulting from washing treatment was confirmed by the lower total and rate of gas production of WCP than those CPE. Nevertheless, washing treatment seemed to remove anti-nutritive effects reflecting by a higher total and rate of gas production from the incubation of WCP than those FCP.

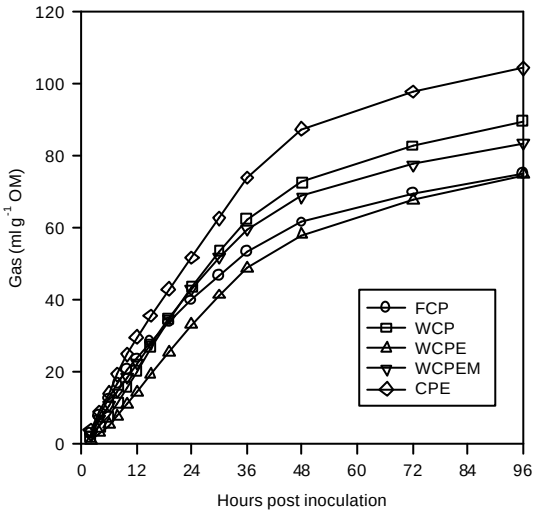

Figure 1. Cumulative gas release

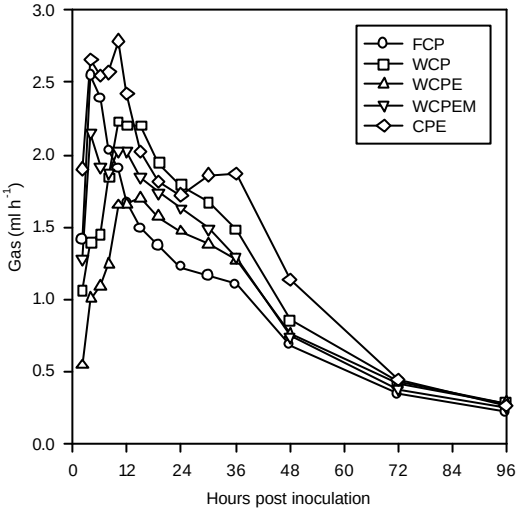

Figure 2. Rate of gas release

Conclusion Ensiling improved the fermentability of coffee pulp. Washing treatment seemed to reduce anti-nutritive effects but under practical situations this treatment ought to be followed by ensiling with an additive such as molasses. Further studies should focus on how the ensiling and washing treatments influence intake and anti-nutritive factors.

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Effect of tropical diets on inocula used on *in vitro* gas production technique

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Introduction. There is much discussion about the effect of the diet of the inoculum donor animal. The ideal diet should supply microrganisms and they should be able to degrade the feed. But when we evaluate several different feeds by a gas production assay, it is very difficult to feed donor animals with a diet composed by all feeds that will be tested. The aim of this work was to investigate the effect of three different tropical diets on inoculum ability to degrade the feeds.

Material and methods. The *in vitro* gas production was conduced according to the Reading Pressure Technique (RPT). Three substrates were tested (Lucerne hay - *Luc*, Tifton hay – *Tif* - and *Brachiaria decumbens* hay - *Bra*). They were chosen for their distinct composition. The same substrates were used as diets to feed Santa Ines sheep. Diets were offered *ad libitum* and only mineral supplementation was added. Inocula were prepared using 50% solid phase and 50% liquid phase of rumen liquor collected from two different animals for each experimental diet and period. *In vitro* degradability data were fitted by Ørskov and McDonald (1979) model and gas production data were adjusted by France at al. (1993) model. The assay was conduced in a complete factorial design (3 inocula X 3 substrates) repeated three times (3 periods) (n=27). The parameters compared were lag time, effective degradability (passage rate of $0.02h^{-1}$), potential gas production and relative gas production (proportion between gas production after 96h and potential gas production) (Bueno et al., 2000).

Results. Period was not significantly different. Both substrate and inoculum were different ($P<0.01$) for all tested parameters. Inoculum*substrate interaction was not significantly different ($P>0.05$) for lag time but it was for other parameters ($P<0.05$). Tables 1 and 2 show the differences between inocula and substrates. *Brachiaria decumbens* hay was nutritionally very poor (CP=30 and NDF=759 g.kg DM^{-1}) and the inoculum prepared with rumen liquor from sheep fed with only this feed also was very poor, resulting on the longest lag time (7.79±0.38h; $P<0.01$) and the worst relative gas production (0.717±0.018; $P<0.01$). Although Lucerne hay was nutritionally the best tested feed (CP=181 and NDF=538 g.kg DM^{-1}), the inoculum prepared with rumen liquor from animals fed with only it could not express its excellence, except for lag time which was the shortest (5.35±0.38h; $P<0.01$).

Table 1. *Effect of inoculum and substrate on lag time and effective rumen degradability.*

inoculum[1]	lag time (h)				effective degradability[3]			
	substrate[2]			mean[4]	substrate[2]			mean[4]
	Luc	*Tif*	*Bra*		*Luc*	*Tif*	*Bra*	
Luc	5.75	4.89	5.41	5.35[b]	0.415	0.300	0.330	0.348[c]
Tif	6.50	5.42	6.30	6.07[b]	0.425	0.335	0.366	0.375[a]
Bra	9.95	6.30	7.13	7.79[a]	0.414	0.329	0.342	0.362[b]
mean[5]	7.40[A]	5.54[B]	6.28[AB]		0.418[A]	0.321[C]	0.346[B]	

[1]inocula collected from sheep fed with: *Luc* – Lucerne hay, *Tif* – Tifton hay and *Bra* – *Brachiaria decumbens* hay; [2]substrates: *Luc* – Lucerne hay, *Tif* – Tifton hay and *Bra* – *Brachiaria decumbens* hay; [3]calculated using a passage rate of 0.02 h^{-1}; [4]means for each inoculum with different superscript (*a*, *b* or *c*) indicate a significant difference (Tukey test; $P<0.05$); [5]means for each substrate with different superscript (*A*, *B* or *C*) indicate a significant difference (Tukey test; $P<0.05$).

Table 2. *Effect of inoculum and substrate on potential and relative gas production.*

inoculum[1]	potential gas production (ml.g DM^{-1})				relative gas production[3]			
	substrate[2]			mean[4]	substrate[2]			mean[4]
	Luc	*Tif*	*Bra*		*Luc*	*Tif*	*Bra*	
Luc	138	150	192	160[b]	0.964	0.769	0.732	0.822[a]
Tif	145	165	194	168[b]	0.939	0.807	0.770	0.839[a]
Bra	139	175	258	191[a]	0.935	0.691	0.526	0.717[b]
mean[5]	140[C]	163[B]	215[A]		0.946[A]	0.756[B]	0.676[C]	

[1]inocula collected from sheep fed with: *Luc* – Lucerne hay, *Tif* – Tifton hay and *Bra* – *Brachiaria decumbens* hay; [2]substrates: *Luc* – Lucerne hay, *Tif* – Tifton hay and *Bra* – *Brachiaria decumbens* hay; [3]proportion between gas production after 96h and potential gas production; [4]means for each inoculum with different superscript (*a*, *b* or *c*) indicate a significant difference (Tukey test; $P<0.05$); [5]means for each substrate with different superscript (*A*, *B* or *C*) indicate a significant difference (Tukey test; $P<0.05$).

Conclusion. For inoculum preparation used on *in vitro* gas production technique, to provide a balanced diet, to meet animal requirements, is more important than the specific feed contained in the diet.

Acknowledgements. This experiment is part of projects supported by FAPESP.

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Urea preserved grain for finishing lambs

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Introduction Urea treatment of moist wheat at harvest preserves the grain, increases its crude protein content, allows it to be fed whole, and results in savings in feed costs when fed to intensively finished cattle (Lewis *et al*., 1999). The objective of this experiment was to evaluate urea treated wheat and barley for finishing lambs.

Materials and methods Moist (700-750 g/kg DM) cereals were treated immediately post-harvest with urea (3% urea on a dry matter basis for wheat, 4% for barley). Preservation of the cereal was achieved by the effect of ammonia released from the urea and sealed within a polythene top cover in a silo. The effect of supplementing urea treated cereals with soya bean meal, which may have a beneficial effect through the supply of amino acids and peptides to rumen microorganisms, was tested. Diets fed were mineralised moist barley (B) or wheat (W) with or without the addition of 5% (by fresh weight) soya bean meal (S) against a control diet (C) based on 675 g/kg dried barley 200 g/kg molassed sugarbeet pellets, 100 g/kg soya bean meal and 25 g/kg minerals and vitamins. Sixty male and 40 female Texel cross Mule lambs aged 8 months (32.6 kg) were randomly allocated to the five dietary treatments: B, BS, W, WS and C and penned in straw-bedded groups of 10 with two replicates (pens) per treatment. They were gradually built up to *ad libitum* intake of experimental diet over two weeks. Performance data was recorded weekly until slaughter at fat class 3L/3H assessed by lumbar palpation. Eleven failed to reach target slaughter condition, 0 on B, 5 on BS, 3 on W, 2 on WS and one on C and their carcase weights were estimated on the basis of final liveweight X 0.45. The data were analysed by ANOVA with specific contrasts for control vs the other treatments, barley vs wheat and soya vs no soya.Information on feed intake and feed conversion was measured on a group basis and with only two replicates was not subjected to statitstical analysis, nevertheless the results are a useful guide to practical application

Results Lambs ate the grain which had a strong ammoniacal smell quite readily, although slowly at first. Lambs on diets C and WS grew significantly faster than those on the barley diets ($p<0.05$). They also had heavier carcases and were fatter and had better conformation at slaughter. Intake of lambs on diet C was markedly higher than the rest at 0.95 vs 0.80 kg DM/d. Lambs on the wheat based diets gained significantly faster than lambs on the barley based diets, 198 vs 145 g/d (sed 20.9 g/d, $p<0.05$) and showed a trend to improved conversion efficiency. The better physical performance and intake of C lambs may have reflected the benefit of a lower starch content and reduced incidence of subclinical acidosis. Feeding additional soya increased gain dry matter and intake on the wheat based diet (NS) but decreased gain dry matter and intake on the barley based diet. Owing to lower costs of feed relative to the control diet, lambs finished with improved margins: B £4.20, BS £3.35, W £3.42, WS £5.44, C £3.15.

Table 1 Analysis of diets and effects on animal performance

	B	BS	W	WS	C	sed	sig
Diet analysis							
DM g/kg	739	744	687	695	835	N/A	N/A
CP g/kg DM	152	174	135	160	153	N/A	N/A
ME MJ/kg DM	12.6	12.6	13.0	13.0	12.5	N/A	N/A
Animal performance							
Dry matter intake (g/day)	780	780	780	840	950	N/A	N/A
Liveweight gain (g/day)	158	133	178	218	225	29.5	*
Feed conversion ratio (kg DM/kg gain)	4.94	5.86	4.38	3.85	4.22	N/A	N/A
Cold carcase weight (kg)	18.34	17.86	18.76	19.06	19.65	0.592	*
Fat class code 2 = 3L, e = 3H	2.00	2.06	2.23	2.45	2.53	0.183	*
Conformation code 2 = U, 3 = R	2.55	2.22	2.55	2.20	2.10	0.169	*

Conclusions Lamb performance on urea treated grain was acceptable. Where wheat was fed addition of 5% soya improved profitability. Results indicated significant cost savings from conserving grain by urea treatment which can be successfully exploited in lamb finishing systems.

Acknowledgements This work was funded by Hydro Nutrition Ltd and Trident Feeds.

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Evaluation of pressed sugar beet pulp ensiled with dried maize distillers grains as a feed for finishing lambs

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Introduction Lamb concentrate costs account for around 30% of the variable costs of an early lambing system and can account for up to 33% of the variable costs for store lamb finishing (MLC, 2000). Over recent years the price for finished lambs has declined. It is against this background of declining lamb prices and reduced gross margins that cheaper alternatives to proprietary lamb concentrates are being sought. The aim of this work was to evaluate an ensiled mix of pressed sugar beet pulp and dried maize distillers dark grains (Praize, Trident Feeds Limited) for finishing lambs.

Material and methods Seventy-two Charollais cross lambs (born December 1999) were used. Lambs were weaned at 8 weeks of age. Post weaning, all lambs were fed a commercial lamb concentrate until the commencement of the trial. At 12 weeks of age, lambs were allocated to one of four groups. Each group was balanced for sex of lamb, rearing status and live weight. Two groups of lambs were fed a barley/soya ration *ad libitum* (Control diet: DM 842 g/kg; 13.3 MJ ME/kg DM; 187 g CP/kg DM). The remaining two groups were fed a 4:1 mix of pressed sugar beet pulp/dried maize distillers dark grains *ad libitum* (Praize diet: DM 373 g/kg; 13.1 MJ ME/kg DM; 174 g CP/kg DM). Lambs were bedded on wheat straw and had free access to fresh water. Individual lamb weights were recorded at the start of the trial and weekly until slaughter. Lambs were selected for slaughter when the target fatness was attained (2, 3L). All lambs were sold on a dead weight basis. Carcass data was obtained from the abattoir. Animal performance data were analysed using analysis of variance. To allow for the analysis of carcass classification data, a numerical value was attributed to each class as follows: Conformation; E=1, U=2, R=3, O=4, P=5; Fat class; 1=1, 2=2, 3L=3, 3H=4, 4L=5, 4H=6, 5=7.

Results There were no obvious health problems associated with either of the trial diets. One lamb on the Praize diet died during the period of the trial. Two lambs in the Praize group did not reach slaughter weight and condition by the end of the trial period (17 May 2000). These lambs were included in the calculation of food conversion and the cost of the treatments. Thirty-six lambs on the Control diet and 33 lambs on the Praize diet were finished for slaughter. Lambs on both treatments had similar live weights at the start of the trial and at slaughter (Table 1). However, lambs in the Control group reached slaughter sooner, and at a younger age than lambs in the Praize group ($P < 0.05$, Table 1). This was due to a higher daily live weight gain (DLWG) for lambs in the Control group (334 *v.* 271 g/d, s.e.d. 17.8). The total amount of weight gained over the trial period was similar for both groups.

Table 1 The physical performance of lambs

	Control (n=36)	Praize (n=33)	s.e.d.	Sig.
Start weight (kg)	27.7	27.1	1.08	NS
Finish weight (kg)	40.0	40.0	0.59	NS
Days on trial	38	48	3.9	*
Age at slaughter (d)	119	129	4.2	*
Total weight gain (kg)	12.4	12.1	0.83	NS
DLWG (g)	334	271	17.8	***

Table 2 Lamb carcass information

	Control (n=36)	Praize (n=33)	s.e.d.	Sig.
Carcass weight (kg)	19.1	18.8	0.29	NS
Killing out %	47.89	47.12	0.546	NS
Conformation	2.81	2.88	0.139	NS
Fat class	2.94	2.79	0.151	NS

Carcass weight and killing out percentage did not differ between the two groups (Table 2). Dietary treatment had no effect on carcass classification. Figures for feed use and food conversion efficiency were calculated on a group basis. Over the trial period, lambs on the Control diet consumed 54.1 kg of food per lamb whilst those on the Praize diet consumed 147.3 kg per lamb. Due to the large difference in the DM content of the diet, food conversion efficiency was calculated in terms of kg of diet DM per kg live weight gain. Lambs on the Control diet used 3.69 kg of diet DM to produce 1 kg of live weight gain. The corresponding figure for lambs on the Praize diet was 4.63 kg.

Conclusions Praize is a suitable feed for finishing lambs over 20 kg. Lambs on the Praize diet had lower DLWG and took longer to finish than those on the Control diet. However, a saving on feed costs of 90p per lamb was made on the Praize diet. Careful planning is therefore required in order to sell lambs at the optimum time in order to take advantage of this saving.

Acknowledgement The financial support of Trident Feeds Limited is gratefully acknowledged.

References

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Comparison of pressed sugar beet pulp ensiled with dried maize distillers grains against a ration based on barley and soya bean meal for fast finishing suckled beef bulls

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Introduction Intensively finished beef cattle have traditionally been fed rations based on rolled mineralised barley with a protein supplement such as soya bean or rapeseed meal. Since feed accounts for 75-85% of the variable costs of intensive beef production systems (MLC 1999) the use of alternative feeds that have a lower cost per unit of energy are worthy of investigation. The objective of this trial was to evaluate feeding pressed sugar beet pulp ensiled with dried maize distillers grains (Praize, Trident Feeds) on the performance of fast finishing continental cross weaned suckled bulls.

Materials and Methods Twenty 7/8th bred Limousin February born weaned suckled bulls with a mean live weight of 336kg were used. The calves had been offered creep feed *ad libitum* prior to weaning. They were weaned in October 1999 and allocated to the following dietary treatments in a randomised block design with 10 cattle per treatment group. One group was fed a rolled barley, soya bean meal and mineral ration (Control: DM 856 g/kg; 13.3 MJ ME/kg DM; 169 g CP/kg DM; 211 g NDF/kg DM; 410 g starch/kg DM; 61g sugar/kg DM). One group was fed an ensiled 4:1 mix of pressed sugar beet pulp and dried maize distillers grains (Praize: DM 394 g/kg; 13.1 MJ ME/kg DM; 175 g CP/kg DM; 358 g NDF/kg DM; 11 g starch/kg DM; 38 g sugar/kg DM). The group fed Praize also received 25g minerals per 100kg live weight per head per day. The treatment rations were fed *ad libitum* to the cattle through to slaughter. The cattle were housed in straw-bedded pens in treatment groups of ten. The cattle had free access to water and barley straw from racks. They were selected for slaughter at MLC fat class 3. To allow for the analysis of carcass classification data, a numerical value was attributed to each class as follows: Conformation; -P=1, P+=2, -O=3. O+=4, R=5, -U=6, U+=7, E=8; Fat class; 1=1, 2=2, 3=3, 4L=4, 4H=5, 5L=6, 5H=7.

Results. The bulls recorded high daily live weight gains (DLWG), slaughter weights, killing out percentage, carcass weights and carcass grades exceeding the performance targets for the system (MLC 1999a). The conformation and fat scores of the carcasses equated to a –U/U+3 grade on the EUROP carcass classification scheme. The mean age of the bulls at slaughter was 429 days (range 377-484). There were no differences between the treatments.

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Table 1 Animal performance

	Control	Praize
Start weight (kg)	336.2	336.4
Slaughter weight (kg)	598.0	587.3
Days to slaughter	175.3	177.4
DLWG (kg)	1.497	1.423
Carcass wt (kg)	356.8	355.1
Killing out %	59.63	60.40
Conformation score	6.40	6.70
Fat score	3.30	3.10

There were no differences in the health or condition of the cattle. Group feed intakes were recorded through to slaughter and feed conversion ratio (FCR) was calculated.

Table 2 Feed Intakes (kg/head)

	Control	Praize
Total feed intake	1602	3435
Total feed intake (DM)	1371	1367
Mean daily feed intake (DM)	7.81	7.71
Calculated FCR (kg DM feed/kg gain)	5.22	5.42

Feed costs per kg live weight gain were calculated based on the feed prices prevailing at the time of the trial which were 60.1 and 55.5p/kg for Control and Praize respectively.

Conclusions Feeding an ensiled mix of pressed sugar beet pulp and maize distillers grains can replace a rolled barley and soya bean meal ration when fed to fast finishing suckled beef bulls without affecting performance.

Acknowledgments Financial support from Trident Feeds is gratefully acknowledged.

References MLC (1999) Beef Yearbook. MLC, Milton Keynes.
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Effect of protein level in cereal based rations for Continental cross Holstein bulls and heifers

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Introduction The use of barley and a protein supplement such as soya bean meal as an *ad libitum* ration for intensively finished male beef calves has been well established and widely used since the conception of the cereal beef system in the 1960's (Preston et al 1963). Rations on commercial beef units rearing Continental cross Holstein beef cattle are usually formulated to contain 175g CP/kg DM. With the recent fall in the price of barley there is increased interest in the cereal beef system for bull calves and late maturing Continental cross Holstein heifers. Increasing the proportion of barley in cereal beef rations can reduce ration costs and provided productivity is maintained, increase profit. There is also a paucity of information on the performance of heifers on the cereal beef system. The objective of this study was to evaluate feeding cereal based rations containing either 140 or 175g CP/kg DM to Continental cross Holstein bulls and heifers.

Materials and Methods A total of 32 Charolais cross Holstein bulls and heifers at approximately 18 weeks old were allocated at random in a 2 x 2 factorial design trial to rations formulated to contain 140 or 175g CP/kg DM. The following rations were fed *ad libitum:* 870g/kg rolled barley, 50g/kg soya bean meal, 50 g/kg molasses, 25g/kg minerals and 5g/kg 'XP' Yeast (Ration 140); 770 g/kg rolled barley, 150 g/kg soya bean meal, 50 g/kg molasses, 25 g/kg minerals and 5 g/kg 'XP' Yeast (Ration 175). The 140 and 175 diets were analysed to contain 135 and 168 g CP/kg DM with an ME value of 13.75 and 13.62 MJ/kg DM respectively. The cattle were housed in four straw- bedded pens in treatment groups of eight. The cattle had free access to water and barley straw from racks. Cattle were selected for slaughter when a target fat class of 3 and 4L was achieved for the bulls and heifers respectively. To allow for the analysis of carcass classification data, a numerical value was attributed to each class as follows: Conformation; -P=1, P+=2, -O=3. O+=4, R=5, -U=6, U+=7, E=8; Fat class; 1=1, 2=2, 3=3, 4L=4, 4H=5, 5L=6, 5H=7.

Results There were marked differences in performance between the bulls and heifers, with the bulls achieving higher daily live weight gains, reduced number of days to slaughter, higher slaughter weights, carcass weights, killing out percentage and a lower fat score. There were no differences in performance between the 140 and 175 rations with either the bulls or heifers. The mean age of the bulls and heifers at slaughter was 373 and 361 days respectively.

Table 1 Animal performance

	Bulls		Heifers	
	140	175	140	175
Start wt (kg)	173.2	173.0	182.4	182.2
Slaughter wt (kg)	525.2	524.0	438.3	449.1
Days to slaughter	261.7	245.1	212.8	209.3
DLWG (kg)	1.36	1.43	1.22	1.29
Carcass wt (kg)	289.6	290.9	229.8	236.4
Killing out %	55.35	55.19	52.66	52.64
Conformation score	4.50	4.87	4.50	4.75
Fat score	3.12	3.12	4.12	4.34

There were no differences in the health or condition of the cattle. Group cereal feed intakes were recorded through to slaughter and feed conversion ratio (FCR) was calculated.

Table 2 Cereal Feed Intakes (kg/head)

	Bulls		Heifers	
	140	175	140	175
Total cereal feed intake	2115	2032	1610	1638
Calculated FCR (kg feed/kg gain)	6.01	5.79	6.29	6.14

Conclusions There were significant differences in performance between the bulls and heifers with the bulls recording higher daily live weight gains, slaughter weights, killing out percentage, carcass weights and reduced number of days to slaughter. The heifers reached slaughter condition of fat class 4L at relatively low slaughter weights in respect of carcass specification for the meat trade. Increasing the crude protein content in the ration from 140 to 175g/kg DM did not improve animal performance, and increased the cost per kg live weight gain. The issue of nitrogen balance and excretion needs to be considered.

Acknowledgments Financial support from Diamond V Mills is gratefully acknowledged.

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Level of supplementary concentrates for Holstein-Friesian young bulls fed silage

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Introduction As a result of the Agenda 2000 increase in the Special Beef Premium and introduction of a slaughter premium for bulls, there is renewed interest in bull beef production especially from low value Holstein-Friesian calves. To evaluate the economics of such a production system further information is required on responses to supplementary concentrates with silage and duration of the finishing period.

Materials and methods Fifty-four winter-born Holstein-Friesian male calves (initial liveweight 51 kg) were reared indoors for 130 days and then turned out to pasture (mean liveweight 160 kg) for a 203 day grazing season. They were then blocked on weight to groups of 9 and assigned to a 3 (feeding levels) x 2 (finishing periods) factorial experiment. The 3 feeding levels were 3 (L) and 6 (M) kg concentrates per head daily plus grass silage *ad libitum*, and concentrates *ad libitum* (H) plus a minimum of 1kg/day of silage dry matter (DM). The two finishing periods were 179 (S) and 272 (E) days. All the animals were housed together in pens of 9 in a slatted shed (2 pens per feeding level) in such a way that each pen had 4 or 5 S and E animals. Group feed intakes (silage for L and M and concentrates for H) was recorded weekly. The concentrate composition (g/kg) was 875 rolled barley, 70 soyabean meal, 40 molasses and 15 mineral/vitamin premix. The silage analysis was (g/kg) DM 202, crude protein 142, *in vitro* DM digestibility 688 and pH 3.8. Estimated metabolisible energy (ME) value of the silage and concentrates was 10.1 and 12.5 MJ/kg DM, respectively. At slaughter, carcasses were weighed and graded according to the EU Beef Carcass Classification Scheme. Feed intake data were analysed by one way analysis of variance with two observations per feeding level. Liveweight data were similarly analysed with block included and the individual animal as the experimental unit. Slaughter data were analysed according to the 3 x 2 factorial design.

Results Daily silage intake decreased ($P < 0.001$) but total intake increased ($P < 0.001$) with increasing concentrate level (Table 1). Intakes were higher after 179 days than earlier. Efficiency of ME utilisation for both liveweight and carcass gains increased with increasing concentrate level up to 179 days but thereafter it tended to decrease. Mean liveweight gain from calf arrival to start of finishing was 764 (s.d. 193) g/day and mean liveweight then was 306 (s.d. 19.8) kg. Mean liveweight gains up to 179 days and from 179 to 272 days were 908, 1164 and 1395 (s.e 39.8) and 961, 1022 and 1200 (s.e 46.0) g/day for the L, M and H feeding levels, respectively. Compared with L, M and H increased slaughter weight by 47 and 96 kg, respectively (Table 2). Corresponding increases in carcass weight were 30 and 64 kg. Across feeding levels the longer finishing period increased slaughter weight by 102 kg. Kill-out proportion increased by 24 g/kg, and there was a 69 kg increase in carcass weight. Conformation improved by 0.52 of a class and fat score increased by 0.13 of a class as a result of later slaughter. Regression of live and carcass weight gains on concentrate level showed that silage alone was capable of supporting a liveweight gain of 0.69 kg/day and a carcass gain of 0.45 kg/day. The response to concentrates was 74 g liveweight and 50 g carcass weight per kg.

Table 1. Feed and energy intakes of Holstein-Friesian young bulls.

	Start to 179 days				179 to 272 days			
	L	M	H	s.e.	L	M	H	s.e.
Silage intake (kg DM/day)	5.05	3.42	1.01	0.163	7.09	5.50	0.99	0.225
Concentrate intake (kg DM/day)[1]	2.48	4.96	8.47		2.58	5.16	10.82	
ME intake (MJ/day)	82	97	116		104	120	145	
Total intake (g/kg liveweight)	19.5	20.4	22.1	0.48	18.7	19.1	19.2	0.61
ME/kg liveweight gain (MJ)	91.9	82.3	84.6		108.1	117.5	121.0	
ME/Kg carcass gain (MJ)	142.6	128.1	128.1		153.1	171.2	183.5	

[1]Fixed allowance for L and M; ME = Metabolisable energy; NS = not significant

Table 2. Slaughter traits of Holstein-Friesian young bulls.

	Feeding level (F)			Finishing period (P)			Significance[2]	
	L	M	H	S	E	s.e.[1]	F	P
Slaughter weight (kg)	514	561	610	511	613	7.3	***	***
Kill-out (g/kg)	531	541	554	530	554	3.4	***	***
Carcass weight (kg)	274	304	338	271	340	4.7	***	***
Kidney & channel fat (g/kg)[3]	27	30	35	27	35	0.001	***	***
Carcass conformation[4]	2.2	2.6	2.8	2.3	2.8	0.15	***	**
Carcass fatness[5]	2.9	3.0	3.2	3.0	3.1	0.08	***	NS

[1]For n = 18; [2]F x P not significant; [3]Of carcass weight; [4] Scale P = 1, E = 5; [5]Scale 1(leanest) to 5 (fattest);

Conclusions Holstein-Friesian bull calves which were 300 kg at the start of finishing produced acceptable carcasses of 300 kg or over when fed silage plus 6 kg concentrates per day for 9 months or concentrates *ad libitum* for 6 months. To take young bulls of 300 kg liveweight to a slaughter weight of 550 kg (carcass weight 300 kg) would require feed inputs of about 4.2 t silage (200 g/kg DM) plus 1.3 t concentrates fed at 6 kg per head daily for 220 days, or 1.3 t silage plus 1.6 t concentrates fed *ad libitum* for 180 days.

Effects of extending the grazing season in beef production systems

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Introduction. The cost of grazed grass is less than half that of grass silage (O'Kiely, 1994) and incomes from beef production are low and largely dependent on EU support schemes. Thus the income from beef production could be incresed by reducing feed costs through increasing the proportion of grazed grass in the diet and optimising the use of the various support schemes. The objective of this two-year study was to examine the effects on the performance of yearling cattle of turnout to pasture three weeks earlier than normal. This was examined within two suckler beef production systems. One was a standard system similar to that outlined by Drennan (1993) and the second was compatible with the Rural Environment Protection Scheme (REPS).

Materials and Methods. The 72 animals used each year were the progeny of spring-calving Limousin x Friesian and Simmental (x Limousin x Friesian) cows, and Charolais and Limousin sires. The animal data for the two years were combined and analysed as a 2 (year) X 2 (production system) X 2 (turnout date) unbalanced factorial design. The two production systems examined differed mainly in stocking and nitrogen (N) application rates. Stocking rates of 0.79 and 0.97 ha/cow plus progeny to slaughter plus replacements and N application rates of 211 and 103 kg N/ha were used in the Standard and REPS systems respectively. There were 20 (20) and 16 (16), and 16 (24) and 12 (20) steers (heifers) on the standard and REPS systems in years 1 and 2, respectively. Half the animals on each system were turned out on March 19 and 22 (early) and the remainder were turned out on April 9 and 12 (late) in years 1 and 2, respectively. Those animals turned out early grazed paddocks intended for silage harvesting. Silage yields and dry matter digestibility (DMD) are presented for year 2. A total of 14 silage paddocks were used for early grazing in year 2. The animals grazed half the area of each paddock and the remainder was ungrazed. Before turnout the animals were weighed on two consecutive mornings. Following turnout of the late group both the early and late groups grazed together within their respective production system and all were weighed 8 days later at assumed similar gut-fill. The steers (heifers) were housed on October 16 (September 9) and November 9 (September 30) and following feeding indoors were slaughtered on March 3 (November 17) and March 6 (November 9) in years 1 and 2, respectively. Carcass data were available for steers only.

Results. The initial liveweight of the animals on the date that the early turnout animals were turned out to pasture was 365 kg (Table 1). Turnout date had a highly significant effect ($P<0.001$) on weight gains in the period from early turnout to 8 days post the late turnout date and there was no effect of production system. There were no effects of turnout date or production system on the weight gains for the remainder of the grazing season (day 8 post the late turnout to housing) or during the finishing period (housing to slaughter) or for the total period (early turnout to slaughter). Neither turnout date or production system had a significant affect on carcass weight, kill-out, conformation or fatness of the steers. When 7 of the paddocks were harvested for silage on May 20, yields from the grazed and ungrazed areas were 3.2 and 5.0 (s.e. 0.20) t DM/ha with DMD values of 794 and 772 (s.e. 7.0) g/kg, respectively. Corresponding values for the remaining 7 paddocks harvested on June 8 were 5.8 and 6.6 (s.e. 0.20) t DM/ha and 734 and 692 (s.e. 7.0) g/kg.

Table 1. Liveweights and weight gains of steers and heifers and carcass traits of the steers.

	Turnout date (T)		Production system (P)			Significance	
	Early	Late	Standard	REPS	s.e.	T	P
Weight at Turnout (kg)	364	365	363	366	6.2	ns	ns
Weight Gain (kg)							
Turnout to 8 days	20.1	4.4	12.2	12.3	1.3	***	ns
8 days to housing	147.4	148.0	149.3	145.9	3.6	ns	ns
Housing to slaughter	79.7	78.0	80.0	77.5	5.5	ns	ns
Turnout to slaughter	247.3	230.4	241.4	235.6	7.8	ns	ns
Carcass traits[a]							
Weight (kg)	384.2	376.3	380.4	380.2	6.9	ns	ns
Kill-out (g/kg)	557	562	559	561	2.8	ns	ns
Conformation[b]	3.3	3.2	3.2	3.3	0.09	ns	ns
Fatness[c]	4.2	4.3	4.3	4.2	0.14	ns	ns

[a]Carcass data for steers only. [b]EUROP classification (P=1,…,E=5). [c]EUROP classification (1=leanest,….,5=fattest).

Conclusion. Turning animals out to pasture 3 weeks earlier in spring had no overall effect on weight gained or on carcass output. Reducing the intensity of beef production by lowering N inputs and decreasing stocking rates had no overall effects on individual animal performance or carcass output. Grazing silage paddocks early in spring significantly decreased yields but the quality (DMD) of the material produced as a result was superior.

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The effects of breed and different levels of dietary protein on store lamb finishing performance

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Introduction Efficient lamb finishing is geared to reaching market at a specific time, this is done by regulating growth rates – this may involve using different feeds – in this case varying in crude protein content or using different breeds – crossbreds rather than purebreds. The objectives of this trial were to determine the effect of different feed crude protein level on lamb performance of two breed types.

Materials and methods 120 spring 1999 born lambs were used for this study, 60 being Swaledale (Sw) and the other 60 being crossbred lambs, these being lambs from the Swaledale ewe crossed with the Blue faced Leicester sire. The 120 lambs were divided into 8 pens, the pens were balanced for weight and breed. One group of lambs (i.e. 4 pens) was fed a diet with 160g/Kg DM of crude protein (HCP) and the others were fed a diet with 140g/Kg DM crude protein (LCP), the diets were balanced in all other aspects. All lambs were weighed fortnightly and assessed for finish and selected at fat class 3, lambs were sold deadweight and carcass information was collected. Feed intakes were recorded and a food conversion efficiency (FCE) = Kg weight gain/Kg food consumed (fresh weight)* 100 was calculated. Data collected were analysed using two-way ANOVA.

Results Table 1: Average results of all groups of lamb on trial

	Sw LCP	Cb LCP	Sw HCP	Cb HCP	All LCP	All HCP	s.e.d. Diet	s.e.d. Breed	Sig. Diet	Sig. Breed
Weight gain (Kg)	7.87	6.17	8.47	6.23	6.99	7.35	0.169	0.171	NS	*
Days on trial	61.1	48.18	60.8	46.43	53.77	53.62	1.273	1.286	NS	***
DLWG (Kg/d)	0.13	0.13	0.14	0.13	0.13	0.14	0.003	0.003	NS	NS
Sale Weight (Kg)	34.91	35.67	33.74	36.22	34.93	34.59	0.825	0.828	NS	NS
Carcass Weight (Kg)	16.5	19.73	16.38	19.95	18.24	18.17	0.429	0.431	NS	**
Killing out value	0.46	0.48	0.46	0.49	0.47	0.47	1.114	1.113	NS	**
Conformation	2.03	2.7	2.07	2.7	2.32	2.39	0.056	0.056	NS	**
Fat Class	2.8	3.15	2.77	3.24	3	3.01	0.071	0.001	NS	NS
FCE	0.12	0.095	0.107	0.098	0.106	0.102	0.003	0.0001	NS	NS

*Conformation score according to following conversion – E=5, U=4.0, R=3, O=2, and P=1. Fat Class being 5=5, 4H=4.2, 4l=3.8,, 3H=3.2, 3L=2.8, 2=2, 1=1. Where significance *, p<0.05, **, p<0.01, ***, p<0.001*

Breed had a significant effect on the performance of the lambs, the Sw had a significantly greater weight gain over the trial period than the Cb lambs, however the Cb lambs were on trial for a significantly shorter period, this however did not affect their carcass weight as this was significantly greater in the Cb lambs compared to the Sw lambs, this was also the case for the killing out value as well as the conformation score and fat class – the Cb lambs performed better than the Sw lambs. From the above table it appears that the diet does not have an effect on performance of finishing lambs. The results also show however that there is some degree of interaction between the breed and level of fed crude protein for some aspects of lamb performance – these being days on trial ($P<0.05$) and conformation ($P<0.05$), with the crossbred lambs fed HCP performing slightly better than the remaining lambs on trial.

Conclusion Within the levels of crude protein used in the trial, lamb performance remained unaltered, however crossbred lambs gave an increased physical performance resulting in a greater carcass yield and shorter time to sale.

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Simple mixes of molassed sugar beet feed, field beans and distillers grains for pregnant March-lambing ewes

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Introduction Feeding of lowland sheep on straw-based systems during pregnancy and in early lactation has shown that ewe and lamb performance can be satisfactory, providing adequate compound supplementation is fed (Davies and Chapple, 1995). Whole barley and soya bean meal has been the standard ration. However, soya bean meal is imported and may not be fully traceable. Maize or barley distillers fed with beans could provide traceable protein to replace soya bean meal in sheep diets. Experiments with housed early-lambing ewes and ewes suckling twin lambs at grass have shown that traceable feeds, based on molassed sugar beet and either maize or barley distillers grains, can replace a barley/soya supplement when fed with straw based diets in late pregnancy or at grass (Chapple *et al*., 1999 and 2000). The objective of this work was to evaluate the effects on ewe and lamb performance of feeding sugar beet feeds with distillers grains and beans to March-lambing ewes on a straw-based system.

Materials and Methods 144 March-lambing North Country Mule ewes, scanned as carrying twins, were housed and divided into four treatment groups (two pens of 18 ewes/treatment) in mid-January 2000. All ewes were fed *ad libitum* barley straw supplemented with either whole barley/soya-bean meal 80:20 (BS), barley distillers/molassed sugar beet feed 70:30 (BD), barley distillers/molassed sugar beet feed and beans 50:30:20 (BDB) or maize distillers/molassed sugar beet feed and beans 50:30:20 (MDB). Supplementary feeding started eight weeks prior to lambing at 0.46 kg/head and gradually increased up to 1.15 kg/head at lambing. After lambing, ewes and lambs were turned out to perennial ryegrass/white clover swards where they were supplemented with 0.5 kg/head of molassed sugar beet feed for eight weeks. Lambs did not receive any creep feed and the experiment finished when they were approximately 10 weeks old. The experiment was a randomised block design and data were analysed using analysis of variance.

Results MDB ewes were significantly lighter ($P < 0.05$) than BD ewes at lambing, but by the end of the experiment (30 May) all ewe were of similar live weight. Ewes on all treatments lost some body condition (0.7 condition score) up to lambing but maintained this condition (score 2.7) to the end of the experiment (Table 1).

Table 1 *Ewe Performance*

	BS	BD	BDB	MDB	s.e.d.
Liveweight (kg) :					
Start weight (12 Jan)	73.2	73.2	73.2	73.2	0.22
Lambing weight (19 March)	68.9	69.6	68.7	66.7	1.06
Final weight (30 May)	60.9	60.4	61.0	59.4	1.34
DM intake (kg/day)	1.98	1.88	1.82	1.82	0.082
ME intake at lambing (MJ/day)	21.0	18.5	19.2	20.1	-

Birth weights of BDB and MDB lambs tended to be lower ($P = 0.06$) than BS and BD lambs. However, overall growth rates from birth to 10 weeks of age were similar for all treatments (Table 2).

Table 2 *Lamb Performance*

	BS	BD	BDB	MDB	s.e.d.
Birth weight (kg)	5.14	5.08	4.87	4.75	0.156
10-week weight (kg)	23.4	23.4	24.5	22.5	0.71
Daily gain: Birth-10 weeks (g)	259	251	263	241	9.0

Conclusion A 70:30 barley distillers and sugar beet mix can replace a whole barley/soya-bean ration when fed to twin-bearing ewes on straw diets without affecting ewe or lamb performance. However, substituting 20% of the diet with beans to replace barley/maize distillers, led to greater ewe weight loss during pregnancy and smaller lambs at birth, but no long term detrimental effects.

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The performance of twin-bearing ewes and their progeny when offered red clover, lucerne and grass silages during late pregnancy

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Introduction Feeding conserved forage in the form of silage to pregnant ewes in winter is common practice in agricultural systems in the UK. However, silage as a sole feed in late pregnancy is unlikely to meet the nutritional requirements of ewes carrying twin lambs, and supplementary feeding is usually required. Evidence suggests forage legumes such as red clover and lucerne offer great scope for improving the supply of protein to ruminants (Frame *et al*., 1998), and feeding silage prepared from these crops could potentially reduce the need for protein supplements. The aim of this experiment was to compare the performance of twin-bearing ewes and their lambs when fed either ensiled red clover, lucerne or ryegrass during late pregnancy.

Materials and methods Twenty four twin-bearing mature Mule ewes, mated to a Suffolk ram, were allocated to one of three forage treatments (*n*=8), with treatment groups balanced for live weight and condition score. The treatments were red clover silage, lucerne silage or ryegrass silage, prepared as large round bales and offered *ad libitum.* The ewes were housed in individual pens eight weeks prior to lambing. The experiment comprised a 14 day adaptation period, followed by a six week measurement period. All ewes received a supplement of molassed sugarbeet shreds (893gDM/kg) plus minerals and vitamins, and were offered water *ad libitum.* Supplementary feeding started six weeks prior to lambing at a rate of 0.25 kg/day/head, and was gradually increased on a weekly basis to a rate of 1.12 kg/day/head at lambing. The amount of sugarbeet offered was similar for all treatments, and was the minimum necessary to ensure all animals met the corresponding energy and DM intake requirements given by AFRC (1993). The ewes were weighed weekly from the start of the experiment until a week before lambing. Amounts and DM contents of forage and sugarbeet offered and refused were recorded daily. Daily sub-samples of silage offered were bulked on a weekly basis and analysed for total nitrogen content (TN) (expressed as crude protein (CP) (TN x 6.25)) and metabolisable energy (ME) content (calculated as digestible organic matter in the dry matter (DOMD) x 0.016). After lambing all the ewes and lambs were turned out onto the same ryegrass paddock, and were offered a standard compound supplementary feed at a rate of 0.5 kg/head until the sward height reached 5 cm. Lambs were weighed at birth and weekly thereafter until 12 weeks post partum. All data collected were analysed using one-way analysis of variance.

Results The silages offered had significantly different DM, CP and ME contents (Table 1). There were significant treatment effects on ewe DM intake, which were reflected in ewe liveweight gain but not in litter weight (Table 1). The type of forage consumed by the ewes prior to lambing had a significant effect on lamb growth rates from birth – 3 weeks and from 4 – 12 weeks. Lamb liveweight at 12 weeks was significantly higher for lambs from ewes that had been offered the red clover treatment.

Table 1. *Effects of forage treatment during the final six weeks of pregnancy on ewe and lamb performance*

	Red clover	Lucerne	Ryegrass	s.e.d	Significance
DM content (g/kg)	305^{a}	367^{b}	314^{a}	7.4	***
CP content (g/kg DM)	215^{a}	240^{b}	182^{c}	5.4	***
ME content (MJ/kg DM)	11.6^{a}	11.1^{b}	10.8^{c}	0.11	***
Ewe DM intake (kg DM/day)	1.33^{a}	1.30^{a}	0.77^{b}	0.112	***
Ewe liveweight gain (kg)	11.6^{a}	11.1^{a}	6.5^{b}	1.10	***
Litter weight (kg)	9.9	9.7	9.5	0.57	ns
Lamb growth rate (g/day)					
Birth – 3 weeks	323^{a}	320^{a}	282^{b}	15.8	*
4 – 12 weeks	277^{a}	250^{b}	276^{a}	11.8	*
Lamb liveweight at 12 weeks (kg)	29.1^{a}	26.7^{b}	27.1^{b}	0.95	*

Means within rows with different superscripts are significantly different (P<0.05),
*Significance: * = P<0.05, ** = P<0.01, *** = P<0.001.*

Conclusions Offering ensiled forage legumes to ewes in late pregnancy can increase DM intake and ewe liveweight gain, leading to increased lamb growth rates in the first three weeks post partum. In the case of ewes offered red clover silage, the benefit in terms of improved lamb liveweight was still evident at 12 weeks post partum.

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Time course of changes in the fatty acid composition of plasma in the milk fed pre-ruminant calf supplemented with a palm/rapeseed oil mixture or fish oil.

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Introduction In pre-ruminant calves the oesophageal groove reflex allows milk to bypass the developing rumen and is a mechanism by which dietary fatty acids can be delivered directly to the small intestine. This experiment was a pilot study carried out to establish the extent and time course of the changes in plasma fatty acid composition in calves fed milk supplemented with oils of differing fatty acid composition and allowed minimal access to roughage (straw bedding). The work was carried out to provide baseline data for a longer study with calves in which rumen development will be encouraged by feeding solid food but the oesophageal groove reflex will be maintained through long term milk feeding and used as a means of delivering polyunsaturated fatty acids (PUFA) directly to the small intestine.

Materials and Methods 8 Jersey bull calves (mean liveweight 32.4 ± 1.38 kg, mean age 15.8 ± 3.5 days) were fed a standard milk replacer (Volac Blossom, g/kg, fat 180; crude protein 230; lactose, 480) (2 x 250g/day) for a period of three weeks to aclimatise them to their new environment. After three weeks the calves (mean liveweight 35.35 ± 1.40 kg) were allocated at random to two dietary treatments; FO - milk replacer plus 25 g/day fish oil ; PRO – milk replacer plus 25g/day of a binary mixture (0.5:0.5 v/v) of palm oil and rapeseed oil. Blood samples were taken at weekly intervals (week 1 – pre-treatment, weeks 2-4 during treatment) and the plasma collected for analysis. Plasma lipids were extracted by the method of Kates (1972) and the fatty acid methyl esters analysed by gas-liquid chromatography. Animals were observed to eat their bedding and at slaughter there was some evidence rumen development. Data were analysed for effects of treatment (T), period (P) and treatment/period interaction (I) by repeated measures ANOVA using the general linear model of Minitab.

Results Introduction of the oil supplements significantly affected the concentration of a number of fatty acids in the plasma. In both groups, total MUFA decreased and total PUFA increased significantly ($p<0.01$) throughout the trial. Total SFA tended decreased in PRO only, mainly due to the significant ($p<0.01$) decrease in the concentration of C16:0 in this group. The concentration of C18:1n-9 decreased significantly ($p<0.001$) and that of C18:2n-6 increased significantly ($p<0.05$) throughout the experiment in both treatment groups. The concentration of C22:6 appeared to have stabilised by week 2, whereas that of C20:5n-3 continued to increase to week 4. The concentration of both fatty acids changed significantly more in FO than RPO.

Table 1 Fatty acid composition of plasma lipids (g/kg total fatty acids)

	FO (n=4)				PRO (n=4)					Significance		
Fatty acids	Week 1	Week 2	Week 3	Week 4	Week 1	Week 2	Week 3	Week 4	SEM	T	P	I
C14:0	5.2	15.5	9.4	5.2	9.0	10.9	6.2	6.2	0.87	NS	*	NS
C16:0	146.5	144.5	137.3	147.9	152.7	121.7	118.0	109.2	3.70	**	NS	**
C18:0	148.2	126.7	119.4	134.7	140.8	125.7	137.3	132.8	3.65	NS	NS	NS
C18:1n-9	282.0	186.5	144.0	93.3	271.4	128.7	104.5	86.8	17.60	NS	***	NS
C18:2n-6	308.8	308.4	351.5	408.6	287.9	393.8	426.6	449.1	15.60	NS	*	NS
C18:3n-3	8.8	15.4	15.4	20.3	12.7	22.9	24.1	23.2	1.28	*	*	NS
C20:1n-9	1.7	2.1	2.6	0.0	1.9	2.6	3.2	0.5	0.32	NS	*	NS
C20:2n-6	4.5	6.6	5.4	0.0	4.9	10.4	10.1	4.5	0.82	*	**	NS
C20:4n-6	25.6	33.7	36.6	28.3	21.9	29.7	30.6	34.4	1.80	NS	NS	NS
C20:5n-3	2.4	39.3	79.4	86.7	4.1	3.3	7.0	10.3	6.39	***	***	***
C22:5n-3	1.2	9.2	12.1	13.0	4.4	4.0	10.5	8.5	1.07	NS	*	NS
C22:6n-3	0.7	20.4	22.4	20.4	2.7	6.0	9.1	5.6	1.78	***	***	**
ΣSFAs	302.0	295.5	267.7	292.8	308.9	262.5	262.8	252.8	6.15	NS	NS	NS
ΣMUFAs	287.7	194.1	152.8	98.4	278.4	134.2	109.5	88.5	17.8	NS	***	NS
ΣPUFAs	370.8	433.0	522.8	577.2	338.6	470.0	518.8	535.5	20.6	NS	***	NS
P/S ratio	1.3	1.5	2.0	2.0	1.1	1.8	2.0	2.2	0.08	NS	***	NS
n-6/n-3 ratio	19.6	4.2	3.1	3.2	14.2	12.7	9.3	10.7	1.03	***	***	***

NS = not significant, * = $p<0.05$; ** $p=<0.01$; *** = $p<0.001$

Conclusions Despite some evidence of rumen development, use of the oesophageal groove reflex to bypass the rumen and deliver PUFA directly to the small intestine offers some potential as seen from plasma fatty acid responses. The time course of the changes in plasma concentration varies between fatty acids.

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Influence of dietary fatty acids on the fatty acid composition of intestinal mucosa in the milk fed pre-ruminant calf.

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Introduction Dietary fatty acids have been shown to affect the activity of the immune system in a variety of species (Calder, 1998) although the exact mechanism by which they influence the nature of the immune response is unclear. The effect of dietary fatty acids on the fatty acid composition of intestinal mucosa is important since this tissue has a rapid turn over and is a major site of antigenic exposure and immune defence. The speed with which changes in dietary fatty acid intake are reflected in the fatty acid composition of cells and tissue of the body varies. In ruminants the development of a functional rumen greatly influences the nature of the fatty acids available for absorption from the small intestine, however, in pre-ruminant animals, milk may be used as a medium to supplement the diet with specific dietary fatty acids. This work was carried out to establish the extent to which different oil supplements could change the fatty acid composition of intestinal mucosa in milk fed pre-ruminant calves.

Materials and Methods 10 Jersey bull calves (mean liveweight 32.4 ± 1.38 kg, mean age 15.8 ± 3.5 days) were fed a standard milk replacer (2 x 250g/day) for a period of three weeks to aclimatise them to their new environment. After three weeks the calves (mean liveweight 35.35 ± 1.40 kg) were allocated at random to two dietary treatments; FO - milk replacer plus 25 g/day fish oil ; PRO – milk replacer plus 25g/day of a binary mixture (0.5:0.5 v/v) of palm oil and rapeseed oil. One animal in each treatment group was slaughtered before receiving the treatment diets (Pre-treatment). Calves were killed after 21 days and intestinal mucosa was collected from three sites along the small intestine (duodenum, medial ileum and terminal ileum). All animals were fasted for 24 hours prior to slaughter. Mucosa lipids were extracted using the 'Folch' procedure and the fatty acid methyl esters analysed by gas-liquid chromatography. Data were analysed by oneway ANOVA using Minitab.

Results There were no differences in fatty acid composition of mucosa from the three different intestinal sites sampled. Supplementation with PRO tended to maintain the mucosa fatty acid composition seen in calves slaughtered prior to treatment (pre-treatment). Supplementation with fish oil had no significant effects on total SFA, MUFA, PUFA or P/S ratio but had a highly significant effect on the ratio of n6/n3 PUFA. The major changes in individual PUFA were significant increases in the concentrations of C18:3n-3, C20:5n-3 and C22:6n-3 and significant decreases in the concentration of C20:2n-6 and C20:4n-6. There were no significant changes in the concentrations of C18:2n-6 and C22:5n-3.

Fatty acid composition of dietary oils and intestinal mucosa (g/kg total fatty acids)

Fatty acids	Fish Oil	Palm/Rapeseed Oil	Pre-treatment (n = 2)	FO (n = 12)	PRO (n = 12)	SED	Significance
C14:0	42.6	4.8	10.8	15.9	12.0	2.80	NS
C16:0	131.9	186.9	183.4	183.7	178.9	9.37	NS
C16:1n-7	49.0	1.7	8.9	10.5	8.0	1.53	NS
C18:0	18.3	26.8	231.3	206.0	202.7	11.42	NS
C18:1n-9	120.8	461.2	194.8	200.8	213.7	19.19	NS
C18:2n-6	14.8	148.7	130.9	135.9	139.9	14.08	NS
C18:3n-3	9.3	55.7	10.3	15.0	9.1	2.44	*
C20:1n-9	-	-	5.5	10.5	4.9	1.41	***
C20:2n-6	0.9	-	6.1	3.1	8.9	1.24	***
C20:4n-6	5.1	-	57.5	55.2	64.4	3.72	*
C20:5n-3	91.7	-	3.2	33.0	4.4	5.83	***
C22:5n-3	4.8	-	24.0	34.9	24.7	5.35	NS
C22:6n-3	128.8	-	5.9	23.3	6.9	2.56	***
ΣSFAs	193.0	219.4	426.4	409.4	395.7	11.75	NS
ΣMUFAs	169.0	462.9	209.1	221.8	226.6	20.18	NS
ΣPUFAs	255.4	204.4	237.8	300.4	258.3	20.65	NS
P/S ratio	1.3	0.9	0.6	0.7	0.7	0.06	NS
n-6/n-3 ratio	0.1	2.7	4.6	1.9	4.8	0.29	***

*= $p<0.05$; *** = $p<0.001$; NS = not significant

Conclusions. In pre-ruminant calves milk is a suitable medium for oil supplementation. FO clearly influenced the fatty acid composition of the gut mucosa within a period of 21 days compared to PRO. The decrease in the n-6/n-3 ratio may have consequences for the development and activity of the gut mucosal immune system.

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Application of a mechanistic model of methanogenesis in the lactating dairy cow. The fate of hydrogen during fermentation and strategies to mitigate methane emissions.

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Introduction Dietary intervention to reduce methane emissions from lactating dairy cattle is both environmentally and nutritionally desirable due to the importance of methane as a causative agent in global warming and as a significant loss of feed energy. This investigation involved the development of a dynamic mechanistic model of whole rumen function (Dijkstra *et al*. 1992), with the objective to simulate whole-animal methane emissions for a range of dietary inputs.

Materials and Methods The model describes the fate of excess hydrogen (H_2) produced during fermentation, from the production of lipogenic volatile fatty acids (VFA) and microbial growth on amino acids. Two moles of H_2 are produced per mol of acetate or butyrate and microbial growth with amino acids yields 0.58 mol H_2 per kg polysaccharide-free microbial growth. One mole of H_2 is utilized per mole of propionate or valerate and microbial growth with non-protein nitrogen (NPN) requires 0.41 mol H_2 per kg microbes. Two moles of H_2 are utilized per mole of unsaturated fatty acid hydrogenated in the rumen. Excess H_2 is utilized during the reduction of CO_2 to yield methane. The model also incorporates a new rumen fermentation VFA stoichiometry (Bannink *et. al.*, 2000). Hind-gut methanogenesis is described by a postruminal sub-model of fermentation in the large intestine. The postruminal sub-model is based on the description of fermentation in the rumen model. Postruminal digestive flow is described by a fractional passage rate yielding a mean retention time in the large intestine of between 9 and 13 h, depending on dry matter intake (DMI). Model evaluation was performed using a data set taken from the literature (Benchaar *et al.*, 1998), and from five calorimetry studies (67 observations) with lactating dairy cows at The University of Reading. The model was written in the Advanced Continuous Simulation Language (ACSL) and the results presented were obtained by running the model until a steady state was achieved.

Results & Discussion Regression analysis showed good agreement between observed and predicted results for data taken from the literature (r^2 0.76, root mean square prediction error (MSPE) 15.4%). Evaluation of model predictions for The University of Reading data showed an under-prediction of mean methane production of 2.1MJ/day (r^2 0.46, root MSPE 12.4%). The diets used in these studies ranged from total mixed rations based on either maize silage, grass silage or whole-crop wheat to zero grazed grass. Application of the model to develop diets to minimise methanogenesis shows a need to limit the concentration of soluble sugars in the concentrate. Complete replacement of sugars in concentrate DM (from 250 to 20 g/kg DM) led to a 3.5% increase in diet metabolisability (metabolisable energy (ME) / gross energy (GE)). On a herd basis, the model predicts that increasing dietary energy intake per cow can minimise the annual loss of feed energy through methane production. Model simulations for lactating cows fed at *ad-libitum* vs. restricted (78% of *ad-lib*) DMI showed a 0.25% decline in the GE lost as methane, equivalent to a saving of 5070 litres methane for a 305 day lactation at 244MJ/day GE intake. Table 1 shows the mean simulated fate of hydrogen within the rumen for our calorimetry trials and the estimates of Czerkawski (1986). The most notable difference between the estimates of Czerkawski (1986) and the model predictions, is the small significance of microbial growth as a hydrogen sink (0.6%). The predominance of microbial growth utilizing amino acids within the model gives rise to a net production of hydrogen for a large part of the microbial population.

Table 1 Mean simulated fate of hydrogen in the rumen for all 5 trials.

	Simulated	Czerkawski (1986)
	% (± SEM) of total hydrogen produced	
VFA	18.5 ± 1.28	33
Microbes	0.6 ± 0.04	12
Biohydrogenation	2.6 ± 0.49	1-2
Methane	78.2 ± 1.31	48

Conclusions These simulations indicate that intensive dairy farming operations designed to fill quota with the least number of cows, produce less methane per litre of milk than regimes that are more extensive. This investigation has also shown the potential for dietary intervention as a means to substantially reduce methane emissions without adverse affects to dietary energy supply.

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The Chemical composition and digestibility of wheat straw treated with urea and white rot fungi

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Introduction In Iran, wheat straw is produced in huge amount and has been used in animal feed. However, the use of straw as an animal feed is limited due to its low available energy as well as its low nitrogen content. Various chemical delignification methods to improve the digestibility of straw have extensively investigated (Sundstol and Owen, 1984). Biological methods of treating straw using fungi such as white-rot-fungi have also been reported (Zadrazil, 1984). The solid-state fermentation (SSF) of wheat straw with white-rot fungi is a complex process, which is influenced by factors such as the species of fungus, substrate, temperature and moisture (Zadrazil, 1984). Rouzbehan et al., (2000) noted that pre-treating the straw with urea and incubation with either pleurotus ostreatus or pleurotus Persian fungi has improved the digestibility of wheat straw. In this study, the effect of urea and another two species of white rot fungi on the nutritive value of wheat straw was tested.

Materials and methods Wheat straw was cut into 3-5 cm lengths and treated with 2% of urea solution (3 kg of dried urea mixed with 100 litres of water). Fifty litres of this solution was added to 100 kg of the straw and kept for three weeks. The treated straw (TS) was then steamed and inoculated with, spawn of either cross between *Pleurotus ostreatus* and Pleurotus sajor caju (TS1) or Pleurotus *of Iranian tissue* (TS2) at a rate of 3 percent (w/w). Five plastic bags, 2 kg each, have TS1 or TS2 were incubated at 25-30o C for four weeks period. All fermented samples were chemically analysed (AOAC, 1984), and the digestibility of DM and OM were measured using *in vitro* technique (Tilley and Terry, 1963). A completely randomised design was used to find out the effect of the tested species on the nutritive value of straw.

Results Table below shows the results. On average, treating wheat straw with urea and either of the two species has significantly ($P<0.05$) decreased the concentration of OM, NDF, and hemicellulose. The increase in the CP content of the treated straw was probably due to the addition of urea. Fermenting the straw with TS2 has led to improve significantly ($P<0.01$) the DM digestibily. Whereas both types of fungi have increased significantly ($P<0.01$) the OM digestibility of the treated straw.

Table 1: Chemical composition and digestibility of wheat straw treated with 3% urea after 4 weeks of solid-state fermentation with two species of fungi *(Pleurotus ostratus xPleurotus sajor caju)* (TS1) and *Pleurotus* of Iranian tissue (TS2).

Treatment	OM	CP	NDF	ADF	HC	Cellulose	Lignin	IVDMD	IVOMD
WS	94.4^{c}	1.6^{a}	75.8^{b}	56.5^{a}	19.3^{c}	43.5	13.0	25.2^{a}	26.0^{a}
UWS	91.9^{c}	2.7^{b}	75.6^{b}	56.9^{a}	18.7^{c}	45.4	11.5	24.2^{a}	27.1^{a}
TS1	89.3^{b}	2.5^{b}	68.4^{a}	50.7^{b}	14.7^{b}	42.4	11.3	28.5a	34.2^{b}
TS2	87.3^{a}	3.2^{c}	67.2^{a}	55.9^{a}	11.4^{a}	44.9	11.0	34.2^{b}	37.1^{b}
SEM	2.1	0.4	2.7	2.3	1.3	1.5	0.5	2.3	2.6
P- value	*	**	*	*	*	Ns	Ns	**	**

WS = untreated wheat straw. UWS=(wheat straw +urea). HC = Hemicellulose. IVDMD=*in vitro* dry matter digestibility. IVOMD=*in vitro* organic matter digestibility. Ns: indicate a non-significant difference.
*($P<0.05$, **($p<0.01$) a, b, c different letters in columns indicate significant differences ($p<0.05$).

Conclusion Incubating the straw with pleurotus ostreatus x Pleurotus sajor caju fungi has improved the IVDMD, however, both species have increased the OM digestibility. More research is needed to evaluate the performance of animal which fed such fungal treated wheat straw.

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The *in vitro* digestion of mature grass hay in the presence or absence of added nitrogen and sugar beet pulp by an equine faecal inoculum using the pressure transducer technique

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Introduction: Traditionally, grass hay fed to horses is low in crude protein (CP) and is unable to meet the animal's nitrogen requirements. This necessitates the provision of a supplemental source of additional nitrogen (N). Sugar beet pulp (SB), which contains *ca*. 100g CP/kg DM and has the potential to fulfil this role. Previous work has indicated that SB CP is poorly digested in the small intestine of the horse, with the majority reaching the hindgut, where it is readily degraded by the microflora (Moore-Colyer, 2000). This experiment sought to determine the effect of added SB and or additional N on the *in vitro* fermentation of hay by a faecal inoculum obtained from a pony fed commercially available chopped hay.

Materials and Methods The technique of Theodorou *et al*. (1994) was used to measure gas produced from mature perennial rye-grass hay (H) and SB when incubated with equid faeces. The feeds used were 100% hay (H), 75:25 hay:sugar beet (HSB1), 50:50 hay:sugar beet (HSB2), 25:75 hay:sugar beet (HSB3) and 100% sugar beet (SB). Six replicates of each feed were incubated without (-N) or with (+N) additional N (trypticase peptone - 0.2g N per 900ml of medium) and three replicates of each treatment were harvested at 49 hours (T1) and 135 hours (T2) post-inoculation, whereupon dry matter loss (DML) for –N and +N and T1 and T2 (DMLT) were determined. Gas production profiles were fitted to the France *et al*. (1993) model, and the fractional rates of gas production (FRGP) and time to produce 50% of the total gas (T_{50}) were calculated.

Results Table 1 shows the total amount of gas produced (A) and the DML from each of the feeds to be significantly ($P<0.05$) different, with SB>HSB3>HSB2>HSB1>H. The FRGP was significantly lower for the H feed than when SB was present. The fitted lag time (Lt) for the HSB feeds were all significantly ($P<0.05$) longer than that noted for the H. The +N or –N treatments had no significant effect on any of the calculated gas parameters for the HSB feeds, however, the + N treatment did reduce the T_{50} for H. Significantly less DMLT occurred from all but SB feed for T1 as opposed to T2.

Table 1 Total gas pool (A), lag time (Lt), rate of gas production (FRGP) and dry matter loss (DML) from five hay:sugar beet food combinations incubated *in vitro* with pony faecal inoculum in the presence or absence of added nitrogen.

	H	**HSB1**	**HSB2**	**HSB3**	**SB**	**s.e.d**
A (-N)	160.3ab	184.0b	218.4c	257.8d	279.0e	8.76
A (+N)	144.1a	171.2b	216.8c	249.5d	287.8e	
Lt (-N)	1.36a	3.80c	3.19bc	3.75c	3.72c	0.480
Lt (+N)	1.51a	3.38bc	2.60b	4.10c	3.68c	
T_{50} (-N)	35.64c	23.27a	22.26a	22.41a	23.68a	1.999
T_{50} (+N)	28.98b	21.15a	21.94a	23.52a	21.96a	
FRGP (-N)	0.0231a	0.0483b	0.0534bc	0.0549bc	0.0530bc	0.00353
FRGP (+N)	0.0294a	0.0528bc	0.0544bc	0.0552bc	0.0566c	
DML (-N)	397.5a	518.8b	619.4c	758.3d	865.8e	24.89
DML (+N)	375.0a	509.3b	608.9c	761.3d	855.2e	
DMLT (T1)	290.2a	446.1b	548.3d	717.6f	847.7h	17.74
DMLT (T2)	482.3c	582.0d	680.0e	802.0g	873.4h	

abc values in the same section not sharing common letters differ significantly.

Conclusion. The addition of N increased the speed of degradation of hay *in vitro* as did the inclusion of SB. This suggests that supplementing H with SB may be a suitable strategy to increase degradation of H by equids. Further studies are required to determine the minimum levels of SB that are required to enhance the degradation of H. The DMLT (T1) values for all feeds examined concur with published *in vivo* values.

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Fibrolytic enzymes increase the hydrolysis and rate of fermentation of pure substrates *in vitro*

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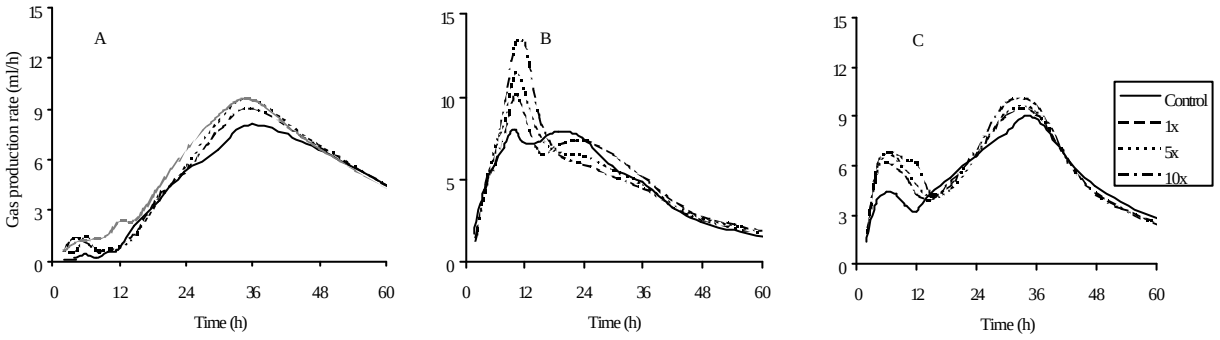
Introduction Treatment of forage with enzyme mixtures can increase rate of degradation *in vitro* (Colombatto *et al*., 2000a). However, the complexity of natural forage makes it difficult to determine what fractions are most affected by enzyme treatment. The use of pure substrates (e.g. cellulose and xylan), provides a way of evaluating enzyme mode of action. Therefore, the present study examined the effects of pre-treating avicel and xylan with an enzyme mixture (EM) on a) the reducing sugars produced during treatment before rumen fermentation and b) the gas production profiles during *in vitro* rumen fermentation using the Reading Pressure Technique (RPT) (Mauricio *et al*., 1999).

Material and Methods The EM (*Liquicell 2500*, Specialty Enzymes and Biochemicals, USA) was characterised biochemically prior to use (Colombatto *et al*., 2000b). Microcrystalline cellulose (Avicel PH-101, Fluka Chemicals), oat spelt xylan (Sigma Chemicals), and a mixture (50:50 w/w) of both were used as substrates. For the reducing sugar (RS) study, triplicate amounts (50 mg) of each substrate were weighed into plastic test tubes. The EM was added at four levels, namely 0, 0.51, 2.55 and 5.1 µl/g substrate DM (Control, 1x, 5x, and 10x, respectively), diluted in distilled water to give a final volume of 5 ml. Treatments were stored at 20°C for 20 h. The RS released were then determined using the dinitrosalicylic acid method, reading the absorbance at 540 nm. For the RPT study, 0.5 g of the same substrates was weighed in triplicate into fermentation flasks. The EM was applied in the same fashion as in the RS study, 20 h prior to inoculation with rumen fluid. Four hours later, 90 ml of anaerobic buffer was added and the flasks stored at 20°C overnight. Rumen fluid was collected pre-feeding (0700 h) from a dry cow fed grass hay (1st replication) or grass silage plus straw (2nd replication). Controls containing rumen fluid and untreated substrates, rumen fluid only, and rumen fluid plus enzyme at the three levels were also included for corrections. Gas readings were taken at 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 36, 48, 72 and 96 h post-inoculation. Rates and extent of gas production were determined. Both studies were replicated twice, and designed as 3 x 4 factorials with substrate and enzyme level as main factors. The data were analysed using the GLM procedures of SAS.

Results Adding EM increased ($P<0.05$) the release of RS from xylan and the mixture of substrates. Increasing the application level greatly increased ($P<0.0001$) the observed effect (0.25, 1.85, 3.35, and 4.24, and 0.25, 1.20, 1.85, and 2.40 mg RS for Control, 1x, 5x and 10x in xylan and the avicel-xylan mixture, respectively). The findings indicate that EM addition hydrolysed complex polysaccharides into simpler molecules. In contrast, no biological effects were detected at any level of EM inclusion with cellulose as a substrate. The RPT study revealed that addition of EM increased ($P<0.05$) the rate of gas production of the three substrates (Figure 1), indicating an increase in the fermentation rate due to enzymes addition.

Figure 1 *Rate of gas production (ml/h) of cellulose (A), xylan (B), or a mixture of both (C)*



Conclusions Addition of a fibrolytic enzyme mixture increased the release of reducing sugars and the rates of gas production in pure substrates. Further research is needed to study if the effects of enzymes are related to direct hydrolysis only or if the sugars released prompted a change in the bacterial populations.

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Rumen microbial degradation of full-fat and defatted palm oil sludge (POS) in a consecutive batch culture system

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Introduction. In Indonesia the production of POS increased rapidly as the areas of palm oil plantation increased. The POS contains high protein (15%), fibre (18%), and fat (20%) and thus likely to have the potential to provide high levels of protein and carbohydrate supplements for ruminant. However, the fast development of POS production in Indonesia is not supported by good researches to examine its nutritive value. High fat content in the diet of steers (Moore *et al*, 1986) has been found to depress the fibre digestibility; it is thought that high fat content in POS could be the limiting factor for the utilisation of the POS by ruminant. Therefore, an experiment *in vitro* was conducted to examine the degradation and fermentation of full-fat and defatted POS by rumen micro-organisms.

Materials and methods. The POS were incubated with mixed rumen microbes in a consecutive batch culture system (CBC) according to the method described by Theodorou *et al* (1987). Samples of POS (T1 to T8) taken from various palm oil plantation in South Sulawesi Indonesia were examined. Approximately 70 mg sample were weighed accurately in duplicate into test tubes. The tubes were autoclaved for 15 min at 121^{o}C. Then 8 ml of nutrient medium and 2 ml fresh rumen liquor were added to each tube containing the samples under a continuous flow of O_2-free CO_2. Two tubes were also prepared for blanks. The CBC was established by sequential transfer of inoculum (2 ml) from one set to another set at 48 h intervals over a period of 12 days. After 48 h incubation, gas production and dry matter loss were measured. The means of dry matter (DM) loss and gas production from POS samples in each replicate over 6 serial incubations were subjected to a ONEWAY analysis of variance using 'Minitab version 10.1'. The standard error of difference between means (SED) and the least significant differences (LSD) were calculated to determine the significance of differences in DM degradation between the seed samples.

Results. The results indicated that there was significant differences ($p<0.01$) in the DM losses of full-fat and defatted POS. The DM losses (g kg^{-1}) of full-fat vs defatted POS were T1 (105 vs 157), T2 (142 vs 245), T3 (97 vs 173), T4 (107 vs 166), T5 (114 vs 182), T6 (137 vs 248), T7 (113 vs 138), and T8 (105 vs 137), respectively. The defatted POS has higher ($p<0,01$, SED =16.4) DM losses than that of full-fat POS. In contrast Ismartoyo *et al* (1997) found that removal of fat from whole cottonseed (WCS) did not increased ($p>0.05$) its degradation and fermentation *in vitro*. Previous studies by the same authors indicated that gossypol (and not fat) in WCS decreased mixed rumen microbes (Ismartoyo *et al*, 1993), protozoal (Ismartoyo *et al*, 1994a), and fungi fermentation *in vitro* (Ismartoyo *et al*, 1995). Although the average (n=8) gas production from defatted POS (1.7 ml) tended to be higher compared to that of full-fat POS (1.6 ml) there were no significant differences ($p>0.05$, SED= 0.12) within and between the two samples. The POS of T2 and T6 appeared to be more degradable and readily fermentable for rumen micro-organisms compared to that of other POS samples. However, the POS are still much less degradable compared to that of oilseeds and legume seeds which found to have DM losses around 400 g kg^{-1} for oilseeds and 500 g kg^{-1} for legume seeds (Ismartoyo *et al*, 1994b).

Conclusion. The low microbial degradation of POS might be due to high content of fat, fibre, and lignin, and possibly also other factors such as antinutrient content and also physical structure of the POS. The fact that removal of fat from the POS increased their fermentation suggesting that the fat could be the main factor for the decreased in the ruminal degradation *in vitro*.

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Effect of particle size and supplemental sugar beet pulp on *in vitro* fermentation of high temperature dried alfalfa incubated with an equine faecal inoculum

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Introduction The fibrous forage high temperature dried (HT) alfalfa has been fed to horses for a number of years because of its consistently high nutritive value. It is common practise in the UK to combine HT alfalfa either chopped or in a ground and pelleted form with sugar beet pulp (SB) as this is regarded as a nutritious feed for horses. Synergistic effects of sugar beet when added to fibre-based diets have been observed in other species (Longland *et al*., 1994) whereby the digestibility of graminaceous forages has been increased. However, such effects have been little examined in horses and there is a lack of information in the literature on the effects of SB on the digestibility of leguminous forages. Thus, the effect of sugar beet on the *in vitro* fermentation of ground and chopped HT alfalfa by an equid hind-gut microflora using the pressure transducer technique of Theodorou *et al*. (1994) was investigated.

Materials and Methods Three replicate samples of HT alfalfa which had either been chopped (A) or ground (G) (to pass through a 1 mm dry mesh screen) were each combined in 125 ml serum bottles with ground molassed sugar beet pulp (SB), in the following ratios (alfalfa either A or G**:**SB); 100:0, 80:20, 60:40, 40:60, 20:80 and 0:100 to give a total sample mass of 1 g per bottle (36 bottles). An equine faecal inoculum was prepared from freshly voided faeces from a horse maintained on a basal diet of hay, SB and A. The above feedstuff combinations were fermented with the faecal inoculum in batch culture. Cumulative gas production was measured according to the method of Theodorou *et al*. (1994), with readings taken at 2, 4, 6, 8, 11, 14, 17, 20, 26, 35, 47, 55, 97 and 120 h post-inoculation. At the end of the incubation period *in vitro* dry matter loss (DML) was determined by lyophilization of the fermentation residues.

Results There was no significant difference between the chopped or ground alfalfa for either total gas production or DML at any of the levels of SB inclusion (Table 1). Total gas production (TG) for G80, G60, G40 and G20 were above those expected if a purely additive effect were to exist through sugar beet supplementation, with increases above predicted values of 5% ($P<0.05$), 8% ($P<0.05$), 10% ($P<0.01$) and 5% ($P<0.05$) respectively. However, this effect was not apparent for the chopped alfalfa where TG was proportional to the relative amounts of sugar beet and alfalfa in the sample. Neither was there an increase in DML above that expected for either G or A at any level of SB inclusion. Furthermore, there were no significant differences in TG and DML between the chopped and ground alfalfa feedstuffs at each corresponding level of SB inclusion.

Table 1: Total gas production and dry matter loss for G and A supplemented with SB

Ratio of HT alfalfa:SB		Total gas production (ml)				Dry matter loss (g kg^{-1} DM)			
HT alfalfa	SB	G	A	Sig.	s.e.d.	G	A	Sig.	s.e.d
100	0	157.06	192.31	NS	6.13	618.3	674.1	NS	1.34
80	20	196.90	204.37	NS	7.16	668.9	700.4	NS	1.10
60	40	228.67	225.60	NS	5.48	727.6	736.9	NS	0.78
40	60	259.01	233.30	NS	4.31	778.0	782.2	NS	1.90
20	80	281.37	275.61	NS	29.46	807.6	793.5	NS	5.63
0	100	304.82	290.24	NS	9.33	876.3	832.0	NS	5.64

Conclusion Particle size of the alfalfa had no effect on either TG production or the extent of DML. Of the parameters presented SB inclusion appeared to enhance TG from ground but not chopped alfalfa. It is clear that both HT alfalfa and SB are highly digestible fibrous feedstuffs and may well be effective partial replacements for hay in horse rations which typically has a digestibility in equids of between 0.3 to 0.4.

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Organic matter degradation of concentrate ingredients determined with the nylon bag and gas production techniques

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Introduction The nylon bag technique is the standard technique used in many feed evaluation systems for ruminants. As the rate and extent of fermentation can also be determined with the gas production technique, this technique offers a potentially good alternative. Cone *et al*. (1998) showed that there was a good relationship between rate of degradation determined with the nylon bag technique and rate of gas production for organic matter and NDF in grass and grass silage. The aim of this study was to determine the possibilities for estimating nylon bag characteristics and calculation of the amount of fermentable organic matter (FOM) of concentrate ingredients with gas production parameters.

Materials and methods Twenty-one concentrate ingredients were investigated, including the formaldehyde treated samples, lupine, solvent extracted (se) rapeseed and soybean. Samples were dried at 70 °C and ground to pass a 1 mm screen. All samples were analysed in duplicate for ash, crude protein (CP), crude fibre (CF), crude fat (Cfat) and sugar. In situ degradation characteristics were determined (Ørskov and McDonald, 1970) in triplicate in 3 dairy cows and residues were fitted to a first-order degradation model to calculate rate of degradation (kd). FOM was calculated using nylon bag parameters, assuming a rumen passage rate of 6 % h^{-1}. Gas production analysis was performed in duplicate in a single run using buffered rumen fluid (32 % rumen fluid) obtained from two non-lactating canulated cows, 2 h after the morning feeding. The cows received 1 kg standard (low in protein) compound feed and ad libitum hay. Gas production profiles were fitted with the model described by Groot *et al*. (1996), used as a mono-phasic and a three-phasic model. Linear regression analysis was done for the nylon bag parameters. Chemical and gas production variables with $p < 0.05$ were added to the regression models.

Results For the 21 samples the washout fraction (W) ranged from 0.5 (± 0.5) % for soybean hulls to 45.8 (± 0.5) % for pea meal. The undegradable fraction (U) ranged from 0.5 (± 0.1) % for maize gluten meal to 35.4 (± 1.9) % for se sunflower meal. Kd ranged from 1.7 (± 0.2) % h^{-1} for protected se soybean meal to 10.5 (± 1.5) % h^{-1} for potato pulp. The FOM calculations ranged from 269 g kg^{-1} for maize gluten meal to 748 g kg^{-1} for citrus pulp. Figure 1 shows the relationship of kd and Bm (time to reach 50 % of maximum gas production using a mono-phasic model) and B2 (time to reach 50 % of maximum gas production of subcurve 2 using a three-phasic model). There was a rather poor relationship of kd with both Bm and B2, as shown by table 1. Using multiple regression analysis D, kd and FOM could be estimated from chemical contents and gas production parameters with a minimum R^2 of 0.70 or more. Degradation rate (kd) was calculated with an exponential model, while the rate of gas production (Bm and B2) was fitted by a sigmoidal model. Use of the natural logarithm of kd improved the relationship with Bm ($R^2 = 0.53$) and B2 ($R^2 = 0.68$).

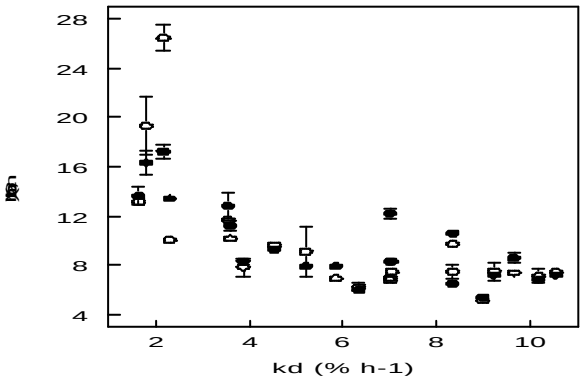

Figure 1 *Relationship between in situ kd and time needed for 50 % of maximum gas production (Bm, O), calculated with a mono-phasic model and time for 50 % of gas production of the non-soluble components (B2, ●), fitted with a three-phasic model.*

Table 1 *Relationships between nylon bag parameters and chemical and gas production characteristics.*

Predicted	variable	R^2	P	RSD
W	sugar, C2	0.50	0.01	9.0
D	ash, C2, GP72	0.72	0.01	7.4
U	CF, GP72	0.57	0.00	6.3
	CF, Cfat, Am	0.66	0.04	5.8
Kd	Bm	0.43	0.00	2.3
	B2	0.58	0.00	1.9
	B2, C1	0.70	0.02	1.7
FOM	Bm	0.50	0.00	10.7
	B2	0.60	0.00	9.6
	CF, Bm	0.64	0.04	5.8
	A1, B2	0.73	0.01	8.1

Conclusions It can be concluded that there was a poor to moderate relationship for organic matter degradability in concentrate ingredients determined with the nylon bag technique and the gas production technique.

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Differentiation of energy supplements using *in vitro* fermentation rates generated with the Reading Pressure Technique

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Introduction The *in sacco* technique has been successfully utilised to differentiate feedstuffs in terms of their rate and extent of degradation. However, the large initial particle losses associated with the requirement to finely divide the substrates prior to incubation and the inability of the technique to examine liquid feed supplements has led to alternative methodologies being adopted. This study examined the ability of the Reading Pressure Technique (RPT) to differentiate a number of minimally processed energy supplements, in terms of their rate of degradation.

Materials and methods Two sources of liquid molasses (cane *CM* and beet *BM*), three cereals (maize *M*, barley *B* and wheat *W*) plus two pelleted sugar beet pulp products (unmolassed, *PL* and molassed *PR*) were examined using the *in vitro* technique of Mauricio *et al*. (1999). The non-liquid feeds were prepared by passing them through a Retsch rotor mill fitted with a 5 x 5 mm screen, so that their resulting physical form resembled that found in the rumen. Fermentation gas release (both cumulative and rate) and organic matter degradation (OMD) profiles were determined over the 96 h incubation period, with approximately 1.0 g substrate added per flask and three replicates together with controls used for each of the seven withdrawal intervals. Total and soluble losses were estimated by soaking 1.0 g samples in 100 ml distilled water for 6 hours at 39 °C, followed by filtration through porosity 1 and 3 sintered glass crucibles, respectively. Particle losses were calculated by difference. As only gas release profiles of *CM* and *BM* could be estimated, four samples of each molasses were examined in triplicate, with approximately 1.3 g added per flask. SAS GLM procedures were utilised to generate LS means and significances of difference between substrates.

Results In general soluble losses were less than 120 g / kg DM with the exception of *PR* (405 g / kg) reflecting the inclusion of molasses, while only *B* and *W* exhibited any particle losses, 80 and 170 g / kg, respectively. Similar rates of fermentation were observed for the two molasses products up to 12 hours post-inoculation. Thereafter that derived from sugar beet produced significantly more gas per g DM incubated than the cane product. These two products produced considerably more gas in the first six hours than any of the other feeds with the exception of *PR* (molassed sugar beet). Significantly different gas and OMD profiles were identified from 6 h post-inoculation for all non-liquid feeds. Lowest rate of gas released and OMD was associated with *M* while *W* had very similar initial OMD and gas production levels to *PR*, suggesting that the large soluble and particle losses identified were also highly degradable. In comparison to the other two cereals barley was ranked *W* >*B* >*M* in terms of both gas release and OMD. A significant difference was also readily detected between the two sugar beet products, with the rate of gas release profile of *PR* exhibiting a double peak at 4 and 12 h post-inoculation in contrast to the single peak (12 h) of the unmolassed product. In all cases gas and OMD were highly significantly correlated, with the fermentation efficiency (OMD, g / gas, ml) of M slightly, although significantly ($P<0.05$), higher than that of the other feeds.

Table 1 *Cumulative gas release and organic matter degradation (OMD) - solid feeds*

	Gas production (ml / g OM)						OMD (g / kg)					
Feed	6 h	12 h	24 h	36 h	48 h	96 h	6 h	12 h	24 h	36 h	48 h	96 h
B	26d[†]	134b	241b	2798b	292b	305ab	276b	533b	762b	854b	884c	911b
M	8e	35d	135c	198c	231c	280c	64d	164d	475c	671c	772d	939ab
W	37b	170a	267a	295a	311a	326ab	408a	620a	847a	881ab	929ab	951ab
PL	30c	105c	235b	276b	298ab	317ab	134c	363c	782b	888ab	888bc	916ab
PR	60a	137b	244b	284b	305ab	332a	387a	592a	844a	913a	939a	958a
s.e.	1.0	2.7	2.9	3.3	4.3	7.2	1.0	2.7	2.9	3.3	4.3	7.2

[†] LS means in columns without similar letters are significantly different ($P<0.05$)

Table 2 *Cumulative gas release – molasses*

Feed	Cumulative gas release (ml / g DM)					
	6 h	12 h	24 h	36 h	48 h	96 h
BM	78a	147a	204a	226a	236a	254a
CM	79a	143a	196b	215b	222b	231b
s.e.	1.4	1.8	2.5	2.8	2.9	3.1
P	n.s.	n.s.	0.05	0.05	0.01	0.001

Conclusions The significant differences observed between *BM* and *CM* clearly indicate that the source of molasses has a considerable effect on product behaviour in the rumen. Equally the significant differentiation between feeds, in terms of OMD and gas release, provides data to allow these feeds to be combined more accurately with respect to synchronised energy release in the rumen. Further studies are to be conducted to investigate the influence of molasses source (cane versus beet) on fermentation efficiency and rumen microbial protein production.

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Towards a continuous culture (Rusitec) model of rumen acidosis: effects of buffer concentration, non-protein nitrogen and concentrate level on pH and feed degradation

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Introduction Rumen acidosis is a problem in many production systems where readily fermentable concentrates are fed. Although acidosis is more common as a subclinical condition that can impair fibre digestion, numerous factors can precipitate the clinical disorder. The objective of the present study was to begin development of a model of acidosis using a rumen simulation technique (Rusitec) and continuous pH monitoring for subsequent evaluation of contributing factors. In the present report the effect of reduced buffer concentration, non-protein nitrogen (NPN) addition and method of NPN provision on pH and feed dry matter disappearance were examined.

Materials and methods Eight Rusitec vessels were inoculated with rumen fluid from a lactating cow and maintained by continuous buffer (McDougall's artificial saliva) infusion and daily addition of 15 g of a mixture of maize silage and wheat (70:30, DM basis) containing 100 g crude protein (CP)/kg DM and assigned to 1 of 4 treatments. Treatments were: control (T1); buffer at 80% concentration (T2); 80% buffer plus NPN (urea to increase feed CP content to 160 g/kg DM plus sodium sulphate) given with feed (T3); and 80% buffer plus NPN continuously infused with buffer (T4). The proportion of wheat added was then increased over three 10-12 d periods (300, 350 and 400 g/kg DM in periods 1, 2 and 3, respectively). Washed grass hay (0.5 g) was incubated in separate nylon bags to determine 48 h DM disappearance (DMD). Measurements of pH before servicing, gas volume and feed DMD were made after 5 days adaptation to each level of wheat. In addition, pH was continuously measured in one vessel for each treatment. Average measurements for each period were analysed as repeated measures and tested for effects of period (wheat level), treatment and treatment by period interaction using mixed models procedures of SAS. Orthogonal contrasts were used to test for linear and quadratic effects of wheat level and separate treatment means. Continuous pH recordings are presented to show patterns of diurnal variation.

Results Increasing wheat level decreased pH, increased feed DMD and decreased hay DMD. In each case the effect was linear ($P < 0.02$), but there was no overall effect of wheat level on gas volume. Reducing buffer concentration decreased pH except when urea was infused continuously (Table 1). While treatment had no effect on feed DMD, hay DMD was greater when NPN was added than for T2 ($P < 0.02$). Overall, gas volume was higher for 100% buffer infusion ($P < 0.01$) and when urea was provided with feed compared to infused ($P < 0.01$), but the effect of buffer concentration on gas volume was not significant at 350 g wheat/kg DM. Gas volumes (ml/d) for T1-T4 were 1076, 733, 1121 and 763 for 300 g wheat/kg DM, 1033, 947, 1130 and 811 for 350 g wheat/kg DM and 1278, 955, 825 and 835 for 400 g wheat/kg DM. The introduction of buffer used to rinse residual feed and the addition of urea with feed increased pH after servicing (Figure 1) and delayed post 'feeding' declines in pH.

Table 1 *Effects of level of wheat and treatments on pH, gas volume and feed and hay DM disappearance (DMD).*

	Wheat, g/kg				Treatment					Probability		
	300	350	400	se	T1	T2	T3	T4	se	Diet (D)	Treatment (T)	D x T
pH	6.25	6.16	6.11	0.03	6.31	6.06	6.08	6.25	0.05	0.02	0.06	0.36
Feed DMD, g/kg	618	631	637	9	642	610	642	621	17	0.01	0.52	0.39
Hay DMD, g/kg	714	686	661	15	708	638	705	697	12	0.06	0.05	0.17
Gas, ml/d	923	980	973	33	1129	878	1025	803	32	0.47	0.01	0.03

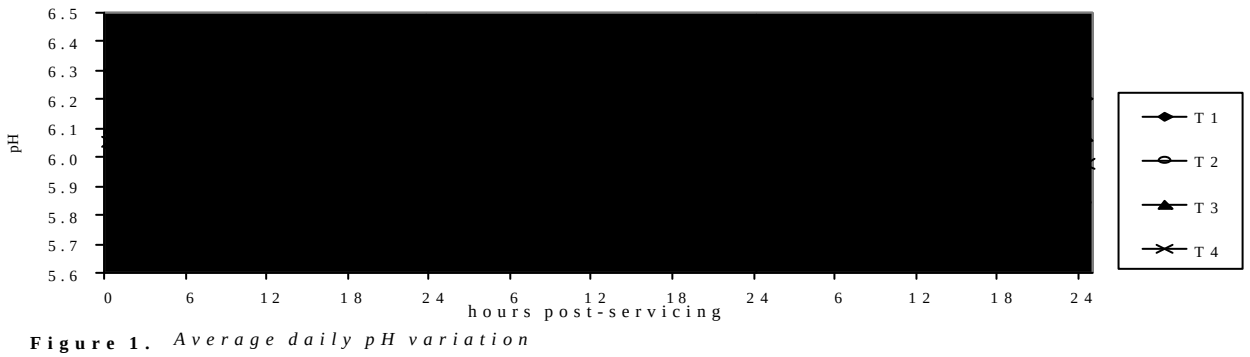

Figure 1. *Average daily pH variation*

Conclusions The effect of urea on pH (Table 1 and Figure 1) may be due to a buffering effect of ammonia. Providing urea by infusion compared to feeding urea increased pH and decreased gas volume without affecting DMD, suggesting greater microbial growth. Reducing buffer concentration had a greater effect on pH than increasing wheat level. With appropriate changes in servicing routine the present model using reduced buffer concentration will allow further evaluation of effects of factors such as NPN addition on interactions between acid load and fibre digestion.

Influence of peptides and amino acids on ammonia assimilation by cellulolytic ruminal bacteria

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Introduction An understanding of the nitrogen compounds required for growth of ruminal bacteria is of importance to optimising ruminal fermentation and in formulating optimal protein requirements of ruminants. Bryant (1973) concluded that cellulolytic ruminal bacteria used only NH_3 as N source for growth. The main species were unable to grow on other nitrogen sources in the absence of NH_3, and the incorporation of radioactive pre-formed amino acids appeared to be minimal. Disappearance of NH_3-N from the growth medium was equal to N incorporation into bacterial protein. As a consequence, the assumption was made in drawing up the Cornell Net Carbohydrate and Protein System (CNCPS) that structural carbohydrate-fermenting bacteria use ammonia as their only source of nitrogen (Russell *et al*., 1992). More recent studies with mixed cultures suggest, however, that fibrolytic bacteria incorporate significant quantities of amino acids (Griswold et al., 1996; Carro and Miller, 1999). The present experiments were undertaken to clarify the N sources for growth of the three main species of cellulolytic ruminal bacteria in pure culture.

Materials and methods Bacteria were maintained on the liquid form of ruminal fluid-containing medium M2. ^{15}N uptake experiments were carried out using basal Hungate and Stack defined medium with 0.6% (w/v) cellobiose in which 40% of the NH_4Cl in the minerals solution was replaced by $^{15}NH_4Cl$ (Sigma; 98% ^{15}N). Various concentrations of pancreatic casein hydrolysate (Trypticase™; Becton Dickinson Microbiology Systems, Cockeysville, MD21030, USA), which contains mainly peptides with a little free amino acids, were added to the medium. Cultures were grown at 39 °C for 24 h, then were centrifuged, pellets were washed once with ice-cold water, and the cells were freeze-dried. ^{15}N enrichment was measured by isotope ratio mass spectrometry (IRMS) and total cell N was measured by a Kjeldahl procedure. ^{15}N enrichment in amino acids was determined by GC/MS of derivatized amino acids. Results are means derived from the analysis of triplicate cultures. The data were compared by analysis of variance. All analysis was carried out using the GENSTAT 5 statistical program.

Results Adding Trypticase to the medium enabled *F. succinogenes* to grow and increased growth of two *Ruminococcus* spp. (Table 1). At 10 g/l Trypticase, substantial amounts of cell N were formed from Trypticase (Table 1), indicating that cellulolytic ruminal bacteria are capable of substantial incorporation of amino acids. At lower concentrations (1 g/l), more typical of ruminal peptide and amino acid concentrations, cell-N derived from $^{15}NH_3$ varied from 87 to 93%. Phenylalanine appeared to be a key amino acid: no *de novo* synthesis occurred in *F. succinogenes*, while in the *Ruminococcus* spp. Phe incorporation was proportionally greater than that of total cell N (Table 1) and of other amino acids (not shown).

Table 1 ^{15}N incorporation and microbial protein synthesis in cellulolytic ruminal bacteria

Species	Bacterial N formed, mg/l				Ppn of microbial N from NH_3				Ppn of microbial Phe from NH_3			
Trypticase, g/l…	0	1	10	SED	0	1	10	SED	0	1	10	SED
Fibrobacter succinogenes BL2	NG	25	83	6**	NG	0.87	0.57	0.02**	NG	0.02	0.02	0.01
Ruminococcus albus SY3	63	76	122	3**	1.09	0.93	0.75	0.03**	0.74	0.65	0.56	0.04*
Ruminococcus flavefaciens 17	21	32	78	2**	1.01	0.93	0.72	0.03**	0.96	0.77	0.59	0.06*

NG – No growth
$^{**}P < 0.001$, $^{*}P < 0.05$

Conclusions These results confirm that peptides and amino acids are important nutrients for optimal growth of cellulolytic ruminal bacteria. They indicate further that phenylalanine is a key amino acid limiting growth of these species.

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Comparison of sheep rumen liquor and Rusitec fluid as inoculum for determining the *in vitro* digestibility of hays

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Introduction One practical disadvantage of some *in vitro* methods used to estimate the *in vivo* digestibility of forages is the need for fistulated donor ruminants to provide the rumen liquor. These are subject to restrictive legislation in many countries and are costly to prepare and maintain. The aim of this study was to investigate whether rumen liquor in the *in vitro* digestibility technique of Van Soest *et al*. (1966) could be replaced by microorganisms derived from Rusitec. This technique involves the incubation of samples with buffered rumen fluid for 48 h followed by an extraction with a neutral-detergent solution.

Material and methods Four rumen-fistulated sheep and a four-vessel Rusitec system were used as a source of microorganisms to determine the *in vitro* digestibility (Van Soest *et al*., 1966) of twelve hays. The *in vivo* dry-matter digestibility (DMD) of the hays had been determined previously and ranged from 503 to 678 g/kg. Each sheep received 1 kg of 80:20 alfalfa hay:concentrate per day offered at 9.00 and 21.00 h for 15 days before starting the *in vitro* digestibility assays. The Rusitec system was inoculated with rumen contents from the same sheep, and each vessel was fed daily 16 g of the same diet for 12 days before doing the *in vitro* digestibility assays. Rumen fluid (from sheep and Rusitec vessels) was obtained 2 hours after morning feeding, filtered through four layers of cheese-cloth and mixed with the buffer solution of Goering and Van Soest (1970) in a proportion 1:4 (v:v). The ANKOM *in vitro* fermentation system Daisy II was used for the incubations. This system consists of a unit containing four glass flasks of 5 L of volume. Samples (500 mg) of each hay (ground to pass a 1.0 mm screen) were weighed in artificial fibre bags (50 x 40 mm; 25 ± 10 µm pore size), which were heat-sealed and placed in the flasks. Each flask was then filled with the buffered rumen fluid of one sheep or one Rusitec vessel. One bag of each hay was included in each flask, so that 4 values of *in vitro* digestibility were available for each feed sample. Duplicates of a reference hay of known *in vitro* digestibility were also included in each flask to detect possible irregularities during the fermentation process. All procedures were conducted under CO_2 with the solutions maintained at 39ºC. Following 48 h of incubation, the bags were boiled in a neutral-detergent solution for 1 h using an ANKOM220 Fibre Analyser unit. Finally, the bags were washed with distilled water and dried at 60ºC for 48 h to determine the *in vitro* DM digestibility. Two incubations were carried out, one with sheep rumen liquor and one with Rusitec fluid. Relationships between *in vivo* digestibility and data obtained with rumen liquor and Rusitec fluid were tested by linear regression procedures.

Results Rusitec fluid as a source of microorganisms resulted in significantly lower (P<0.01) DM digestibility values than sheep rumen liquor for all hays, with differences ranging from 52 to 162 g/kg. However, *in vitro* DMD (g/kg) using sheep rumen liquor (*y*) was highly (R^2 = 0.858; P<0.001) related to digestibility using Rusitec fluid (*x*) by the following linear equation: *y* = 127 + 0.954 (s.e. 0.1229) *x*; RSD = 32.5. There were significant relationships (P<0.001) between the *in vivo* DMD of hays and the *in vitro* DMD determined with both types of inoculum (Figure 1). Judged by the size of the RSD (20.6 and 21.9 for rumen liquor and Rusitec fluid, respectively) and the coefficient of determination (0.899 and 0.896), the precision of the estimates of *in vivo* DMD were similar for both types of inoculum.

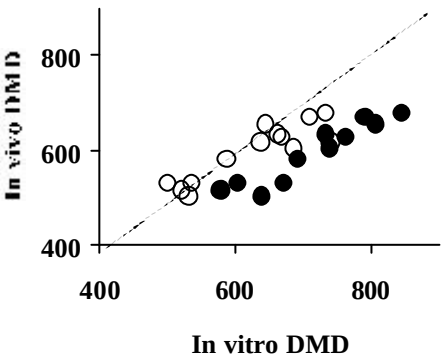

Figure 1 Relationship between the *in vivo* DM digestibility (DMD; g/kg) of twelve hays and their *in vitro* DM digestibility (IVDMD; g/kg) determined using rumen liquor (●) or Rusitec fluid (❍) as inoculum. Regresion equations were: DMD = 83.2 + 0.713 (s.e. 0.0756) $IVDMD_{Rumen\ liquor}$ (R^2 = 0.899; P<0.001; RSD = 20.6) and DMD = 144 + 0.729 (s.e. 0.0829) $IVDMD_{Rusitec\ fluid}$ (R^2 = 0.886; P<0.001; RSD = 21.9).

Conclusions In spite of the scarce number of samples, the results of this study suggest that vessel fluid from Rusitec could be used as a source of microorganisms for determining the *in vitro* digestibility of hays. Use of Rusitec would obviate the need to use fistulated animals, as rumen liquor to commence Rusitec could be obtained at abattoirs.

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Non-additivity of feedstuffs examined *in vitro* and the influence of incubation medium pH

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Introduction Non-additivity occurs when the nutritive value of a mixture of feedstuffs differs from that of the sum of its components. It is most commonly observed when one dietary constituent influences, either positively or negatively, the apparent digestibility of another under conditions where components such as nitrogen and sulphur are non-limiting. In general negative effects occur due to the depression of rumen pH or substrate competition, while positive effects have been identified when readily fermentable fibre sources such as sugar beet pulp have been included in rations containing poorly fermented forages such as cereal straw. With the increasing use of *in vitro* systems, not just to examine feed degradation characteristics but to derive parameters such as microbial protein yield, the following study was conducted to determine whether such interactions could be identified *in vitro*.

Materials and methods The fermentation characteristics of three feeds - grass hay (*H*), wheat (*W*) and molassed sugar-beet pulp (*SBP*) - were examined alone and with either *W* or *SBP* in combination with *H* (65:35 DM basis). The influence of incubation medium was assessed by the inclusion of citric acid (Mould *et al*., 2000) to provide initial pH values of 6.74, 6.56, 6.31 and 5.86. A 5 x 4 factorial design was used with three replicates, plus appropriate controls, for each treatment combination at each of the five fermentation periods. The Reading Pressure Technique (Mauricio *et al*., 1999) was applied to obtain gas production yields and to assess organic matter degradation (OMD) dynamics over the 96 h incubation period. Rumen fluid inoculum was obtained from a dry cow offered grass hay *ad libitum* plus 1.0 kg concentrate daily. Incubation pH of each flask was measured at the termination of fermentation. Non-additive effects were identified by comparing observed (*O*) values with those calculated (*C*) by proportional summation of values from the substrates examined alone. An estimate of fermentation efficiency (FE) was obtained by relating OMD to gas release at 96 h. SAS procedures were utilised to generate LS means and identify significant difference both between treatments and observed and calculated values. Only feed combination data are presented in this summary.

Results Decreasing the fermentation medium pH depressed OMD and the quantity of gas released, with the magnitude varying inversely with the pH and was greatest with mean pH values below 6.0. Observed and calculated cumulative gas values for *W-H* combinations were similar at 24, 48 and 96 h post-inoculation. However significant negative associative effects were identified at nearly all incubation interval x pH levels with OMD values depressed below those calculated by as much as 67 g/kg. In contrast while no differences were observed at the highest incubation pH levels there was an increasing tendency, as pH decreased, for the *SBP-H* combinations to produce significantly more gas than calculated. These positive associative effects were also identified with OMD and especially at shorter time intervals and lower pH levels (e.g. at 24 h and pH 5.65 calculated and observed OMD values were 355 and 463 g/kg respectively). The strong inverse relationship identified between FE and incubation pH may result from a shift in VFA production towards C_3 from C_2 and C_4 due to the adverse conditions existing for fibre degradation.

Table 1 *Non-additivity of feed combinations: cumulative gas production, OMD and fermentation efficiency (EF)*

Feed	Mean pH[†]	Gas production (ml / g OM)						OMD (g / kg)						FE
		24 h		48 h		96 h		24 h		48 h		96 h		96 h
		O	*C*	*O*	*C*	*O*	*C*	*O*	*C*	*O*	*C*	*O*	*C*	
W-H	6.57	223^{a}	218	254^{a}	250	259^{a}	259	798^{a§}	825§	845^{a}	853	880^{a}	881	3.34
	6.34	205^{a}	209	250^{a}	244	248^{a}	257	797a	808	848^{a}	851	875^{ab‡}	886‡	3.47
	5.87	166^{b}	161	182^{b}	191	208^{b}	204	704^{b‡}	756‡	803^{b¶}	837¶	860^{b}	849	4.23
	5.61	132^{c}	125	137^{c}	139	125^{c§}	155§	628^{c}	642	720^{c¶}	752¶	724^{c¶}	791¶	5.40
SBP-H	6.62	211^{a‡}	201‡	258^{a}	246	249^{a}	259	788^{a§}	812§	838^{b}	841	882^{a§}	868§	3.49
	6.40	203^{a‡}	185‡	237^{a}	234	259^{a}	254	806^{a§}	771§	867^{a¶}	850¶	876^{ab}	876	3.45
	5.95	170^{b‡}	142‡	211^{b}	192	239^{b‡}	212‡	679^{b¶}	599¶	831^{b‡}	817‡	865^{a¶}	838¶	3.77
	5.65	115^{c‡}	88‡	144^{c‡}	115‡	167^{c‡}	144‡	463^{c¶}	355¶	676^{c¶}	578¶	728^{c¶}	668¶	4.41

[†] Mean of pH estimates taken over entire 96 h incubation period.
‡§¶ Contrasting O / C values with these symbols are significantly different at *P<0.05, P<0.01* and *P<0.001*, respectively.
Means in columns within feeds without similar letters are significantly different (*P<0.05*)

Conclusions Positive and negative non-additive effects were readily identified, with their magnitude varying both with incubation interval and fermentation medium pH. The results suggest that the use of fermentation parameters such as gas production or OMD derived from single feeds to generate similar estimates, microbial protein yield or ATP production for feed combinations is incorrect.

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Relationship between the production of short chain fatty acids and gas when proteins are incubated *in vitro*

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Introduction The *in vitro* gas production technique is a means of measuring the dynamics of fermentation. If Wolin (1960) stoichiometry is assumed, and the amount of short chain fatty acids (SCFA) produced during an incubation are measured, the volume of gas produced can be predicted (Blümmel *et al*., 1997). When carbohydrate rich feeds are incubated, observed and predicted gas volumes are well related (Getachew *et al*., 1998). Blümmel *et al*. (1999) also observed a good relationship between observed and predicted gas volumes across a wide range of feeds. However, it was suggested by Cone and van Gelder (1999) that when proteins were incubated *in vitro*, the relationship was poor, which would suggest that the fermentation of proteins *in vitro* does not follow Wolin (1960) stoichiometry. The objective of this study was to investigate the relationship between observed and predicted gas volumes when protein rich feeds were incubated *in vitro*.

Materials and methods Air dried, ground (1 mm screen) samples of 15 protein-rich feeds were incubated in triplicate for 8 or 48 h with buffered rumen fluid. Feeds included sunflower seed meal, maize gluten, extracted cottonseed, distillers' dark grains, soyabean meal, rapeseed meal, palm kernel meal, extracted linseed meal and maize distillers' grains. The amount of SCFA produced at the end of the incubations was measured, and expressed in terms of mmol/g DM. The cumulative gas volume was also measured, using the apparatus described by Cone *et al*. (1996). This was expressed in terms of ml/g DM. For each sample, predicted gas volume was calculated using the equations described by Blümmel *et al*. (1997). Predicted gas volumes (PGV) at 8 and 48 h were then compared with observed gas volumes (OGV) by regression analyses.

Results At 8 h, a good relationship was observed between predicted and observed gas volumes, but by 48 h this relationship was lost. The details of the regression analyses were:
At 8 h, R^2=0.701, s=11.8, P<0.001; OGV = 16.1($\pm$12.11,ns)+ 0.820($\pm$0.1488, P<0.001)xPGV
At 48 h, R^2=0.166, s=33.9, ns; OGV = 126($\pm$40.2, P<0.01) + 0.363($\pm$0.2255, ns)x PGV

Conclusions When protein concentrates are incubated *in vitro*, their initial fermentation follows Wolin (1960) stoichiometry. However, at later stages of the incubation, the fermentation does not follow this stoichiometry. This may be because, initially, it is the carbohydrate fraction of the feed that is being fermented, and that when the protein is fermented, it does not follow Wolin (1960) stoichiometry. However, it may also be because, with a more limited supply of fermentable (although not necessarily degradable) material, microbial recycling begins sooner with protein concentrates. When using the gas production technique to estimate the dynamics of protein fermentation, therefore, 48 h may be too long an incubation period.

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The effect of sodium supplementation of pregnant cows on the preference of their calves for concentrate with added sodium

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Introduction The sodium appetite of cattle can be increased by feeding supplementary sodium in the first six weeks of life (Phillips *et al*., 1999). It has also been observed that the offspring of rats given supplementary sodium during pregnancy have an enhanced sodium appetite (Contreras and Kosten (1983), which may be due placental transmission of aldosterone and angiotensin, the regulators of sodium appetite. An experiment was conducted to determine whether the sodium intake of pregnant cows affected the sodium appetite of their calves.

Materials and methods Twenty-two Friesian cows were offered a complete diet (680g silage, 220g distillers' grains, 70g molasses, 20g barley and 3g minerals/kg DM) *ad libitum* during pregnancy. For the final two months they were allocated by predicted calving date to either treatment A, with supplementary sodium, or treatment B, no sodium. Sodium chloride (70 g/cow/d) was mixed daily with the diet of cows in treatment A. Following weaning at 2 days of age, the calves from these cows were offered the choice of concentrate with or without 5 g Na/kg, added as NaCl, during the first six weeks of life, together with 2 l/d of whole milk. The intake of the two concentrates was determined daily and the results analysed by a linear model that included cow treatment and calf concentrate as factors and calf live weight as covariate.

Results Cows in treatment A had increased plasma sodium concentration before calving (Table 1). The calves born to treatment A cows (A calves) were heavier than those born to treatment B cows (B calves). The concentrate intake of B calves was greater than A calves and they tended to gain weight faster. There was no difference in final weight of A and B calves. B calves consumed more of the concentrate without Na (B calves 507 g/d, A calves 407 g/d, SED 47.8, P = 0.05) and tended to consume less of the concentrate with Na (B calves 255 g/d, A calves 315 g/d, SED 30.0, P = 0.06). The total sodium intake and sodium concentration in the faeces were greater for B calves, probably as a result of their increased concentrate intake.

Table 1 The weight gain, intakes and sodium concentrations in cows and their calves in treatment A (supplementary sodium given to cows during pregnancy) and treatment B (no supplementary sodium).

	Treatment			
	A	B	SED	Probability
Cows				
Plasma Na (meq/l)	106	93	6.7	0.05
Calves				
Initial weight (kg)	44.9	40.6	2.06	0.05
Weight gain (kg/d)	0.57	0.65	0.040	0.06
Final weight (kg)	69.6	67.0	3.33	0.44
Concentrate intake (g/d)	342	392	22.0	0.03
Na intake (g/d)	38.6	43.6	2.42	0.04
Faecal Na output (mg/kg DM)	2.3	3.0	0.30	0.03

Discussion and Conclusion

Adding salt to herbage increases the intake of cattle (Chiy *et al*., 1993), and it seems possible that this led to the increased initial weight of A calves. The increase in plasma sodium of the cows with sodium supplementation suggests that the cows in treatment B were deficient in sodium. The selection by B calves of more concentrate without sodium and less with sodium may have been due to the pre-natal influence of angiotensin or aldosterone. It could also have been due to an attempt by the A calves to increase their sodium intake to requirements, but as the sodium content of the unsupplemented concentrate was already well in excess of calf requirements, this seems unlikely. We conclude that the sodium appetite of calves can be conditioned by the sodium intake of their dams during pregnancy.

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Plasma inorganic iodine values in beef cows following rumen bolus or dietary mineral supplementation

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Introduction Plasma inorganic iodine concentrations (PII, ng/ml) are increasingly used in preference to plasma thyroxine concentrations (T_4) for diagnostic purposes. PII represents current dietary iodine intake and responds rapidly (within a very few days) to increases and decreases in dietary iodine (I) intake by cows (Rogers and Mee, 1966). This study measured changes in PII in response to different I intakes supplied by contrasting methods to beef cows during late pregnancy and early lactation.

Materials and methods Twelve housed in-calf Hereford x Friesian cows were given a basal diet of hay plus a sugar beet pulp/soya bean meal mix providing 3.4mg I /day for 17 days when plasma samples were obtained from all the cows. The cows were randomly divided into three groups each of four cows and given the same diet for the remaining 6-8 weeks of pregnancy. Following typical practice, as each cow calved the sugar beet pulp/soya allocation was doubled so that the basal diet I intake was 4.5mg total I /day. Group 1 cows (NIL) continued to receive no I supplement throughout. Group 2 cows (MIN) were given 24mg I /day as calcium iodate in a mineral supplement incorporated in the sugar beet pulp/soya mix. This allowance increased to 48mg I /day after calving. Group 3 cows (BOL) were given two High Iodine All-Trace boluses (Agrimin Ltd. DN20 0SP) after the initial 17 days with no supplementary I. These contained 3.4g total I (0.72 proportion as calcium iodate and 0.28 proportion as potassium iodide) with other trace elements and vitamins. Further plasma PII concentrations were assessed after 1 and 5 weeks (before calving) and again after 13 weeks when all the cows had calved. The daily I release (mg /day) from BOL (as measured separately in rumen-cannulated cows) was 113 at week 1, 24 at week 5 and 13 at week 11 and thereafter for their anticipated life of about 8 months. Statistical evaluation of the mean PII concentrations for the three groups at each occasion was by paired t-tests.

Results There were no indications of iodine insufficiency in the cows. Overall mean (± sem) plasma T_4 concentrations (nmol /litre) were 111 ± 2.9 at week 0, 115 ± 2.8 at week 1, 78 ± 3.9 at week 5 and 63 ± 3.3 at week 11 after calving. There were no significant differences or even trends between dietary treatments at any stage.

Table 1 Mean (±sem) plasma inorganic iodine values (PII, ng/ml) and supplementary iodine intakes (mg I /day)

Weeks	Group 1 NIL		Group 2 MIN		Group 3 BOL	
	Intake	PII	Intake	PII	Intake	PII
0	0	24 ± 3.1	0	16 ± 0.7	0	23 ± 0.9
1	0	29 ± 3.3	24	237** ± 27.6	113	403** ± 82.2
5	0	23 ± 2.1	24	165*** ± 10.0	24	164** ± 13.8
11	0	69 ± 7.6	48	306** ± 23.2	13	137* ± 22.3

Both MIN and BOL supplements significantly increased mean PII concentrations above that of the NIL group of cows. The NIL cows also had significantly higher PII values at week 11 (69ng/ml) than before calving (23-29ng/ml) when the basal diet provided marginally less total iodine. For all the observations there was a significant correlation (r=0.92, P<0.001) with the regression PII (ng/ml) = 59.3 + 3.59 x supplementary I intake (mg/day). McCoy *et al*. (1997) considered that 'the lower normal limit for PII in pregnant cows is 80 ng/ml'. This would be satisfied with a daily intake of 5.7mg supplementary I /day. The MIN supplement appeared to provide an unnecessarily large amount of iodine. The mean I release from two boluses was 13mg /day after 11 weeks and this would be sustained (separate studies in rumen-cannulated cows) for about five further months. This would be associated with a fully adequate PII concentration of 106 ng/ml. The much higher initial rate of release from the boluses would be useful to correct any pre-existing iodine inadequacy in cows. Whilst iodine supplementation may often be commenced in the last 2-3 months of pregnancy when suspect calves are born, it is perhaps possible that thyroid abnormalities could be initiated at an earlier stage. Administration of sustained-release ruminal boluses gives some flexibility in timing.

Conclusion A mineral supplement, following typical practice, providing 24mg I /day in late pregnancy and 48mg /day in early lactation to beef cows resulted in plasma inorganic iodine concentrations which were apparently generous. Two iodine-reinforced trace element-vitamin sustained release boluses, after initially higher release rates, provided about 13mg I /day for about five months and the resulting PII concentrations may be considered to be in the normal range.

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A new source of Magnesium and Phosphorus for dairy heifers fed a grass silage based diet

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Introduction Magnesium (Mg) and Phosphorus (P) are essential for various metabolic functions of dairy heifers and thus any dietary imbalance may be detrimental for growing heifers. The most frequently used source of Mg is calcined magnesite (MgO), which has an availability of only 0.20 and is unpalatable to animals. Other inorganic sources of minerals are usually expensive, may be difficult to mix into a premix and can be unpalatable. This study compared the effect of feeding fixed amounts of two mineral mixes that had almost identical mineral levels but differed in sources to supply Mg and P, on palatability, growth and health status of growing heifers.

Materials and methods The study compared two minerals, Standard v New, involving dairy heifers consuming a total mixed ration (TMR) indoor followed by grazing on a perennial ryegrass sward outdoor. Forty-one Holstein-Friesian heifers initially weighing 341±12 kg were distributed into Treatment (New mineral, n=21) and Control (Standard mineral, n=20) groups. Each group was balanced for initial age, live-weight, condition score and genetic potential (Profit Index, PIN) and loose housed on straw in an open shed. The two mineral-vitamin mixes had almost identical chemical composition and contained, either MgO and dicalcium phosphate (Standard) or $MgHPO_4 x\ 3H_2O$ (New). About 62g of a mix was fed to the relevant heifer group daily plus either fixed amounts (kg DM) of a TMR (6.1 including 2.1 concentrate) and big bale grass silage (2.7) or at turn out *ad lib* grazed grass plus 0.5kg crushed wheat. Diets supplied Ca, Mg and P needs of heifers, assuming a daily growth rate of 750g (AFRC 1980, 1991). Each kg concentrate contained 503g crushed wheat, 308g molassed sugar beet pulp, 56g soybean meal, 56g rapeseed meal and 77g molasses and 806g DM, 3.5g Ca, 2.2g P and 1.1g Mg. Blood samples from each heifer were collected on three occasions. Firstly, on 13th December 1999, about 8 weeks after the start of the TMR feeding (Time 1), secondly, on 31st March 2000, about 2 weeks prior to their turnout (Time 2) and thirdly, on 23rd June 2000, about 9 weeks after turn out (Time 3) and analysed for various minerals and metabolites (Table 1). Daily liveweight gain (DLWG) /heifer was calculated by using liveweights of corresponding heifers being recorded initially on 16th October 1999 (Time 0) and then at Time 1, Time 2 and Time 3. Each heifer was conditioned scored at only Time 1 and Time3. The data were statistically analysed to compare the effect of minerals (diet), time and diet x time on the growth and metabolic status of heifers by using GLM in SAS. Daily DM intake (DMI) /heifer /week of concentrate and silage was also calculated.

Results and Discussion There were no refusals of TMR and any small refusals by heifers of big bale silage were ignored. Overall daily DMI (kg) /heifer of clamp and big bale silage, concentrate and minerals remained uniform (4, 2.7, 2.1 and 0.062 respectively) across both groups. Results are shown in Table 1. During indoor feeding, the Treatment (Treat) group had 0.04kg more LWG /heifer in Time 1 but 0.15kg less LWG /heifer in Time 2 than the Control group (Table 1). However, the overall indoor LWG /heifer for Treatment (n, 38) was only 0.055kg less ($P>0.05$) than that for Control (n, 36). Despite a significantly lower growth rate during outdoor grazing (Time 3) for both groups, the Treatment heifers had a far greater LWG than the Controls ($P<0.001$). This poor performance by the Control group was perhaps due to the lower biological availability of Mg in MgO and limited grass availability during a wet and cold spring. Both groups maintained their health throughout and any variation in blood profiles were of no biological importance. The P and Mg portion of each mineral mix used in this study cost £50 /tonne.

Table 1 Live weight gain, condition score and blood profiles of heifers fed different minerals at various times.

	Time 1 (TMR feeding)			Time 2 (TMR feeding)			Time 3 (Grazing)		
	Control	Treat	SEM	Control	Treat	SEM	Control	Treat	SEM
Live weight, *kg/heifer*	393	385	11.95^{ns}	513	489	11.94^{ns}	516	534	12.51^{ns}
DLWG, *kg /heifer*	1.02	1.06	0.068^{ns}	1.05	0.90	0.033^{**}	0.02	0.43	0.058^{***}
Condition score, *0-5 scale*	2.76	2.57	0.070^{ns}	ND	ND	ND	2.60	2.69	0.073^{ns}
Blood plasma profiles, mM /L									
β-Hydroxybutyrate	0.39	0.41	0.024^{ns}	0.32	0.34	0.024^{ns}	0.50	0.60	0.055^{ns}
Urea	1.15	1.12	0.061^{ns}	1.82	1.74	0.054^{ns}	1.91	2.76	0.136^{***}
Magnesium	1.10	0.99	0.015^{***}	0.97	0.97	0.014^{ns}	0.92	0.89	0.015^{ns}
Phosphorus	2.84	2.58	0.083^{*}	2.53	2.63	0.047^{ns}	2.65	2.36	0.076^{**}

ND, not determined; SEM, standard error of means; Liveweights (kg /heifer) at Time 0 were 344±13 (Control) and 338±12 (Treatment).

Conclusion Overall daily LWG /heifer, averaged over Time 1, Time 2 and Time 3, was 84g greater ($P<0.05$) for the Treatment than the Control group. The heifers consuming new mineral maintained their health as indicated by their metabolic profiles and general appearance and management. Both mineral mixes were palatable to heifers.

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Phosphorus pollution by dairy cows and its mitigation by dietary manipulation

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Introduction Phosphorus (P) is a key mineral in energy metabolism and is essential in nearly every biochemical aspect of dairy cow metabolism. Therefore, P needs to be supplied in sufficient quantity to optimize animal performance. However, dairy cows only use 30 – 45% of their dietary P intake and the rest is excreted mainly in faeces. Excess faecal excretion can lead to P accumulation and saturation in the soil and filter into groundwater or remain in surface water (Tamminga, 1996), which is known to cause eutrophication. It is therefore desirable to formulate P rations according to the requirement of the animals and thereby reduce P pollution. The objective of the present study was to develop a dynamic model of P metabolism in dairy cows and use that to identify and quantify trends of P excretion as a function of P intake and investigate effects of energy supplementation on P utilisation.

Materials and methods Data from two experiments conducted at the University of Reading were used to develop the model. The trials were conducted with six multiparous Holstein/Friesian dairy cows in early or mid lactation fed grass silage diets with different levels of P and energy supplementation.

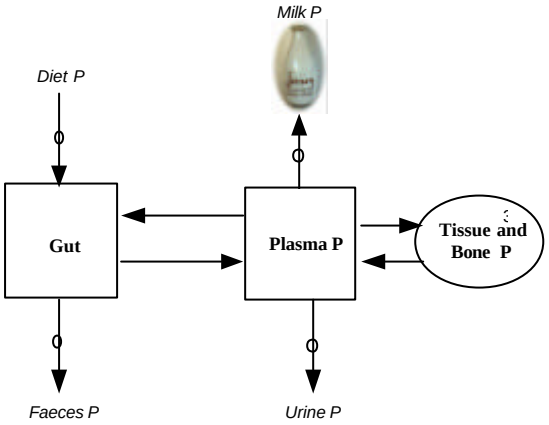

Figure 1 Schematic representation of the dynamic model of phosphorus metabolism in a lactating dairy cow

The cows consumed an average of 190 and 210 MJ/d in the first and second experiments respectively. Based on the two experiments and the literature, a dynamic model of P was developed (Fig. 1). The model contains three pools, and arrows represent inputs and outputs to and from the pools (Fig. 1). For simplicity, P in blood and saliva are combined in one pool (Plasma P). The model was programmed in the Advanced Continuous Simulation Language (ACSL, 1996) and a fourth-order Runge-Kutta method was used for numerical integration. The model was run until a steady-state was achieved and the fluxes optimized were P excreted in feces and urine, secreted in milk and endogenous P contribution to the gut.

Results In proportion to dietary P intake, similar quantities of P were released into the gut (mostly as saliva P) in both experiments. Simulation of P utilisation in the cow using the model showed that there was a linear relationship between P intake and faecal P output (Fig. 2). Due to significant differences in parameter estimates for faecal P output, faecal excretion was reduced from 55 to 39% of the total P in the gut (74 to 60% of dietary P intake) due to increased energy intake. However, the rate at which faecal P was excreted was similar in both experiments.

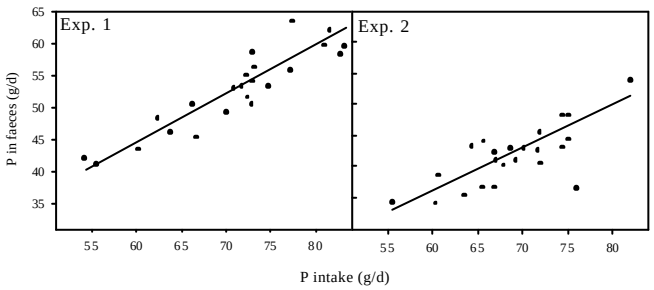

Figure 2 Observed (symbols) and predicted (lines) P output in faeces for two experiments with different energy intakes.

The model predicted that there was a linear relationship between P secreted in milk and P intake in Exp. 1 (data not shown). However, in Exp. 2, there was a linear relationship until intakes of 70 gP/d, after which, there appeared to be saturation (data not shown). Above P outputs of 70 g/d the relationship was predicted to be curvilinear and increased at a lower rate once it reached 25 gP/d secreted in milk. Higher energy intake affected not only the amount of P directed towards milk but also the rate at which P is incorporated in milk. As dietary P intake increases, the benefit to milk P output decreased.

Conclusions Simulation of P utilization by the lactating dairy cow predicted that increasing energy intake by 10% reduced P excretion by 20% and increased P secretion in milk by 13%. Reducing P intake by 10% of the recommended rate (AFRC, 1991), while keeping energy at 210 MJ/d, reduced faecal P output by a further 10% and milk output by just 2%. Therefore, for a 600kg cow, at a production level of 20-25kg milk/d, it is suggested that P should be supplemented at a rate of 68 g P/d which is predicted to be optimally utilized and reduce P pollution substantially.

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The effect of different levels of inorganic sulphur on the rumen parameters of Raini goat

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Introduction Added sulphur (S) in the diet of ruminants has an equivocal effect on the ruminal fermentation. For example, Hegarty et al. (1994) reported an increased total VFA concentration in the rumen of sheep fed a high S diet versus those offered a low S diet (<0.25%, DM basis). Moderated high percentages of S (0.4-0.6%) in the diet of sheep have generally had no effects on ruminal VFA and ammonia-nitrogen concentrations (deOliveira et al., 1996). Working with Angora goats, Qi et al. (1992) noted that total ruminal fluid VFA concentration ranged from 76.7 to 79.1 mM and was not affected by added S (0.16-0.4%). There is limited information related to the influence of supplemental S on the metabolic responses in the rumen of Cashmere goat. Therefore, an experiment was conducted with an Iranian native breed of Cashmere producing goat, Raini, to evaluate the metabolic responses in rumen parameters to S supplementation.

Methods and materials Three rumen fisulated male Raini kids with an initial body weight of 18 (s.d. 1.5) Kg were used in a completely randomized design (CRD) to evaluate rumen metabolic of these animals to S supplementation. Goats were fed ad lib an isocaloric (9.57 MJ/Kg DM) and isonitrogenous (84 g metabolism protein/Kg DM) diets which containing either 0.14, 0.22 , 0.28 , 0.34 or 0.4 % of S on the DM basis. The source of S supplement was CaSO4. Goats were housed in individual pens. Each period lasted 3 weeks, 2 weeks as an adaptation to the diet and 1 week for sampling the rumen fluid. For each diet, the rumen fluid samples were obtained in two days consecuetively at pre-feeding, 1, 3 and 6 h postprandial. Rumen pH, ammonia-N and total volatile fatty acids (VFA) were measured. The data were subjected to analysis of variance to compare the effect of five diet means on the rumen parameters.

Results Table 1 shows the results of the mean values of the two days sampling. Biologically, ruminal pH was not affect by S level of the different diets. Nevertheless, the pH of rumen fluid of kids fed the first diet was significantly ($p<0.05$) higher than those offered diet 6. The ammonia-N concentration was significantly ($p<0.05$) lower in animals fed diet 3 in comparison to other diets. Goats given diets 4 and 5 had significantly ($p<0.05$) higher total VFA concentration than those fed other diets.

Table 1 Effect of different level of dietary S on the ruminal parameters

	Sulphur Inclusion (%)						
	0.14	0.22	0.28	0.34	0.4	SEM	Sig.
pH	6.6[a]	6.4[ab]	6.4[ab]	6.5[ab]	6.2[b]	0.038	*
Ammonia-N mg/l	218[a]	216[a]	152[b]	230[a]	264[a]	8.78	*
Total VFA mM	59.2[a]	80.4[a]	77.5 [a]	136.1[b]	123[b]	4.9	*

* indicates significant at 5%.

Conclusions The results of the present study show that diets 4 and 5 had increased the concentrations of ruminal ammonia-N and VFA, which may be as a consequent of higher microbial activity in the rumen, when compared with other diets. For Raini goat breed, it seems that the optimum requirement of ruminal microbes for dietary sulphur have been met by iether 0.34 or 0.4 percent.

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The effect of molybdenum, iron and sulphur supplementation on growth rate and copper status of lambs

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Introduction For sheep and cattle, primary copper deficiency may occur due to a lack of copper within the feed or soil, whereas secondary copper deficiency may occur in the presence of a combination of high dietary levels of molybdenum (Mo), sulphur (S) and/or iron (Fe). This is due to the anaerobic interactions within the rumen (Phillippo *et al.*, 1987) resulting in thiomolybdate production. Recent work suggests that iron may play a significant role in copper absorption. Mackenzie *et al.* (1997) proposed that a caeruloplasmin to plasma copper ratio may provide a more accurate biochemical indicator of copper status than other current techniques of assessment. A low ratio may suggest that thiomolybdate is being absorbed into the blood which reduces activity of the copper enzymes. The objective of this experiment was to assess the effect of molybdenum and iron in the presence of sulphur on the copper status and performance of intensively reared lambs, and to predict the accuracy of this ratio when assessing the effects of molybdenum or iron on copper metabolism.

Materials and Methods Forty-eight individually penned Charolais cross female lambs approximately ten weeks of age with an initial mean live weight of 23.2kg (s.e.d. 0.89) were randomly allocated to one of four dietary treatments (twelve lambs per treatment). They were fed a complete diet (1.5kg/day) during a seven day adaptation period prior to the start of the trial based on 350g/kg straw pellets, 247 g/kg whole barley, 153 g/kg rapeseed meal, 100 g/kg citrus pulp, 50 g/kg soyabean meal, 60 g/kg molasses and 40 g/kg mineral/vitamin pre-mix (ME 10.6 MJ/kg DM: CP 148.7 g/kg DM:5.47 mg/kg Cu), but containing no additional mineral supplementation (control), treatment group two received 500 mg/kg iron and 2 g/kg sulphur (Fe group), group three received 5 mg/kg molybdenum and 2 g/kg sulphur (5 Mo group) and group four received 10 mg/kg molybdenum and 2g/kg sulphur (10 Mo group). Lambs were slaughtered after ten weeks. Lambs were weighed and blood sampled by jugular venepuncture once weekly. Plasma copper (Pl-Cu) concentrations was determined by atomic absorption spectrophotometry (µmol/L) and the caeruloplasmin (CP) activity was determined on a Cobas Mira (Roche)(mg/dL). The ratio of CP:Pl-Cu was also assessed. Statistical analysis was performed using ANOVA and repeated measures using Genstat version 4.1.

Results The results are presented in Table 1 and 2.

Table 1 Effect of treatment on CP:Pl-Cu ratio

Wk	Control	Fe	5 Mo	10 Mo	s.e.d.	Sig
0	1.39	1.26	1.25	1.27	0.101	NS
1	1.08	1.05	0.96	0.74	0.059	***
2	1.21	1.02	1.03	0.87	0.098	**
3	1.14	1.06	0.89	0.71	0.121	**
4	1.04	0.92	0.76	0.63	0.051	***
5	1.00	0.97	0.82	0.70	0.074	***
6	1.38	1.27	1.02	0.88	0.056	***
7	0.88	0.80	0.66	0.57	0.032	***
8	0.98	0.78	0.70	0.60	0.039	***
9	0.91	0.80	0.69	0.57	0.045	***
10	0.95	0.89	0.60	0.71	0.051	***

There were significant differences ($p<0.01$) for the CP:Pl-Cu ratio between treatments from week one onwards (Table 1). There was an overall trend for the Control group to have a higher CP:Pl-Cu ratio than the Fe, 5 Mo and 10 Mo treatment groups respectively (Control > Fe > 5 Mo > 10 Mo). The CP:Pl-Cu ratio was significantly lower in the 10 Mo than the control treatment groups from week 1 to week 10 ($p<0.05$) Repeated measures analysis indicated a significant treatment x time effect ($p<0.01$) for the CP:Pl-Cu ratio. There were no significant effects of treatment on the daily liveweight gain (DLWG), total dry matter intake (DMI) or food conversion efficiency (FCE).

Table 2 Effect of treatment on lamb performance

	Control	Fe	5 Mo	10 Mo	s.e.d	Sig
DLWG (g/day)	0.27	0.27	0.28	0.28	0.017	NS
Total DMI (kg)	110.8	110.7	108.0	114.4	4.23	NS
FCE	0.17	0.17	0.18	0.17	0.008	NS

Conclusion The CP:Pl-Cu ratio confirmed that lambs receiving a high molybdenum supplementation had a lower ratio compared to the other three treatment groups and therefore this method of assessing copper status may be more useful than other current methods. The ratio decreased over the ten-week trial period confirming the theory of Mackenzie *et al.* (1997) that the ratio is sensitive to dietary copper antagonists. A high molybdenum concentration within the diet may therefore exacerbate the production of thiomolybdates, making copper unavailable to the animal.

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Effects of short and long term sodium supplementation on copper accumulation in sheep

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Introduction The presence of high concentrations of sodium in the mammalian small intestine plays a major role in facilitating copper absorption (e.g. Wapnir and Stiel, 1987). An experiment was conducted that investigated the medium and long-term effects of adding sodium to the diet of sheep on the copper accumulation in body tissues.

Materials and methods Twenty-four Welsh Mountain mature ewes were allocated to be fed either a sodium supplement for six (treatment 6) or nine (treatment 9) weeks, or a Control treatment which received sodium for the first three weeks only. In the first part of the study, sodium chloride was added in solution to grass silage at a rate of 7 g Na/kg DM and fed *ad libitum* to twelve ewes. Silage without sodium was fed to twelve control ewes. After three weeks six ewes that had been fed silage with sodium (treatment 6) and six of the control ewes were fed silage with the sodium supplement for three weeks. The remaining twelve ewes remained on their original diets that they had been fed in the first three weeks. The first 12 ewes were then slaughtered and body tissues (bone, brain, hoof, liver, kidney, spleen, uterus, aqueous humour of the eye, rumen, blood) extracted for the assessment of mineral concentrations (15 common metals, boron and phosphorus). Ewes in treatment 9 and the remaining six control ewes were then also fed silage with the sodium supplement for three weeks, after which they too were slaughtered. The metal concentrations were determined by Inductively Coupled Plasma Emission Spectroscopy after acid digestion. A blood serum sample was collected before slaughter. Statistical analysis was by Anova of three linear models with medium and long term exposure analysed separately and both medium and long term exposure together as main effects.

Results The changes in copper concentrations in body tissues are presented as several tissues were affected by short or long term sodium supplementation The concentration of copper in the brain and liver were increased by the long period of exposure to a high sodium diet, but not by the short period of exposure (Table 1). The concentration in the aqueous humour of the eye was increased by overall exposure, and particularly short-term exposure. Hoof copper concentration also tended to be increased by short-term exposure. The concentrations of copper in the uterus and the blood were reduced by short-term exposure.

Table 1 The effects of short or long periods of sodium exposure on copper accumulation in the brain, aqueous humour, hoof, uterus and blood of sheep

	Medium sodium exposure			Long sodium exposure				
Weeks of Na exposure	3	6	S.E.D.	3	9	S.E.D.	Overall S.E.D.	Interaction S.E.D.
Brain (mg/kg DM)	22.6	18.1	5.33	19.8	32.3	5.96	4.00	5.66*
Liver (mg/kg DM)	170	155	73.8	153	342	68.1*	56.3	79.6
Aqueous humour of eye (µg/kg?DM)	70	150	11.6*	78	93	18.0	15.5*	26.5†
Hoof (mg/kg DM)	1.4	3.4	0.94†	3.5	3.7	1.23	0.81	1.22
Uterus (mg/kg DM)	3.7	2.6	0.36*	3.4	3.5	1.1	0.54	0.75
Blood (mg/l)	1.28	0.94	0.14*	1.11	1.00	0.14	-	-

† $P < 0.10 > 0.05$, * $P < 0.05$

Discussion The increase in copper accumulation in the brain, aqueous humour of the eye and possibly the hoof may reflect enhanced uptake of copper. The enhanced accumulation of copper in some body organs during short-term exposure may have created a temporary reduction in copper concentration in the blood and the uterus, which was diminished or eliminated by the long-term exposure.

Conclusion Exposure of sheep to sodium enhances copper concentrations in some organs, but blood concentration was reduced by sodium in the short-term.

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Effect of Ca: P ratio on grower-finisher pig performance and mineral excretion.

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Introduction The level of calcium (Ca) in the diet affects the utilisation of phytic acid-phosphorus (P) through the formation of insoluble Ca phytate and/or reduction of phytase activity (Larsen 1993). High Ca: total P ratios (Ca: tP) (1.5:1 to 2.0:1) in corn-soya bean meal diets supplemented with microbial phytase decreased the utilisation of P for weanling (Lei *et al*., 1994) and growing-finishing (Liu *et al*., 1998) pigs. However, it is important to note that the majority of research using supplemental microbial phytase and Ca:P ratios was carried out using maize-soyabean meal based diets. The objective of this experiment is to examine the effect of Ca: tP ratio on the efficacy of microbial phytase and its effects on pig performance, nutrient digestion and mineral metabolism using wheat, barley and soya bean meal based diets.

Materials and Methods A 2 x 2 factorial arrangement of treatments was used to evaluate the response of grower – finisher pigs (34 to 90 kg; 70 days duration) to two supplemental phytase levels (0 and 750 FYT/kg) and two Ca:tP ratios (1.66:1 and 1:1). All diets were formulated using standard values for ingredients to have similar concentrations of digestible energy (13.8 MJ DE/kg) and lysine (11.5 g/kg) and contained wheat (406 g/kg), soya bean meal (312 g/kg), barley (250 g/kg), tallow (10 g/kg) and minerals and vitamins. All diets were steam pelleted. Forty eight entire male pigs (n = 12) averaging 33.5 kg in live weight were offered individually food containing 4.3 g/kg tP, 1.4 g/kg available (a) P and 7.0 g/kg Ca (T1), 4.3 g/kg tP, 1.4 g/kg aP and 7.0 g/kg Ca and 750 FYT/kg of Innozyme phytase (T2), 4.3 g/kg tP, 1.4 g/kg aP and 4.3 g/kg Ca (T3) and 4.3 g/kg tP, 1.4 g/kg aP and 4.3 g/kg Ca and 750 FYT/kg of Innozyme phytase (T4). Twelve entire male pigs averaging 52 kg in live weight were randomly allocated to the same diets in a digestibility study for two collection periods.

Results The effect of Ca: tP ratio and microbial phytase on pig performance and nutrient and mineral digestibility are given in Table 1. Lowering the Ca: tP ratio from 1.66:1 to 1.0:1 increased the apparent digestibility of the dry matter (DM) and gross energy as well as the digestible energy (DE) content of the diet ($P < 0.05$). The inclusion of phytase increased the digestibility of the DM, gross energy, nitrogen and Ca as well as the DE content of the diet ($P < 0.05$). There was a significant ratio by phytase interaction ($P < 0.5$) in P digestibility. The inclusion of microbial phytase significantly increased P digestibility when added to the 1.0:1 ratio, however phytase had no effect when added to the 1.66:1 ratio. The inclusion of microbial phytase increased feed intake (2.16 v 2.0 kg/day; sem 0.05; $P < 0.05$) and live weight gain (0.893 v 0.818 kg/day; sem 0.022; $P < 0.05$). Lowering the Ca: tP ratio resulted in a significant improvement in food conversion ratio (FCR) (2.32 v 2.40, sem 0.03; $P < 0.05$) and bone Ca content ($P < 0.001$). In conclusion the beneficial effects of microbial phytase supplementation of pig diets are adversely affected by a wide Ca: tP ratio.

Table 1: Effect of P level and phytase inclusion on pig performance, nutrient and mineral digestibility

Treatment	T1	T2	T3	T4				
Phosphorus (g/kg)	4.2	4.2	4.2	4.2		Significance		
Calcium (g/kg)	7.0	7.0	4.3	4.3		Ca: tP ratio	Phytase	Ca: tP ratio x Phytase
Phytase inclusion (FTU/kg)		750		750	s.e.m			
Nutrient and mineral digestibility								
Dry matter	0.866	0.883	0.884	0.892	0.006	*	*	ns
Energy digestibility	0.851	0.872	0.870	0.880	0.008	*	*	ns
DE content (MJ/kg)	14.03	14.34	14.49	14.60	0.115	*	*	ns
N digestibility	0.818	0.865	0.849	0.885	0.018	ns	*	ns
P digestibility	0.348	0.338	0.337	0.385	0.017	ns	ns	*
Ca digestibility	0.396	0.429	0.354	0.415	0.020	ns	*	ns
Pig Performance								
Feed Intake (kg/day)	2.04	2.10	1.95	2.21	0.076	ns	*	ns
Weight gain (kg/day)	0.831	0.860	0.845	0.926	0.034	ns	*	ns
FCR (kg/kg)	2.43	2.37	2.29	2.36	0.041	*	ns	ns
Bone measurements								
Ca content	24.9	25.4	25.9	26.0	0.205	***	ns	ns
P content	11.3	11.3	11.3	11.5	0.077	ns	ns	ns

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Influence of Natuphos® phytase and organic acids on the performance of growing/finishing pigs

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Introduction Numerous studies have clearly shown that the digestibility of phosphorus in pig diets can be improved 20-30% by supplementation with Natuphos® phytase allowing the phosphate content of the feed to be reduced without the P-intake becoming deficient. It is also well accepted that organic acids can significantly improve performance of pigs, particularly up to 30kg liveweight, and recently that synergistic benefits result when both Natuphos® phytase and organic acids are included in the same ration. The objective of this study was to investigate the influence combining Natuphos® phytase (replacing calcium and phosphorus) and organic acids (liquid v dry product) on growing/finishing pig performance.

Materials and methods Two hundred and forty white type hybrid pigs were randomly allocated to one of four treatments; A) conventionally formulated standard diet; B) reduced Ca, P diet (replacing 1.0 kg Ca; 1.15 kg P/tonne) containing 100g/tonne BASF Natuphos® 5000G (providing 500 FTU phytase/kg feed); C) diet B reformulated to include 7, 6, 5, 4 and 3kg BASF Luprocid® liquid (638g/kg Formic acid; 250 g/kg propionic acid) in each of the respective diet stages; and D) diet B reformulated to include 10, 8, 6, 5 and 4kg of BASF Lupromix® Dry UK (280g/kg formic, 300g/kg fumaric, 230g/kg propionic) in each of the respective diet stages. Five diet stages were fed across treatments (creep 8.9g/kg Ca, 7.5g/kg P, starter 8.5g/kg Ca, 7.3g/kg P, link 8.0g/kg Ca, 6.8g/kg P, grower 7.0g/kg Ca, 5.5g/kg P and finisher 7.0g/kg Ca, 5.5g/kg P) formulated to be typical of UK specifications and ingredients and of similar nutritional value across treatments. Pigs we fed *ad-libitum* throughout and housed in groups of 5 pigs/pen for creep and starter stages and 15 pigs/pen for remaining stages. Data were subjected to analysis of variance using individual pig records for growth rate, carcass data, and on a pen basis for feed intake, FCR and subjective scour score (1=very clean; 5=very dirty).

Results With the exception of weight after the grower diet, there were no significant differences between treatments during the creep, grower or finisher phases, or for carcass quality (Table 1). Performance during the starter and link phases however was significantly improved when Natuphos® was added to the diet, and further improved when organic acids were added. There were no significant differences in pig performance between the two acids, although an improvement in subjective scour score was seen with Lupromix® Dry.

Table 1 Pig performance on each diet

	A Control	B Natuphos	C Natuphos + Luprocid	D Natuphos + LuproMix Dry	s.e.d	P value
Number of animals	60	60	60	60		
Start wt. (kg)	8.1	8.1	8.1	8.1	0.24	NS
Creep - Final wt (kg)	9.7	9.8	9.9	9.8	0.28	NS
- Daily gain (g)	227	246	258	244	22.0	NS
- FCR	1.13	1.15	1.02	1.04	0.07	NS
Starter - Final wt (kg)	16.1	16.7	17.2	17.0	0.50	NS
- Daily gain (g)	488^{e}	536^{f}	557^{f}	554^{f}	21.5	P<0.01
- FCR	1.08	1.07	1.05	1.07	0.016	NS
Link - Final wt (kg)	38.2^{a}	40.1^{ab}	42.1^{b}	41.7^{b}	1.05	P<0.001
- Daily gain (g)	652^{a}	686^{ab}	734^{c}	722^{bc}	20.9	P<0.001
- FCR	1.64	1.65	1.57	1.63	0.029	NS
Grower - Final wt (kg)	66.5^{a}	70.4^{ab}	73.2^{b}	73.5^{b}	2.13	P<0.01
- Daily gain (g)	629	675	691	701	32.4	NS
- FCR	2.62	2.51	2.54	2.53	0.055	NS
Finisher - Final wt (kg)	89.3	89.2	88.8	89.7	2.02	NS
- Daily gain (g)	725	706	699	708	38.8	NS
- FCR	3.12	3.14	3.17	3.18	0.200	NS
Backfat thickness (P + P_3) mm	20.0	20.7	20.8	21.9	0.98	NS
Lean %	59.2	58.8	58.8	58.2	0.51	NS
Subjective scour score	1.9^{a}	1.9^{a}	1.9^{a}	1.5^{b}	0.17	P<0.05

Conclusion This experiment proved that Natuphos® phytase can be used with confidence to replace inorganic phosphorus in diets with a resultant increase in performance, particularly in younger pig diets. Addition of either Luprocid® or Lupromix® Dry to starter and link diets further improves pig performance.

Growth performance and bone strength of piglets fed Natuphos â Ô

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Introduction Concerns over pollution from intensive animal production has lead to developments in enzyme technology to improve the utilisation of minerals such as phosphorous (P) from the diet, and to reduce levels of added inorganic minerals. BASF Natuphos® (registered trademark of Gist-brocades) is a formulation of microbial phytase which improves digestibility of P from plant origin. The aim of this study was to determine whether reducing levels of inorganic P (Dicalcium Phosphate-18%P) and including quantities of Natuphos® would effect have any effect on growth performance and bone strength.

Materials and methods A total of 40 weaned piglets were used, male and female, about 26 days of age and 8.5kg liveweight. Pigs were randomly allocated to one of two treatments (control or Natuphos®) and housed in groups of 5 in a flat deck until Day 26, then combined in treatment groups in a grower building until slaughter at 30 kg. A creep diet was fed for the first 7 days, then a starter ration for 14 days and finally a link ration. Composition of the diets and inclusion of additives are shown in Table 1. Feed and water were available *ad libitum.* Pigs were weighed at the start, at each diet changeover and prior to slaughter. Feed intake was not recorded for individual pigs. After slaughter (electrical stunning and exsanguination), data were collected on cold carcass weight, backfat and estimated lean content. The front forequarter was cut off and placed in deep freeze at -20^{o}C for about 4 weeks. Each foreqarter was then allowed to thaw at room temperature and dissected to yield the 3rd metacarpal bone and the humerus. The metacarpal and humerus were weighed and subjected to a three-point flexure test using an Instron test rig, (span fixed at 25 and 70 mm respectively). Data were recorded on load and deformation at the peak and linear limit, and subsequently a value for flexural stiffness (FS) calculated. Levels of ash, Ca and P were determined by placing the bones in a furnace set at 550^{o}C for 15 hours. Ash samples were digested with nitric acid/hydrogen peroxide before determination of calcium and Phosphorus level by I.C.P. Data were analysed by analysis of variance (Minitab, V 9.2; Minitab Inc., USA), the values for average daily gain being adjusted for liveweight at the start of each stage.

Table 1 Diet composition at each stage and inclusion of Natuphos® (NP) or Dicalcium Phosphate (DP)

	Diet composition (g/kg)			Control			Natuphos		
Stage	Crude Protein	Oil	Lysine	NP (g/kg)	DP (g/kg)	Ca:P	NP (g/kg)	DP (g/kg)	Ca:P
Creep	218	90	13	0	6.71	1.38	100	0.31	1.39
Starter	229	86	13	0	5.90	1.07	100	-	1.18
Link	203	56	10	0	5.52	1.21	100	-	1.18

Results Pigs given the Natuphos â diets tended to be heavier at the start of the trial (P=0.06) than the control pigs (Table 2), and were significantly heavier the creep and starter stages (P<0.01 and P<0.001 respectively). There was however no difference in liveweight after the link ration. Pigs fed Natuphos â diets had greater daily gain than control-fed pigs at all three stages, though this was significant only for the creep stage (P<0.05). The relative increase in growth rate was 49, 16 and 5 % for creep, starter and link stages respectively. There was no significant effect of treatment on carcass quality, any of the physical estimates of bone strength or ash content. Levels of phosphorous in the humerus were however significantly higher (P<0.001) for pigs fed diets containing Natuphos â .

Table 2 Growth performance, physical and mineral characteristics of bones in control and Natuphos® (NP) rations

Category	Control	NP	s.e.d.	P value	Category	Control	NP	s.e.d.	P value
Liveweight (kg) and carcass data					*Metacarpal*				
Initial	8.1	8.8	0.36	P=0.06	Weight (g)	12.0	12.0	0.40	Ns
Creep	9.1	10.3	0.42	P<0.01	Peak Load (KN)	0.45	0.43	0.03	Ns
Starter	14.9	17.3	0.69	P<0.001	FS (KNmm^2)	53	48	3.4	Ns
Link	31.4	30.5	0.97	Ns	Ash (g/kg)	346.7	347.5	8.48	Ns
Cold wt. (kg)	20.7	20.2	0.73	Ns	P (g/kg ash)	196.4	197.1	1.80	Ns
Fat (P_1+$P_{3\ mm}$)	14.4	13.3	0.83	Ns	*Humerus*				
Lean (%)	59.0	59.6	0.46	Ns	Weight (g)	104.8	102.5	3.07	Ns
Daily gain (g)					Peak Load (KN)	1.89	1.80	0.072	Ns
Creep	145	216	32.4	*	FS (KNmm^2)	1665	1483	121.8	Ns
Starter	424	493	32.7	P=0.06	Ash (g/kg)	478.4	494.1	13.35	Ns
Link	635	664	29.3	Ns	P (g/kg ash)	190.5	195.1	1.08	P<0.001

Conclusions The growth performance of weaned piglets was improved by the addition of Natuphos®, particularly in the creep and starter stages. There were no deleterious effects of Natuphos® on bone strength, ash or phosphorous content of selected bones from the forelimb. Therefore this study has demonstrated that replacing the inorganic source of P though the inclusion Natuphos® resulted in improved performance, without any adverse effect on bone strength.

Ruminal peptide-N concentrations in Iranian Balochi lambs fed diets containing lucerne hay or silage

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Introduction Peptides are intermediates in the conversion of ingested protein to ammonia in the rumen and their accumulation depends upon the nature of diet (Mesgaran & Parker,1995). Transient accumulation of peptides occurs after feeding and then their concentrations declines. In addition, it suggests that the production of peptides in the rumen was not altered by the protein supplements when diets provided similar effective rumen degradable protein (ERDP)(Mesgaran & Moosavi, 1999). The objective of the present experiment was to investigate the effect of diets, with similar ERDP, containing lucerne hay or silage on the ruminal peptide-N concentrations.

Materials and Methods Four Iranian Balochi lambs weighing 33.7 ± 0.34 Kg, each with a permanent rumen fistula, were assigned to a balanced 2^2 Latin Squares and fed as TMR twice daily. The diets consisted of a basal diet of ground barely and sugar beet pulp (180 and 120 g DM d^{-1} $lamb^{-1}$, respectively) which was supplemented with lucerne hay (LH) or silage (LS), 300 g DM d^{-1} $lamb^{-1}$. The diets provided similar ERDP (98.4 g Kg^{-1} DM). Samples of rumen contents were taken, by suction, at 0.0, 0.5, 1, 2, 3 and 5 hours after the morning feed. Ruminal fluid was prepared for peptide analysis using sulphate-tungstate method described by Chen et al. (1987). Tugstate acid-precipitate nitrogen was assayed by a standard macro-Kjeldahl procedure. The data were analysed using a repeated measures design; with sheep as a block, the effect of treatments repeated on time was tested against sheep x treatment and the effect of time was tested against the residual.

Results The peptide-N concentrations at each sampling time are shown in the Table. The ruminal peptide-N concentrations in lambs fed diet containing LH was notably higher than the other group in each sampling time. Peptide-N concentrations showed linear and quadratic significant responses to time ($p<0.05$). Peptide-N concentrations increased after the feeding and declined at 5 hours after that.

Table Peptide-N concentrations (mg $liter^{-1}$) in the rumen fluid of Iranian Balochi lambs fed the experiment diets containing lucerne hay or silage

Time(h)	Treatments		SEM (time)	Statistical Significant		
				LH vs LS	Time	
	LH	LS			L	Q
0.0	34.620	46.500	21.4	NS	*	*
0.5	103.775	69.525				
1	121.550	74.975				
2	146.925	91.100				
3	141.125	93.333				
5	111.525	81.025				

*: $p<0.05$

Conclusion The data related to the present study showed that the ensiling of lucerne may influence the nitrogen metabolism in the rumen. The increasing of peptide-N concentrations in LH compared to LS confirmed that Lucerne hay protein was more susceptible to rumen degradation, or, the peptide composition resulted from LH fermentation are more resistant to rumen microbial breakdown (Mesgaran & Parker, 1996). Peptide-N concentrations increased from 0.5 hour after the feeding and declined at 5 hours. However, the concentrations of peptide-N at 5 hours after the feeding were still considerably higher than those of before feeding. So, from the physiological aspects, the determination of the composition of such peptides may be important in ruminant nutrition.

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The potential of urinary metabolites of plant compounds as indicators of botanical composition of the diet of goats

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Introduction: Knowledge of the nutrition of free-ranging herbivores and the impact on their environment is often limited by lack of information concerning the botanical composition of their diets. For herbivores grazing heterogeneous vegetation environments, current methods for estimating the botanical composition of their diets are limited in their scope and accuracy. The purpose of this study was to establish whether urinary metabolites, occurring as a result of ingestion of different individual plant species, have the potential to be used as markers to estimate diet composition.

Materials & Methods: Thirty goats housed in metabolism cages were offered a basal diet of dried grass pellets and hay supplemented with individual plant materials for 3 days and total output of urine was collected for 5 days. Animals were randomly allocated to groups of 3, with each group receiving a different treatment during each of three 5-day experimental periods separated by at least 2 days. Plant material was offered from the following species: *Acer pseudoplatanus* (AP), *Aesculus hippocastanum* (AH), *Alnus glutinosa* (AG), *Anthriscus sylvestris* (AS), *Betula pendula* leaves (BL) and twigs (BT), *Calluna vulgaris* (CV), *Chamnaerion angustifolium* (CA), *Crataegus monogyna* (CM), *Cytisus scoparius* (CS), *Erica cinerea* (EC), *Fraxinius excelsior* (FE), *Juncus effusus* (JE), *Luzula sylvatica* (LS), *Pinus sylvestris* (PS), *Populus nigra* (PN), *Quercus robur* (QR), *Rubus idaeus* (RI), *Rumex obtusifolius* (RO), *Salix caprea* leaves (SL), twigs (ST) and leaf and twig mixture (SM), *Sambucus nigra* (SN), *Sorbus aucuparia* (SA), *Ulex europaeus* (UE), *Ulmus glabra* (UG), *Urtica dioica* (UD) and *Vaccinium myrtillus* (VN). Urine samples were analysed for metabolites associated with ingestion of individual plant materials by high performance liquid chromatography (HPLC) to detect aromatic compounds and gas chromatography (GC) to detect organic acids, aromatic compounds and additional compounds extractable from acidified urine into diethyl ether (as tert-butyl, dimethylsilyl derivatives). Patterns of metabolites detected in urine were subjected to principal co-ordinates (PCO) analysis to determine whether patterns were different for individual plant materials ingested.

Results: The HPLC method detected around 40 individual metabolites and the GC method detected around 200. Due to the nature of the compounds that were detected by each method, it is unlikely that HPLC analysis provided any additional information from that provided by GC analysis. The metabolites of interest disappeared from urine within 48 hours after feeding the plant materials. PCO analysis results showed that ingestion of each diet resulted in a different pattern of metabolites in urine. Figure 1 shows the results of PCO analysis of GC data plotted in 2 dimensions. Patterns of metabolites which were similar are shown as being close together, greater distances between points indicate less similarity between patterns of metabolites detected in urine from goats fed that diets.

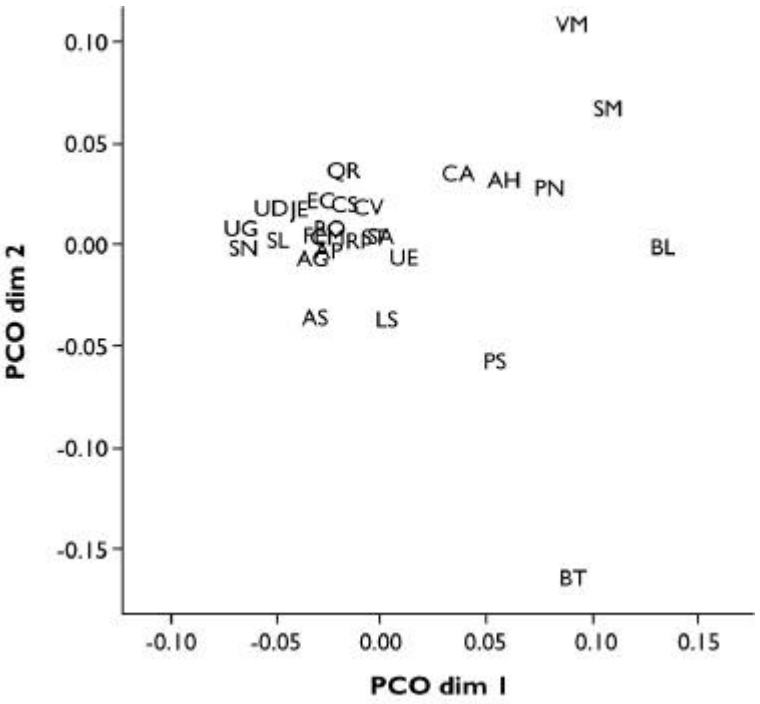

Figure 1 Two-dimensional plot from principal co-ordinates analysis of metabolite patterns detected in urine from goats consuming different plant materials.

Conclusions: The results of the present study indicate that the pattern of metabolites excreted in urine by goats often varies according to the plant species ingested. It is possible that the different patterns of urinary metabolites detected after ingestion of different plants have potential to be used as qualitative indicators of the ingestion of these plant materials by ruminants. The results of this study support the possibility of using urinary metabolites of plant secondary compounds as markers to estimate the diet composition of free-ranging herbivores but further work is required to develop a successful technique. For quantitative estimations, recoveries of metabolites relative to the intakes of individual plants need to be determined. Furthermore, simple methods of sampling and measuring urine output from free-ranging animals would be required. However, perhaps the greatest potential of such urinary metabolites is as qualitative markers to be used in combination with quantitative faecal markers, such as plant-wax hydrocarbons and fatty alcohols.

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The biohydrogenation of *n*-3 polyunsaturated fatty acids determined *in-vitro*

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Introduction Unprotected *n*-3 PUFA supplements fed to ruminants are subject to lipolysis and biohydrogenation in the rumen (Wachira *et al*. 1998). Improving the *n*-3 PUFA content of ruminant products therefore requires some form of protection of dietary lipid from microbial activity in the rumen. The *in-vitro* incubation of PUFA sources offers the opportunity of rapidly determining the level of protection offered against ruminal biohydrogenation. The objectives of the current experiment were therefore to determine the biohydrogenation of a number of sources containing a-linolenic acid using the *in-vitro* gas production technique.

Materials and methods Eleven sources of *n*-3 PUFA were incubated *in vitro* using a modification of the manual gas production technique described by Theodorou *et al.,* (1994). The sources were (a) Linseed oil (Control), (b) linseed, (c) linseed heat treated with xylose for 30 minutes, (d) linseed heat treated with xylose for 45 minutes (c and d supplied by Borregaard UK Ltd), (e) formaldehyde treated linseed, (f) vermiculite soaked with linseed oil, (g) NaOH/formaldehyde treated linseed, (h) formic acid/formaldehyde treated linseed, (i) ammonium tetraformic/formaldehyde treated linseed, (j) Omega-3 (e to j supplied by Trouw UK Ltd) and (k) extracted palm kernals soaked with linseed oil (UFAC UK Ltd). Dried grass (2.032g) and de-fatted linseed were added to provide similar amounts of fermentable organic matter in each vessel. Rumen fluid was collected from two sheep fed 500g/d of concentrate with hay offered *ad-libitum.* The rumen fluid was blended, strained and 200ml (rumen fluid 10:90 anaerobic buffer) added to equal amounts of fat (300mg) previously weighed into 300ml bottles. The gas production was measured manually and the fermentation terminated at 6h, 12h, 24h and 48h (3 replicates per time point). The culture was freeze-dried and fatty acids analysed by gas-liquid chromatography. Data were analysed using analysis of variance as a randomised block design.

Results The cumulative gas production increased with time and was similar for all the PUFA sources (Table 1). The biohydrogenation of linseed oil (a) and linseed (b) was rapid with approximately 0.9 of a-linolenic acid being lost at 24h (Table 2). The biohydrogenation of a-linolenic acid in the vermiculite/linseed oil (f) was high at 6h (0.62) but increased relatively slowly to a final value of 0.83 at 48h of incubation. The products with the best protection of a-linolenic acid were NaOH/formaldehyde treated linseed (g) and formic acid/formaldehyde treated linseed (h) with 0.74 and 0.61 of a-linolenic acid being lost at 48h respectively. The biohydrogenation of the other sources of a-linolenic acid was high.

Table 1 Cumulative gas production (PSI).

Time	a	b	c	d	e	f	g	h	i	j	k	s.e.d.
6h	12.9	12.1	11.4	11.8	11.6	14.1	9.5	11.4	11.9	13.7	11.4	1.01
12h	26.7	26.7	25.1	25.8	23.8	29.9	22.2	24.1	22.9	28.6	24.2	1.72
24h	40.3	43.0	40.3	41.9	38.8	45.6	36.3	38.9	38.8	42.7	42.9	2.26
48h	55.8	59.6	56.0	54.7	62.0	59.8	51.6	54.8	54.0	55.1	62.2	5.53

Table 2 The proportion of a-linolenic acid biohydrogenated *in-vitro*.

Time	a	b	c	d	e	f	g	h	i	j	k	s.e.d.
6h	0.25	0.22	0.22	0.14	0.17	0.62	0.01	0.11	0.13	0.66	0.25	0.066
12h	0.61	0.49	0.56	0.47	0.41	0.69	0.10	0.28	0.24	0.77	0.64	0.052
24h	0.92	0.89	0.87	0.79	0.75	0.79	0.42	0.57	0.70	0.93	0.86	0.038
48h	0.97	0.98	0.94	0.89	0.93	0.83	0.61	0.74	0.91	0.96	0.91	0.021

Conclusions The gas production profiles indicate extensive fermentation of all the diets. Despite this, compared to the Control, NaOH/formaldehyde and formic acid/formaldehyde treated linseed both had a significantly lower biohydrogenation of a-linolenic acid at all time points and therefore represent a source of protected *n*-3 PUFA.

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Rumen microbial breakdown of plant secondary compounds in ruminants consuming mixed diets.

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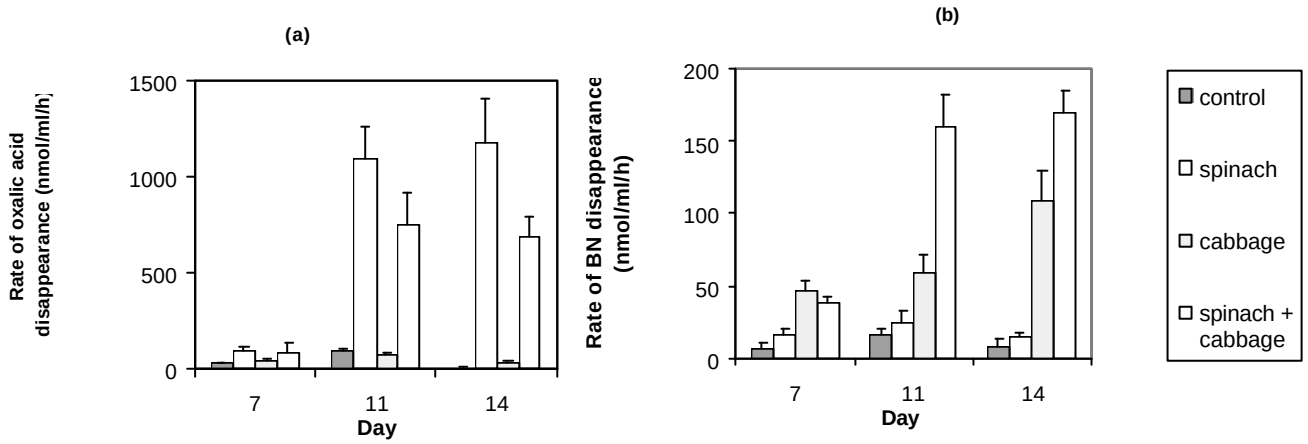
Introduction Ruminants foraging under free-ranging conditions include a diversity of plants in their diet. A wide range of plant secondary compounds are broken down under microbial action in the rumen thus protecting the host animal from their otherwise toxic effects. For example, oxalic acid (OA), found in many tropical grasses, is effectively degraded by *Oxalobacter formigenes* following a period of adaptation of the rumen micro-flora (Allison *et al*, 1985). Similarly, butenyl nitrile (BN), a metabolite of glucosinolates, found in brassica plants, has been shown to degrade under rumen microbial action (Duncan & Milne, 1992). The purpose of this experiment was to investigate whether adaptation to the plant secondary compounds found in one food type might influence the degradation of other, unrelated secondary compounds and *vice versa.*

Methods Twenty-four goats were offered combinations of spinach, which contains OA, and cabbage, which contains BN. The experiment was conducted as a 2 x 2 factorial design with factors consisting of the presence of spinach (0.0 or 0.3 x maintenance metabolisable energy intake (M)) or cabbage (0.0 or 0.3 x M) in the diet. The background diet of fresh grass was fed so that the total intake of the goats matched their estimated maintenance requirements for metabolisable energy. Rumen samples were collected by stomach tube on days 7, 11 and 14 following commencement of the experimental diets. Rates of breakdown of OA and BN were measured using established methods (Allison *et al*, 1985; Duncan & Milne, 1992).

Results Inclusion of spinach in the diet led to a marked increase in the rate at which OA was broken down by rumen micro-organisms ($P<0.001$; Fig 1a). The increase was evident at day 7 but was more marked on days 11 and 14. Similarly, breakdown of BN increased significantly in rumen fluid from cabbage-fed goats ($P<0.001$) with the rate of breakdown increasing as the experiment progressed ($P<0.001$; Fig 1b). Inclusion of both spinach and cabbage in the diet led to enhancement of BN breakdown ($P<0.05$ for interaction) and suppression of OA breakdown ($P<0.05$ for interaction; Fig 1a and b) when compared to breakdown rates in animals receiving only one feed. Rates of breakdown of secondary compounds were minimal in control animals.

Figure 1. Rate of breakdown of oxalic acid (a) and butenyl nitrile (b) in the rumen of goats on control (fresh grass),



spinach, cabbage, and spinach and cabbage dietary treatments.

Discussion Consumption of plants containing specific secondary compounds led to the expected increase in the rate of their breakdown in the rumen under microbial action. Adaptation to BN led to a suppression of the rate of oxalic acid breakdown in the rumen when both plants were offered possibly as a result of the direct action of BN on OA-degrading bacteria. The enhancement of BN breakdown when OA was also consumed was unexpected. The mechanisms underlying the interaction between the two degradation pathways is the subject of further research using continuous batch culture techniques.

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Effects of fibre level and particle size on rumen microbial fermentation and protein metabolism using liquid and solid associated bacteria

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Introduction. The effects of fibre level (F) and forage particle size (S) on ruminal fermentation profile is often mediated through changes in feed intake, rates of digestion or passage, ruminal pH and/or bacteria population. Therefore, most *in vivo* studies have confounded the direct effect of F or S with changes in the rumen environment. *In vitro* systems allow to control several fermentation conditions independently (pH, flow rates, intake). Total, bacterial and dietary nitrogen (N) flows are generally calculated using liquid associated bacteria (LAB), although solid associated bacteria (SAB) represent about 80% of total bacterial population in the rumen (Olobobokun and Craig, 1988). The objective of this experiment was to study the effects of F and S on microbial fermentation and N metabolism using LAB or SAB values in a dual flow continuos culture system.

Materials and methods. Eight dual flow (1326 ml) continuous culture fermenters (Hoover *et al*., 1976) inoculated with strained ruminal fluid were used in two consecutive periods (7 d for adaptation and 3 d for sampling). Treatments were arranged in a 2 x 2 factorial, being F (high=HF (67% alfalfa hay, 33% concentrate); low=LF (39% alfalfa hay, 61% concentrate)) and S (large=LS (≥3mm); small=SS (≤1mm)) the main factors. Diets (95 g of DM) were fed in four equal portions throughout the day. Temperature (39 ºC), pH (6.4), and liquid (10 %/h) and solid (5 %/h) dilution rates were maintained constant. During sampling days, collection vessels were maintained at 4 ºC and a composited sample of the 3 sampling days was taken for analyses (DM, OM, volatile fatty acids (VFA), total, ammonia and non-ammonia N, NDF, ADF and purine bases). The LAB and SAB were isolated on the last day of each experimental period. The LAB was obtained by filtration and differential centrifugation, and SAB was obtained by using a combination of detachment procedures (Whitehouse *et al*., 1994). Data were analyzed by the GLM procedure of SAS (1988, SAS Inst., Cary, NC).

Results. Apparent DM and OM digestibilities were higher in LF (44.6 and 35.5 %) compared to HF (41.6 and 31.2 %, $P<0.05$). There were no effects of F or S on true DM or OM, NDF or ADF digestibilities. Total VFA production was higher ($P<0.05$) for LF (122 mM) compared to HF (102 mM). Higher proportion of acetate was observed for HF (63.5%) compared to LF (58.2%). When SS was fed instead of LS, there was a reduction ($P<0.05$) in the acetate proportion (63.4 vs 58.3 %, respectively) and in the acetate to propionate ratio (3.13 vs 2.41, respectively). There were no F or S effect in ammonia production or in total, ammonia and non-ammonia N flows. Bacterial N flow was higher in SS compared to LS when LAB were used for calculations. In contrast, when SAB were used, these differences were not significant (Table 1). Efficiency of microbial protein synthesis (EMPS), expressed as g of bacteria N per kg of OM truly digested (g/kg OMTD) was affected by S when LAB were used for calculations (Table 1). However, the EMPS was affected by F when SAB were used. There were no differences in dietary N flow or protein degradation due to F or S.

Table 1. *Effects of fibre level and particle size on rumen microbial nitrogen metabolism*

Item		Diet[1]				SEM	P^2 <	
		HL	HS	LL	LS		F	S
Dietary N flow, g/d	LAB	1.43	1.21	1.48	1.36	0.10	NS	NS
	SAB	1.22	1.11	1.47	1.34	0.15	NS	NS
Bacterial N flow, g/d	LAB	0.94	1.33	0.97	1.02	0.08	NS	*
	SAB	1.14	1.43	1.00	1.04	0.13	NS	NS
Protein degradation, %	LAB	48.4	55.4	44.9	48.9	3.58	NS	NS
	SAB	55.9	59.1	45.4	49.5	5.32	NS	NS
EMPS, g/kg OMTD	LAB	26.8	34.9	27.2	27.7	1.91	NS	*
	SAB	30.7	35.8	26.9	26.7	1.88	**	NS

[1] Diet: HL= high fibre level, large particle size; HS= high fibre level, small particle size; LL= low fibre level, large particle size; LS= lowfibre level, small particle size. [2] Interaction F x S was not significant ($P>0.05$)

Conclusions. Diet LF resulted in higher apparent DM and OM digestibilities and total VFA compared to HF. Acetate proportion was higher in HF compared to LF. The acetate percentage and the acetate to propionate ratio were lower in SS compared to LS. These effects were independent of diet, pH, intake or dilution rates. Estimates of bacterial N flow and EMPS were dependent on the type of microbial population used for calculations and may result in different estimates of bacterial and dietary N flows to post-ruminal sites.

Acknowledgments. Financial support from CICYT (AGF97-0444) is acknowledged

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Flow of microbial and non-microbial N fractions entering the omasal canal in dairy cows

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Introduction In order to provide accurate estimates of protein supply for lactating dairy cows, the effect of feed ingredients on ruminal protein degradability as well as microbial protein synthesis must be determined. The omasal sampling technique coupled with a triple marker method allows the determination of N flow from the reticulorumen with relatively small contribution from endogenous sources (Ahvenjärvi et al., 2000). The objective of the current study was to assess the contribution of microbial and non-microbial fractions to the total flow of N leaving the rumen of lactating dairy cows fed typical feed ingredients.

Materials and methods Supplementation of grass silage with barley and rapeseed meal (RSM) was studied in a 4 × 4 latin square experiment using four lactating ruminally cannulated Finnish Ayshire dairy cows. Four experimental diets comprised grass silage fed alone (diet S) or that supplemented with 6 kg/d of barley (diet B), 2.1 kg/d of RSM (diet R) and 6 + 2.1 kg/d of barley and RSM, respectively (diet BR). Digesta flow entering the omasal canal was determined based on sampling of digesta from the omasal canal utilising a triple marker method based on indigestible NDF, Yb-acetate and LiCoEDTA (Ahvenjärvi et al., 2000). This procedure allowed calculation of chemical components entering the omasal canal associated with particle and liquid phases, the latter also comprised of small particulate matter. Ammonium sulphate (10% enrichment of ^{15}N) was continuously infused into the rumen to label rumen microbes with ^{15}N isotope. Enrichment of ^{15}N was determined in liquid (LAB) and particle associated bacteria (PAB) and protozoa (LAP). Non ammonia N (NAN) entering the omasal canal was fractionated into microbial NAN associated with LAP, PAB and LAP as well as non microbial NAN associated with particle and liquid phases. To determine LAP-NAN flow entering the omasal canal, LAP were isolated from a recorded volume of omasal canal digesta, and daily LAP-NAN flow was calculated based on total volume of digesta entering the omasal canal. Flow of PAB-NAN was calculated based on ^{15}N enrichment in PAB and particle phase, whereas LAB-NAN flow was calculated based on ^{15}N enrichment in LAB and liquid phase unaccounted for by ^{15}N in LAP.

Results Supplementation of grass silage diet with barley increased total NAN, LAB-NAN and LAP-NAN flow entering the omasal canal, but decreased non microbial NAN flow associated with large particle phase. Barley had no effect on apparent ruminal N digestibility, whereas true ruminal digestibility of N increased. Supplementation of grass silage diet with RSM increased total NAN flow and non microbial NAN flow in large particle phase. In addition, RSM increased apparent ruminal N digestibility, but had no effect on true ruminal N digestibility. Supplementation of grass silage diet had no discernible effects on the efficiency of microbial N synthesis.

Table 1. Effect of supplementation of grass silage diet with barley and RSM on the intake and omasal canal flow of N fractions

Item	Diet				SEM	*P*-value		
	S	B	R	BR		B	RSM	B×RSM
Intake, g/d	333	401	458	482	3.9	<0.001	<0.001	0.001
Omasal canal flow, g/d								
Total NAN	292	360	349	389	15.3	0.011	0.030	0.40
PAB-NAN	49	56	54	48	2.7	0.90	0.62	0.064
LAB-NAN	100	159	123	173	12.4	0.005	0.19	0.72
LAP-NAN	9	25	9	20	4.4	0.021	0.53	0.66
Non microbial NAN								
particulate phase	50	44	72	59	3.0	0.022	<0.001	0.35
liquid phase	83	76	91	89	6.5	0.54	0.15	0.76
Ruminal digestibility, g/g								
Apparent	0.120	0.100	0.234	0.198	0.0319	0.41	0.016	0.80
True	0.596	0.700	0.642	0.695	0.0181	0.005	0.30	0.21
Microbial efficiency								
Apparent†	21.8	24.6	23.2	24.3	1.79	0.32	0.77	0.65
True‡	17.6	19.2	18.5	19.2	1.15	0.35	0.74	0.71

†Microbial NAN g/kg OM apparently digested in the rumen
‡Microbial NAN g/kg OM truly digested in the rumen

Conclusions Supplementation of a grass silage diet with barley and RSM markedly increased the flow of NAN entering the omasal canal. For barley, the increase was attributable to an enhanced synthesis of LAB and LAP, whereas the effect of RSM could be accounted for by an increase in non-microbial NAN flow.

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Effect of fibre source on the efficiency of microbial synthesis by mixed microorganisms from the sheep rumen *in vitro*

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Introduction The efficiency and rate of microbial protein synthesis in the rumen depend on several factors, of which the chemical and physical properties of the plant fibre are among the most important. Estimation of microbial yield and fermentation rate can be obtained from *in vitro* experiments, which combine gas production and substrate degradability measurements and/or use microbial markers. The aim of this study was to investigate the effect of different fibre sources on microbial protein synthesis in a batch culture system *in vitro*.

Material and methods Neutral-detergent fibre (NDF) was prepared from 5 sources: cotton seed, bean straw, rye straw, citrus pulp and ryegrass hay by repeated washing in detergent. The chemical composition of the NDF varied, with acid-detergent fibre contents of 802, 820, 735, 812 and 660 g/kg NDF, and lignin concentrations of 217, 223, 108, 185 and 126 g/kg NDF for the cotton seed, bean straw, rye straw, citrus pulp and ryegrass hay NDF, respectively. NDF samples (500 mg) were incubated *in vitro* with 35 ml of buffered rumen fluid. Rumen fluid from four rumen-fistulated sheep fed a mixed diet was strained through two layers of nylon-cloth to remove particles and large protozoa, and incubated with buffer solution (1:2) at 39ºC under CO_2. 10 mg of NH_4Cl and 1 mg of $^{15}NH_4Cl$ was added to each bottle. A total of nine bottles were incubated for each fibre source. Gas production was measured in three bottles at 2, 4, 6, 8, 10, 12, 18, 24, 30, 48, 72 and 96 h using a pressure transducer. In the remaining bottles, gas production was measured after 24 h; the fermentation was then stopped by cooling the bottles in ice. The contents were transferred into centrifuge tubes and centrifuged at 20,000 g for 15 min. The supernatant was used for volatile fatty acids (VFA) and ^{15}N enrichment analysis. The incubation residue was freeze-dried and analysed to determine its NDF concentration, non-ammonia N content and the ^{15}N enrichment of the residue. Gas production values were fitted with time to an exponential model. The proportion of microbial N in the non-ammonia fraction of the incubation residue was estimated, based on the assumption that all microbial N was formed from ammonia, by dividing the total ^{15}N incorporation in the incubation residue by the ^{15}N enrichment of the supernatant. Biomass yield was also calculated as proposed by Blummel *et al* (1997) assuming a stoichiometrical relation between substrate fermented and the production of gas and VFA. Data were analysed by one-way ANOVA with NDF source as the only treatment factor.

Results Fermentation rates, as estimated from gas production data, differed between the five fibre sources, with the lowest value ($P<0.05$) obtained with cotton-seed NDF and the highest value ($P<0.05$) with NDF extracted from the citrus pulp and ryegrass hay (Table 1). Gas production, NDF degradability and total VFA production after 24 h of incubation followed a similar pattern. Microbial synthesis estimated from the gas production data and microbial N values obtained from ^{15}N were in good agreement, with the lowest synthesis recorded with cotton-seed fibre ($P<0.05$). Efficiency of microbial synthesis varied from 21.0 to 28.3 mg N/g DM fermented for the cotton-seed and the bean straw, respectively.

Table 1. Effect of fibre source on gas production and microbial synthesis parameters

	Cotton -seed	Bean straw	Rye straw	Citrus pulp	Ryegrass hay	s.e.d. (d.f.=10)
Fermentation rate (h^{-1})	0.023^{d}	0.044^{c}	0.053^{b}	0.072^{a}	0.070^{a}	.00186
Gas production 24 h (ml)	20.1^{d}	37.2^{c}	62.1^{b}	79.5^{a}	82.6^{a}	1.14
NDF degradability	0.260^{d}	0.381^{c}	0.491^{b}	0.727^{a}	0.705^{a}	.0090
Total VFA (µmol)	540^{e}	780^{d}	1375^{c}	1743^{b}	1952^{a}	54.5
Microbial contamination of incubation residue (mg)(1)	116^{c}	120^{c}	111^{c}	186^{a}	159^{b}	5.0
Microbial DM (mg) (2)	84^{d}	110^{c}	117^{c}	231^{a}	178^{b}	5.2
Net microbial N (mg) (3)	2.75^{c}	5.47^{b}	5.95^{b}	8.10^{a}	9.23^{a}	.373
Efficiency microbial synthesis (mg N/g DM fermented)	21.0^{b}	28.3^{a}	24.1^{ab}	21.9^{b}	26.0^{ab}	1.56

(1) DM washed out with the neutral-detergent solution (2) Microbial biomass estimated according to the calculations of Blummel et al. (1997) (3) Total ^{15}N incorporation = Total N in incubation residue x ^{15}N enrichment in incubation residue; Microbial N in incubation residue = Total ^{15}N incorporation /^{15}N enrichment in supernatant

Conclusions The results of this study show that the source of plant fibre has a profound effect on its rate of fermentation by rumen microorganisms. The efficiency of microbial protein synthesis *in vitro* also varied with the source of the fibre, but in a manner that was not correlated with the fermentation rate.

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In vitro microbial growth as affected by the type of carbohydrate and the source of nitrogen

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Introduction The nitrogen (N) requirements for optimum growth of ruminal micro-organisms are still a matter of controversy. Whereas the results of some experiments indicate that peptides and amino acids stimulate the growth of mixed rumen bacteria in comparison to ammonia, no effect due to the N form has been found in other studies. Therefore, it has been suggested that growth of rumen micro-organisms would be stimulated only when the rate of provision of energy permitted (Cruz Soto *et al*., 1994). The aim of this study was to investigate the effect of two N sources (ammonia and isolated soyabean protein) on the *in vitro* fermentation of two substrates (starch and cellulose) differing in their rate of fermentation.

Material and methods Four rumen-fistulated sheep fed on an alfalfa hay:barley (70:30) diet were used as rumen liquor donors. Rumen fluid from each sheep was strained through two layers of nylon-cloth (40 µm pore size) to remove particles and big protozoa, and mixed with buffer solution in a proportion 1:4 at 39ºC under continuous flushing with CO_2. In order to stimulate the growth of cellulolytic bacteria, isobutyric, isovaleric and valeric acids were added to the buffered rumen fluid. Portions of 500 mg of starch or cellulose were accurately weighed into 125-ml serum bottles. Isonitrogenous solutions of ammonia (NH_4Cl) and a mixture ammonia + isolated soyabean protein (50:50) were prepared, and 2 ml of the corresponding solution (containing 15 mg of N) were added to each bottle. The addition of N took place at the beginning of the incubation (1 ml) and after 9 h (1 ml) in order to assure the warranty of preformed amino acids. ^{15}N was used as a microbial marker. For each N treatment and substrate a total of 12 bottles were incubated at 39ºC with 50 ml of the buffered rumen liquor of each sheep. After 24 h for starch and 36 h for cellulose, the following parameters were determined. Gas production was measured in three bottles, the fermentation was then stopped, and the contents were transferred into centrifuge tubes and span at 20,000 g for 30 minutes. Volatile fatty acids (VFA) and ammonia-N concentrations were determined in the supernatant, and the incubation residue was freeze-dried to determine the apparent digestibility of substrates. The content of the remaining bottles (9) were homogenised and used to obtain bacterial pellets (by differential centrifugation of *c.* 300 g of the mixture) and total digesta (obtained after freeze-drying of 150 g of the mixture). The proportion of microbial N in the non-ammonia fraction of total digesta was estimated by dividing the 15N enrichment of total digesta by the enrichment of bacterial pellets. True digestibility of substrates was calculated considering the bacterial DM in each treatment estimated from 15N calculations. Within each substrate differences between N treatments in all measured parameters were assessed by analysis of variance, with N source and sheep as main effects.

Results The replacement of ammonia by soyabean protein resulted in a greater ($P<0.05$) VFA production for both substrates (Table 1). The presence of protein increased ($P<0.05$) gas production when starch was incubated and a tendency ($P<0.10$) in the same sense was detected for cellulose. The lower ($P<0.05$) ammonia-N concentration observed when starch was incubated with soyabean protein could be due to an incomplete degradation of the protein or to a greater utilisation of the protein by the rumen micro-organisms. Both microbial growth and its efficiency (mg microbial N/g DM truly fermented) were increased when starch was incubated in the presence of protein, but no differences ($P>0.05$) due to the type of N were detected for cellulose.

Table 1 *Effect of N source on* in vitro *fermentation of starch and cellulose (*a,b: $P<0.05$; †: $P<0.10$)

	Starch			Cellulose		
Item	NH_4Cl	Protein	s.e.d.	NH_4Cl	Protein	s.e.d.
VFA (mmol)	4.20 [a]	4.64 [b]	0.052	4.39 [a]	4.89 [b]	0.016
Gas (ml)	85.0 [a]	89.6 [b]	2.93	76.9	79.2 †	2.33
NH_3-N (mg/l)	316 [b]	249 [a]	13.3	384	366 †	16.2
True digestibility (g/kg)	905 [a]	922 [a]	4.6	747	755	2.6
Bacterial N (mg)	17.9 [a]	19.1 [b]	0.12	14.0	14.1	0.13
Efficiency of bacterial growth (mg bacterial N/g DM truly fermented)	39.6 [a]	41.4 [b]	0.40	36.9	37.4	0.34

Conclusions The results of this study would indicate that N forms other than ammonia are needed for maximum activity of rumen micro-organisms growing *in vitro*. However, the response of rumen micro-organisms to non-ammonia N forms seems to be affected by the characteristics of the fermented substrate.

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Variation between feedstuff degradabilities assessed using short-term *in vitro* incubations and a comparison with *in sacco* derived values

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Introduction While end-point *in vitro* techniques provide no information regarding the kinetics of feedstuff degradation they are generally simpler to conduct and require fewer inputs especially with respect to labour. In contrast systems, which permit degradation dynamics to be assessed, require that multiple measurements are made and be continued for a sufficient period to ensure that asymptotic values are reached. This study was therefore conducted to examine the variability that could be expected between short-term end-point *in vitro* degradability techniques and to compare some of the values with *in sacco* derived estimates.

Materials and methods Two sets of feeds were examined. The first, milled to 2 mm, consisted of grass hay (*H*), sugar-beet pulp (*SBP*), wheat straw (*WS1*) and maize silage (*MS1*) and the second, obtained from NSRU ADAS and for which the *in sacco* degradabilities were known, comprised two wheat straws (*WS2, WS3*), two maize silages (*MS2*, *MS3*) and grass silage (*GS*). The ADAS feeds had been milled to pass a 1 mm screen. Four techniques were utilised to estimate organic matter degradation (OMD) 48 h post-inoculation of the first set of feeds: the Reading Pressure Technique (*RPT*, Mauricio *et al*., 1999), the ANKOM technique (*ANK*, Mould and Nordheim, 1998) and the Tilley and Terry technique of Minson and McLeod (1972) both with (*TT+*) and without (*TT–*) inclusion of the final acidified-pepsin phase. A similar buffer solution was used in all techniques. The study to determine RPT estimates for the ADAS feeds was conducted separately but included the first set of feeds as controls. Rumen fluid inoculum was obtained from a dry cow offered grass hay *ad libitum* plus 1.0 kg concentrate daily. SAS procedures were utilised to identify significant differences between techniques and to generate correlations and regression equations.

Results and discussion Although significant differences between systems in terms of OMD were identified, all ranked the feeds similarly and were highly correlated (Tables 1 and 2). However, relative to the other systems and with the exception of *SBP*, the ANKOM technique significantly underestimated degradability. This is probably a direct result of either bag pore size restricting both microbial access and wash-out of degradation end-products or to the production of a micro-environment within the bag inhibiting fermentation. The difference between RPT and TT is likely to be a consequence of the smaller sample size (1.0 and 0.5 g, respectively) and both the different sample weight : buffer ratio and buffer : inoculum ratio used. The effect of including the acidified-pepsin stage in the TT analysis was variable and non-significant. No significant differences were found between the RPT analyses indicating good repeatability and a highly significant regression between RPT and *in sacco* values was observed (*in sacco* = 0.786*RPT* + 0.163, r^2 = 0.911, *P> 0.001*)The slightly lower RPT values probably result from the different incubation conditions and to greater feed particle losses with the *in sacco* technique.

Table 1 *Variation in OMD estimation (g / kg) due to technique*

Feed	ANK	TT-	TT+	RPT1‡	RPT2‡	*in sacco*
H	375b†	512a	525a	533a	511a	-
MS1	571c	679b	734a	765a	743a	-
SBP	927ab	897b	894b	951a	933a	-
WS1	221c	332b	320b	459a	454a	-
WS2	-	-	-	-	402	459
WS3	-	-	-	-	477	547
MS2	-	-	-	-	703	688
MS3	-	-	-	-	599	676
GS	-	-	-	-	610	643

† LS means in rows without similar letters are significantly different (*P<0.05*)
‡ RPT1 and RPT2 – first and second RPT analyses, respectively

Table 2 *Correlation analyses - in vitro techniques*

	TT-	TT+	RPT1	RPT2
ANK	0.978	0.954	0.976	0.975
TT-	1	0.991	0.983	0.967
TT+	0.991	1	0.976	0.967
RPT1	0.983	0.976	1	0.997
RPT2	0.976	0.967	0.997	1

R values, *P> 0.001*

Conclusions Despite the range of variables within each *in vitro* system, the OMD values obtained 48 h post-inoculation were remarkably consistent and highly correlated. The significant regression between *in sacco* and RPT values suggests that *in vitro* techniques have the potential to replace techniques which require numerous surgically modified animals.

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The effect of diet on rumen chitin content in sheep

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Introduction Measurement of *in situ* fungal populations is needed in order to determine the extent of their contribution to the ruminal digestive process. Since chitin is present in the cell wall of rumen fungi (Orpin, 1977), measurement of this polymer can be used to estimate fungal biomass and their growth *in vitro* (Rezaeian, 1996) and *in vivo* (Argyle and Douglas, 1989). The objective of this experiment was to study the effect of diet composition on the chitin content of both ruminal fluid and digesta solids as an index of the fungal population in the rumen.

Materials and methods Four rumen fistulated sheep (live weight 35- 40 kg) were given 150 g hay and 500 g lucerne pellets defined as low hay content diet (LHC) and 500 g hay and 550 g lucerne pellets defined as medium hay content diet (MHC) for two consecutive 6-week experimental period respectively. Samples of rumen content were taken just before feeding (0 time) and at 2, 8 and 16 h after the morning feed. They were then strained and samples of rumen fluid (SRF) and separated plant particles (PLP) were collected for chitin analysis using the feed samples as the control material. Microscopical examination were also performed on the SRF samples taken 2 h after morning feeding to determine the presence of the fungal zoospores. The chitin content of samples was determined from the glucosamine hydrochloride equivalent resulting from hydrolysis as described by Chen and Johnson (1983).

Results Motile zoospores were observed in all of the SRF samples taken from the four experimental animals. In general, the increase in the proportion of hay to pelleted Lucerne in the diet resulted in an increase in the level of chitin in the both SRF and PLP fractions of ruminal digesta. The mean values of those in medium hay composition diet (MHC) was 14.4 and 4.92 mg/g DM in SRF and PLP samples respectively compared to low hay composition diet (LHC) which was 12.4 and 3.33 mg/g DM (Table 1). These differences were statistically significant ($p<0.05$) at different sampling times. The chitin component (mg/g DM) of SRF samples was also higher than that of PLP samples in both diets. The ratio of chitin concentration in SRF samples when compared to PLP samples was 3.7:1 and 2.9:1 for the LHC and MHC diets respectively.

Table 1 *Chitin component (mg/g DM) of the strained rumen fluid and the plant particles in sheep fed a diet with either low hay content (LHC) or medium hay content (MHC) twice a day.*

Time after	strained rumen fluid				plant particles			
Morning	Diet types				Diet types			
Feeding (h)	LHC	MHC	SE	Sig.	LHC	MHC	SE	Sig.
0	12.6	15.1	0.34	**	3.08	5.22	0.281	**
2	12.2	13.6	0.37	*	3.25	4.72	0.273	**
8	12.3	14.5	0.53	*	3.51	5.09	0.357	*
16	12.7	14.4	0.50	*	3.49	4.64	0.286	*
Mean	12.4	14.4	0.35	**	3.33	4.92	0.267	**

Values are means of four sheep. Sig. = Significant *= P<0.05 **= P<0.01 SE = Standard error of mean.

Conclusion Significant differences of the chitin content of both SRF and PLP samples between two different diets not only showed the effect of the diet on the level of this fungal marker in the rumen but also indicates the validity of the assay to demonstrate this difference. Therefore, the increase in the level of rumen chitin in the MHC diet compared with the LHC diet must be due to the increased proportion of hay in the diet, which seems to favour fungal development over pelleted lucerne. Whether the differences in the levels of chitin in the rumen of animals fed different diets is a consequence of the indirect effect on fungal population or antagonists needs to be elucidated.

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Effect of zeolite nutrition on rumen ecosystem in dairy cow

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Introduction Feeding dairy cows diet formulation by zeolite has been reported improved the efficiency of milk yield (Roussel ,1992). Lupez et al (1992) reported that efficiency of ion exchange in rumen was improved When animals fed with diets containing zeolite. The objective of this study was to determine the effect of zeolite concentration on rumen pH, liquid smell, bacterial flora, protozoa number, methylene blue reduction time and the sedimentation and flotation period.

Materials and methods Ninety holestein dairy cows are randomly received one of the 3 diet in a completely randomized design. The composition of diets (A, B, C) has been showed in Table 1 and formulated based on NRC (1989) and had predicted NE_l contents 1.5 Mcal/kgDM and CP 170g/kgDM .Each diet had different amount of zeolite .The period of the present study was 21 days .The rumen fluid of ten cows of each treatment was obtained by stomach tube.The effect of zeolite was determined on rumen pH liquid smell, bacterial flora, protozoa number, methylene blue reduction time and the sedimentation and flotation period by using Dirksen (1987) assay.

Table 1 The compositional of the experimental (g/kgDM)

Ingredients	A	B	C
Lucerne	400	400	400
Barley	228	228	228
Wheat Bran	207	190	180
Cottonseed meal	138	138	138
Zeolite	0	18	30
Vit & Min	27	26	24

Results When zeolite in diet of cow there was used 18g/kgDM in diet of cow , there was no significant effect on rumen pH, while there was a significant decrease ($p<.05$) in rumen pH in the cow was fed with 30g/kgDM zeolite(Table 2).Zeolite had no considerable effect on rumen content smell and bacterial flora and methylene blue reduction time.The sedimentation and flotation time was increased significantly due to zeolite ($p<.05$),but there was no significant difference between the 2 zeolite levels.

Table 2 Effect of zeolite level on rumen ecosystem[1]

Item	0	1.8%	3%	SE
Bacteriology	gr-	gr-	gr-	-
Liquid smell	natural	natural	natural	-
Protozoa ($\times 10^6$ /ml)	14.5	16.71	13.85	0.7
PH	6.87^a	6.71^a	6.34^b	0.11
Sedimentation and Flotation time (sec)	170^b	350^a	352.86^a	11.71
Methylene blue reduction time (sec)	191.25	139.29	175.5	27.09

1- $P<.05$

Conclusion zeolite utilization in the dairy cow diet had no negative effect on the rumen pH, liquid smell, bacterial flora , protozoa number, methylene blue reduction time and the sedimentation and flotation. Therefore, in practical terms the determination of optimum level of feeding zeolite in dairy cow needs to further research.

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Characterisation of proteolytic activity of rumen microbes and commercial proteases

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Introduction Commercial proteases yield more reproducible estimates of extent of rumen protein degradation than either in situ or rumen in vitro methods. Although extents of protein digestion determined using commercial proteases have been positively correlated to extents of in situ protein degradation, these values have not proven reliable predictors of rates of rumen protein degradation. This is because no protease-based assay has yet been developed that successfully mimics the specificities of the organisms involved in protein degradation in the rumen. If the proteolytic activity of rumen microbes and commercial proteases can be correctly characterised, then it would be possible to elaborate protease blends that match the protein degradative activity of mixed rumen microbes. The research reported here is an extension of earlier work as a prelude to preparing enzyme mixes with "protease fingerprints" similar to rumen microbes.

Materials and Methods Three separate incubations were conducted using rumen contents from two cows fitted with permanent rumen cannulae and fed a diet of (DM basis) 40% lucerne silage, 20% maize silage, 29% high moisture maize, 10% soyabean meal plus 1% mineral-vitamin supplement. Digesta was strained through four layers of cheese cloth to yield strained rumen fluid (SRF). Equal SRF volumes from each cow were mixed and the mix diluted with an equal volume of 0.1 M sodium phosphate buffer (SPB; pH 7.0). Glucose (1 g/100 ml) and 2.0 mM mercaptoethanol were added to SPB-SRF and held under CO_2 at 39°C until incubations began (< 15 min. later). Nineteen commercial proteases also were tested in two separate incubations. Fifteen protease substrates, all *p*-nitroanilide (pNA) derivatives of amino acids or peptides, were incubated. Each pNA substrate was dissolved at 0.30 mM in SPB containing 20% ethanol. Incubation tubes contained 0.30 ml of SPB plus 0.30 ml of a substrate solution; in blanks, 0.3 ml of carrier (SPB plus 20% ethanol) replaced substrate solution. Tubes were warmed in a 39°C room; incubations were begun by adding 0.30 ml of 39°C inoculum (either SPB-SRF or commercial protease dissolved in SPB). Tube headspace was gassed with CO_2 (SRF only), tubes capped and incubated with shaking at ~150 RPM for 0 or 40 min. in the 39°C room. Incubations were stopped by adding 0.20 ml of 100% TCA (final concentration, 18% TCA); after 30 min., tubes were centrifuged (30 min.; 10,000 x g). Incubation tubes were run in triplicate with SRF and in duplicate with commercial proteases. Extent of substrate hydrolysis was determined from net release of free pNA in TCA supernatants by reaction with 2-dimethyl amino cinnamaldehyde using pure pNA standards. Absorbance was read at 530 nm in 96-well plates using a plate reader. Rates of hydrolysis of each substrate were expressed as micromol/h per ml for each enzyme source; a relative hydrolysis rate, expressed as a proportion of the rate observed for SRF, was computed for each substrate for all commercial proteases. Significance for relative rates of hydrolysis of each substrate due to source of commercial protease was determined with a one-way analysis of variance model using the GLM of SAS; differences among means were determined by LSD at alpha = 0.05.

Results Mean rates of hydrolysis by SRF varied greatly among the 15 pNA substrates, ranging from about 0 (for Glu-pNA and N-Succinyl-AlaAlaAla-pNA) to nearly complete hydrolysis (for Leu-pNA and Pro-pNA) over 40 min. incubations. Because their rates of hydrolysis were very slow, Glu-pNA and N-Succinyl-AlaAlaAla-pNA were excluded from further tests; the SRF fingerprint from the remaining 13 substrates was used to characterise activity of the 19 commercial proteases. Relative rates of hydrolysis of GlyPro-pNA did not differ due to protease source (P = 0.60). Nine commercial proteases (collagenase, ficin, pepsin, Aspergillus saitoi, Bacillus licheniformis, Bacillus polymyxa, Bacillus thermoproteolyticus, bacterial protease, and Rhizopus species) had very little activity toward any of the remaining 12 substrates. No mean relative rate of hydrolysis was greater than the LSD(0.05) for any of the 12 substrates; thus, none of these nine had activity that was significantly greater than zero. The low activity of ficin was surprising in view of its extensive application for assessing feed protein degradability. Four proteases had significant activity toward only one substrate: chymotrypsin (Benzoyl-Tyr-pNA activity only), Proteinase K (AlaAlaAla-pNA), Streptomyces caespitosus (Leu-pNA), and trypsin (Benzoyl-Arg-pNA); other proteases had significant (P < 0.05) activity toward 2-10 substrates. A complementary pattern was noted for pancreatin and Aspergillus oryzae protease. Two activities missing or very low in Aspergillus oryzae, plus activity toward GlyPro-pNA, were present in substantial amounts in pancreatin; together, these proteases hydrolysed significant amounts of all substrates. Lowest relative activity was toward Pro-pNA: Aspergillus oryzae hydrolysed it at only 9% of the rate of SRF. Activity toward all of the remaining substrates was >30%, often >100%, of SRF activity. Aspergillus oryzae was the only commercial protease tested that cleaved >1% of the Pro-pNA hydrolysed by SRF. Whether this activity is important in determining protein degradation rate in the rumen is unknown. Mixing enough Aspergillus oryzae and pancreatin to yield a blend in which the lowest relative activity for each equaled 100% of that in SRF also would result in several activities ranging from about 5 to 30 times that in SRF.

Conclusions These preliminary results show the promise of characterising proteolytic activity of SRF and commercial proteases for preparing protease blends that mimic mixed rumen microbes. The next step is to test mixes of Aspergillus oryzae and pancreatin proteases for effectiveness for predicting rates and extents of rumen protein degradation.

The impact of hexose partitioning in sheep *in vivo*

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Introduction Methane production represents an important sink for hydrogen within the rumen Beever (1993) suggested that the partitioning of fermentable dry matter (DM) between microbial synthesis and fermentation products would alter the pattern of hydrogen production and hence methanogenesis. This hypothesis was investigated *in vitro* using a range of diets varying in carbohydrate source (Moss *et al*., 2000). Methane production (moles) increased as the proportion of DM fermented to short chain fatty acids (SCFA) increased and this was related to decreasing water soluble carbohydrate (WSC) to cell wall (NDF) ratio of the diet. The objectives of the current study was to design diets with a range of WSC:NDF ratios and to measure the impact on hexose partitioning and methane production in sheep *in vivo*.

Materials and methods Four diets comprising on a DM basis 0.25 grass hay, 0.05 rolled barley and either 0.70, 0.47, 0.23 or 0.00 molassed sugar beet shreds (MSBF), with the remaining portion as unmolassed sugar beet shreds (UMSBF) were offered at the maintenance plane of nutrition to wether sheep in two equal feeds at 08.45 and 16.45h in a 4 x 4 Latin square design. Fresh water was available at all times. The study comprised four 21d periods with 10d acclimatisation to the diet, 7d collection of faeces and urine followed by four 24h measurements of methane production determined in open-circuit respiration chambers. The respective diets were also incubated in duplicate *in sacco* at 0, 2, 5, 8, 12, 24, 48 and 72h to provide an estimation of rumen degradable OM. SCFA profiles were determined on day 11 at 0, 0.5, 1, 2, 4, 6, 8, 8.5, 9, 10, 12 and 14h postfeeding. Feeds were analysed for DM, ash, crude protein, starch, WSC and NDF. An index of microbial protein output was estimated by determining daily urine allantoin and was used as a measure of hexose partitioning. The data were analysed by analysis of variance using Minitab.

Results The diets contained a relatively constant amount of starch (34 to 37 g kg^{-1} DM) with 408, 452, 498, 543 g kg^{-1} DM NDF and 234, 187, 138, 89 g kg^{-1} DM WSC for 0.70, 0.47, 0.23 and 0.00 MSBF diet respectively. The ratio of WSC:NDF ranged from 0.57 to 0.16 for the high and zero MSBF diets respectively. The apparent digestibility of OM, the 24h rumen degradable OM (*in sacco*), methane production and urine allantoin output are shown in Table 1.There was no significant effect of diet on total SCFA concentration but prior to the morning feed there was a significant difference ($P<0.05$) in total SCFA concentration between the high and zero MSBF diets (50.4 and 58.1 mmol l^{-1}) suggesting that more of the rumen degradable OM has been fermented.

Table 1 *Apparent digestibility of organic matter (OM), 24h in sacco rumen degradable OM and methane production from diets containing 0.700, 0.47, 0.23 and 0.00 molassed sugar beet feed*

Measurement	Treatment, inclusion of molassed sugar beet feed				
	0.70	0.47	0.23	0.00	SED
Apparent digestibility of OM	749	757	741	753	17.1
Rumen degradable OM at 24h (*in sacco*)	751	641	589	532	14.5***
Methane (l d^{-1})	28.3	27.7	27.1	26.4	0.44***
Methane (l kg^{-1} rumen degradable OM)	60.5	72.0	74.8	83.7	2.45***
Urine allantoin output (mmol per day)	20.2	22.9	12.2	11.0	2.09***

*** $P<0.001$

Conclusion The partitioning observed *in vitro* were confirmed *in vivo*. It can be concluded that the partitioning of hexose into fermentation end-products (including methane) and microbial biomass in the rumen (*in vivo*) is influenced by the dietary carbohydrate source as observed *in vitro* (Moss *et al*., 2000). Increasing the proportion of WSC in the ration from 0.089 to 0.234 decreased methane production (l kg^{-1} rumen degradable OM) by 20.2 which is a significant reduction. As methane production expressed per kg OM apparently digested was unaffected by diet further work is required in productive animals to confirm that the improved rumen efficiency is reflected in increased animal productivity and that methane production per unit product is reduced.

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The effects of lysine:energy density on performance and nitrogen balance of 50, 65 and 80 kg pigs

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Introduction: Pig producers are under pressure from cost production and are having to become increasingly environmentally aware. Phase-feeding has been suggested as an alternative feeding strategy, which may have the potential to improve productivity and reduce nitrogen excretion in the growing-finishing period. The objective of this study was to evaluate the effect of mono- versus tri-phase feeding on pig performance and nitrogen balance.

Materials and Methods: Six boars and 6 gilts were randomly allocated to 1 of 3 treatments; high lysine (lys): digestible energy (DE) (H), low lys:DE (L) and tri-phase (P). Pigs were grouped by sex in partially slatted pens of six with a space allowance of 1.4m^2. Treatment H was fed a 0.89 g lys:MJ DE (g:MJ) diet from 38 kg to slaughter, treatment L was fed 0.7 g:MJ from 38 kg to slaughter and treatment P was fed 0.89 g:MJ from 35 to 55 kg (Early Grower, EG), 0.80 g:MJ from 55 to 75 kg (Late Grower, LG) and 0.70 g:MJ from 75 kg to slaughter (Finisher, F). Performance was measured in terms of average daily gain (ADG, $g.d^{-1}$), average daily feed intake (ADI, $kg.d^{-1}$) and feed conversion ratio (FCR). Carcass protein deposition rate (PDR, $g.d^{-1}$) was estimated by the method of Moughan *et al*. (1995). Rate of carcass gain (DCG, $g.d^{-1}$) and carcass FCR (CFCR) was calculated. For the nitrogen balance experiment, groups of five boars fed one of the three dietary treatments corresponding to those in the performance trial, were placed in individual crates. Collection was conducted over 5-day periods commencing at 50, 65 and 80 kg. Faeces and urine were collected separately and analysed for nitrogen content to facilitate the estimation of; daily rate of nitrogen retention (NR) per metabolic live weight ($W^{0.75}$) ($NW^{0.75}$, $g.kg.d^{-1}$), daily rate of nitrogen output per $W^{0.75}$ (NO, $g.kg.d^{-1}$) and the ratio between NR and absorbed nitrogen (AN) (NR:AN). Pigs were slaughtered at 98.1 kg and lean meat percentage (LM, g/kg) was recorded. Data was analysed using the SAS general linear model.

Results:

Table 1. Effect of feeding regime on nitrogen retention, output and efficiency (l.s.m.±.s.e.m.)

	H	P	L	s.e.m.
$NR_{50\ kg}$	1.33		1.30	0.173
$NO_{50\ kg}$	1.56[a]		1.29[b]	0.083
$NR:AN_{50\ kg}$	0.534		0.589	0.040
$NR_{65\ kg}$	1.62[b]	1.57[b]	1.30[a]	0.081
$NO_{65\ kg}$	1.52	1.51	1.41	0.126
$NR:AN_{65\ kg}$	0.583	0.580	0.595	0.029
$NR_{80\ kg}$	1.43	1.50	1.32	0.124
$NO_{80\ kg}$	1.85	1.60	1.60	1.604
$NR:AN_{80\ kg}$	0.495	0.536	0.573	0.032

Table 2. Effect of feeding regeime on pig performance and carcass characteristics (l.s.m.±.s.e.m.)

	H	P	L	s.e.m.
ADG	906	852	849	24.8
ADI	2.20	2.28	2.17	0.053
FCR	2.45[b]	2.69[a]	2.57[ab]	0.059
LM	587.8	568.6	572.1	9.013
DCG	669.3[b]	636.0[ab]	616.9[a]	20.19
CFCR	3.27[b]	3.69[a]	3.65[a]	0.118
PDR	158	147	144	0.060

([abc] results sharing the same superscript are not significantly (P = 0.05) different.)

Conclusions: The results of the performance experiment suggest that the tri-phase (P) feeding offered no significant benefit in terms of ADG, indeed FCR was adversely affected. The results of the nitrogen balance support this view and lead us to draw the following opinion: Feeding high lysine:DE in the early and late grower periods results in superior performance. However pigs fed low lysine:DE appeared to compensate for this shortfall in early growth by maintaining a higher ratio of nitrogen utilisation (expressed as NR:AN) into the finisher (80 kg) period. It would appear that this sustained efficiency, while not significantly different, facilitated the pigs on the low lysine:DE treatment maintain similar overall performance and carcass characteristics as those supplied with a high lysine:DE diet. When multiple diets of reduced lysine:DE density were supplied, growth performance was constrained before there maximum needs for nitrogen retention at 70.8 kg proposed by Van Lunen and Cole (1998) was met, and compared to L pigs, P pigs were not been 'primed' with a low lysine:DE diet to allow them to achieve the same efficiency of nitrogen utilisation over the final phase. Thus if a phase feeding approach is to be successfully employed, it is important that no reduction in dietary density occurs before the pigs maximum rate of protein deposition has been reached and they have truly entered the 'finishing' period.

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The relationships between plasma glucose and insulin concentrations, and growth performance in German Pietrain and Large White porcine genotypes

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Introduction Insulin is a central regulatory factor in the control of nutrient partitioning during growth and development. It has been demonstrated that the rate of protein deposition is elevated in porcine muscle following insulin infusion (WrayCahen 1998), and that the degree of adiposity is positively associated with plasma insulin concentration (Wood *et al.*, 1977; Polonsky 1995). The objective of the study was to examine the effect of plasma glucose and insulin concentrations on growth performance in two porcine genotypes.

Materials and Methods Commercial sows were artificially inseminated with semen from either German Pietrain (P) or Large White boars (LW), and a random sample of their subsequent progeny (P n=11: LW n=13) were entered into the study at 3 months of age (Body weight; P: 27.2±0.6; LW: 27.5±0.6 kg; mean±SEM). Pigs were individually fed *ad libitum* (Growlean OP meal, 14 MJ kg^{-1}, BOCM, UK) using FIRE (Food Intake Record Equipment) feeders, and were humanely slaughtered at 5-6 months of age (Body weight; P: 96.8±2.7; LW: 100.2±2.4 kg; mean±SEM). At 24 h prior to slaughter, eye muscle and backfat thickness were measured using ultrasound scanning, and a jugular blood sample was obtained from each pig for determination of plasma glucose (Glucose GOD/PAP, Randox Laboratories Ltd, UK) and insulin concentrations (Insulin ELISA kit, Diagnostic System Laboratories Inc., UK). Differences between genotypes were assessed using the General Linear Model of the Minitab program version 13. Regression analysis was used to determine whether there was a relationship between growth performance parameters and plasma concentrations of insulin and glucose.

Results Feed intake ranged between 1400 – 2200 g/day during the experimental period and was similar between the two groups. Growth rate ($P<0.05$), circulating glucose levels ($P<0.01$) and plasma glucose:insulin ratio ($P<0.05$) were significantly higher in the LW group (Table 1). There were no differences in eye muscle depth, backfat thickness or plasma insulin concentrations between the two porcine genotypes. However, backfat thickness was related ($P<0.01$) to insulin concentration in LW pigs but this association was not apparent in the P genotype. The regression equations were:

LW: Backfat thickness = -79.7 + 49.9 insulin (R^2=46%)
P: Backfat thickness = -9.7 + 11.7 insulin (R^2=9%)

Table 1 Growth performance parameters, plasma glucose and insulin concentration in two porcine genotypes

	Pietrain (n=11)	Large White (n=13)
Growth rate (kg/d)	0.90±0.04*	1.07±0.05
Eye muscle depth (mm)	62.4±1.2	62.7±1.9
Backfat thickness (mm)	11.8±0.6	11.7±0.9
Plasma glucose concentration (mM)	5.10±0.27**	5.90±0.28
Plasma insulin concentration (µIU/ml)	1.84±0.02	1.83±0.01
Glucose:insulin	2.77±0.15*	3.22±0.16

Values are means±SEM. *P<0.05, **P<0.01.

Conclusions In conclusion, the growth rates observed may partly be attributed to alterations in glucose metabolism. Further work is required to explain the difference in the relationship between circulating insulin levels and carcass backfat thickness observed in P and LW pigs.

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Use of soluble spray dried porcine plasma in the water supply enhances piglet growth and intestinal integrity post weaning.

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Introduction A number of antibiotic growth promoters commonly used in EU pig diets have recently been banned following increasing public concern about antibiotic resistance and residues in meat products. Consumer and retail pressure has led to calls for research to identify alternatives to antibiotic growth promoters. It is therefore appropriate to investigate the use of feed ingredients which promote growth and intestinal health for the newly weaned piglet. Piglets which receive diets containing spray dried porcine plasma (SDPP) in the first week after weaning usually show improved feed intakes and growth rates when compared to piglets receiving conventional skim milk based starter diets (Toplis and Miller, 1999). The aim of this experiment was to determine whether similar improvements in piglet performance could be achieved when piglets received a soluble form of SDPP in their water supply instead of in the feed. In addition we investigated whether supplementation of the water supply of piglets already receiving a plasma containing diet further improved their post weaning performance. Although commonly used in Europe SDPP is currently excluded from piglet diets in the UK.

Materials and methods One hundred and twenty eight crossbred piglets (62.5% Large White, 25% Landrace, 12.5% Duroc) were weaned into fully slatted flat deck pens. The piglets were weaned at an average of 25.1 days of age (sd = 4.22) at a mean weight of 7.8 kg (sd = 1.91). Piglets had not received creep feed prior to weaning.. Eight piglets were allocated to each pen (1.37 m x 1.43 m) on the basis of litter, liveweight and sex. There were four treatments fed until day 14 after weaning: A Control - no plasma, B plasma in water, C plasma in feed, D plasma in water and feed. The soluble plasma product was included in the water of designated groups of pigs for 5 days at a concentration of 2.4 %, then for a further 5 days at 1.2 % and for a further four days at 0.7 %. Four pens were randomly allocated to each of the four treatments. All diets contained zinc oxide and were formulated to contain 16.25 MJ DE/kg, 16.5 g total lysine/kg. From day 14 onwards all piglets received the same diet (15.0 MJ DE/kg, 15.0 g total lysine/kg) and no supplementation in the water. Piglets were individually weighed at weaning and 7, 14 and 20 days after weaning. Food and water were provided *ad libitum* throughout the 20 day trial period. Data were analysed using the GLM procedure of Minitab 12.2.

Results Piglet performance during the experiment is given in Table 1. Growth rate and feed conversion ratio were significantly better for all plasma supplemented piglets than control piglets in week 1 ($P<0.05$). There was no additional benefit from providing plasma in both feed and water. End weights of plasma fed pigs were numerically greater than those of control pigs but this failed to reach significance. Feed intake over the 20 days of the trial was significantly greater for piglets which received plasma in the feed only than for any other treatment ($P<0.05$).

Table 1 *Piglet start weights, overall average daily gains (DLWG, g), average daily feed intakes (ADFI, g), feed conversion ratio (FCR) and weights at the end of the trial for piglets receiving plasma in feed and or water or neither for the first 20 days after weaning.*

	Start weight (kg)	Week 1 FI	Week 1 DLWG	Week 1 FCR	End weight (kg)	Overall FI	Overall DLWG	Overall FCR
Control	7.7	140	117^{a}	1.50^{a}	13.9	337^{a}	291	1.16
Plasma in water	7.7	163	184^{b}	0.90^{b}	14.4	345^{a}	319	1.08
Plasma in feed	7.9	188	184^{b}	1.13^{b}	14.8	391^{b}	345	1.13
Plasma in both	7.8	168	185^{b}	0.92^{b}	14.3	354^{a}	321	1.11
SEM	*0.35*	*10.8*	*14.0*	*0.07*	*0.31*	*9.1*	*13.6*	*0.03*
Significance	NS	NS	*	**	NS	*	NS	NS

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

Conclusions All three plasma treatments were equally effective in stimulating piglet performance during the first week of the trial. Therefore it appears that including plasma in the water is as effective as providing it in the diet. There was no further advantage to having plasma in both diet and water. A surprising result was that there appeared to be a negative carry over effect of feeding both plasma in the diet and plasma in the water during the third week of the trial when all piglets were on the same diet. These piglets grew more slowly in the third week than those which had received plasma in the feed only.

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The effects of liquid feed on the small intestine mucosa and performance of finishing pigs at different water to feed ratios

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Introduction Liquid feeding of growing pigs is believed to increase feed intake and growth performance compared to a dry diet. There is little information on the comparative feed conversion efficiency of pigs on the two forms of diet or the underlying physiological basis for the differences and how liquid diets affect the absorptive mucosa of the small intestine. This study was designed to investigate differences in feed utilisation and mucosal structure in modern genotype pigs offered equal amounts of feed in dry and liquid form. Additionally, the effect of reducing the pH of the liquid diet was considered.

Materials and methods Sixty four Large white x Landrace boars with a mean liveweight of 47.2 (s.e. 0.54) kg were randomly allocated to pens of eight pigs and bedded on straw and fed in individual feeders. The trial was arranged as a completely randomised design. The four treatments were based on the same diet (14 MJ DE/kg, 19% CP, 11.5g lysine/kg) and offered either as dry pellets, or soaked in water for four hours at water to feed ratios of 1.5:1 or 3:1 and 3:1 with a lactic acid inclusion to achieve a pH of 4 (3:1-4). Equal weights of dry pellet were offered in each treatment three times a day and the daily allowance was increased weekly from 1.45 kg to 2.65 kg of air dry feed. This scale was designed to be just less than the *ad libitum* intake of comparable pigs and in practice all the feed was consumed. The pigs were weighed at the same time each week and backfat recorded fortnightly. At slaughter, four pigs from each treatment were killed and tissue samples taken from the small intestine at sites 2, 20 and 50% (A, B, C) along its length from the gastric pylorus. The samples were immediately fixed in gluteraldehyde and sections prepared for examination by light and scanning electron microscopy. Ten well orientated villi and their adjoining crypts were measured and the mucosa examined and graded on a scale of zero (all finger villi) to seven (flat mucosa). The gastrointestinal components were measured and weighed. Differences in performance, villus height and grade were assessed by analysis of variance using Genstat 5.4 and significance between treatments determined by least significant difference.

Results Table 1 shows that pigs offered the 3:1 and 3:1-4 diets had significantly (P<0.05) higher average daily gain (ADG) than groups on the dry diet but they consumed marginally less fed. There was a highly significant difference (P<0.001) in FCR between the groups and all liquid diets were superior to dry pellets but the 3:1-4 group was also significantly better than 1.5:1. There were no differences in backfat levels between the treatments groups but the ratio of digestible energy intake to liveweight gain was significantly higher in the dry fed pigs. At each section of the small intestine sampled, villus height was significantly greater in all the liquid fed groups. At position B there were also differences between the three liquid fed groups with the 3:1 group having shorter villi. The grades indicate that differences in villus form were identified throughout the sections of the intestine but these were greatest in the duodenum and early jejunum.

Table 1 Effect of liquid feed on performance

	Dry	**1.5:1**	**3:1**	**3:1-4**	**s.e.d.**
DFI kg/day	2.00^{b}	2.00^{b}	1.94^{a}	1.94^{a}	0.187***
ADG g/day	0.96^{b}	1.04^{ab}	1.05^{a}	1.09^{a}	0.040*
FCR	2.09^{c}	1.94^{b}	1.87^{ab}	1.79^{a}	0.073***
Backfat mm	10.3	10.4	10.6	10.7	0.6 NS
DE/lwt gain	28.8^{b}	26.5^{a}	25.5^{a}	25.0^{a}	1.0**

Table 2 Villus height in liquid and dry fed slaughter pigs (μm)

Section	**Dry**	**1.5:1**	**3:1**	**3:1-4**	**s.e.d.**
A Villus ht	219^{b}	308^{a}	348^{a}	318^{a}	33.8***
Grade	4.8^{c}	4.4^{bc}	3.0^{ab}	2.1^{a}	0.77**
B Villus ht	209^{d}	338^{b}	261^{c}	383^{a}	24.7***
Grade	5.0^{c}	3.75^{b}	3.1^{b}	2.0^{a}	0.42***
C Villus ht	249^{b}	402^{a}	352^{a}	395^{a}	35.9***
Grade	4.7^{b}	4.6^{b}	4.3^{ab}	3.1^{a}	0.54*

**P<0.05 ** P<0.01 *** P<0.001
mean values with the same superscript are not significantly different (P<0.05)

Conclusions In the absence of differences in daily feed intake, liquid feed was utilised more efficiently by pigs during the growing finishing phase. The water content of the liquid diets did not appear to affect pig performance between the liquid fed groups and there was no clear advantage of acidification. Villus height and form clearly deteriorated in the dry fed animals implying a much reduced digestive and absorptive surface area in the small intestine which may explain in part, the better fed utilisation of the liquid fed groups. As feed intake was largely equal, the differences in mucosal morphology appear to result directly from the physical form of the diet. Observations with scanning electron microscopy revealed increased levels of villus tip erosion and disruption to the brush border in the dry fed pigs.

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The effects of liquid feed on the small intestine mucosa and performance of piglets at 28 days postweaning.

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Introduction It is well established that villus atrophy and crypt hyperplasia occur at weaning reducing the digestive and absorptive capacity of the small intestine in piglets. Liquid feeding appears to improve intake and growth in piglets but little is known about the effects of liquid feed on the small intestine mucosa. Deprez *et al* (1987) reported higher villi 11 days after weaning in piglets fed a liquid diet but there is little evidence that these changes persist in older pigs or are specifically due to diet form. This study is part of a series evaluating the effect of liquid feed on pig performance and adaptive changes in the absorptive mucosa of pigs from weaning to slaughter.

Materials and methods Thirty-six crossbred male piglets with a mean liveweight of 9.6 (s.e. 0.18) kg were weaned randomly into two pens of eighteen piglets. The piglets received creep feed prior to weaning. All piglets received a starter diet (18 MJ DE/kg, 25% CP, 17.5g total lysine/kg) for the first two weeks and a second stage starter diet (16.15 MJ DE/kg, 22.5% CP, 16 g total lysine/kg) for the following two weeks. Treatments comprised *ad libitum* feed offered as dry pellets from a trough or as a meal freshly mixed with water and dispensed from a Pioneer feeder at a water to feed ratio of 1.24:1. On the last day of the trial four animals from each treatment group were killed and tissue taken from the small intestine at sites 2, 25, 50 and 75% along its length from the gastric pylorus. The samples were immediately fixed in gluteraldehyde and sections prepared for examination by light and electron microscopy. Ten well-orientated villi and adjoining crypts and 10-20 microvilli at two or more sites were measured in each pig and the surface area of each microvilli calculated. The mucosa was also examined by scanning electron microscopy. Differences between treatments were assessed using the GLM procedure in Minitab 13.

Results Piglet performance is shown in table 1. Piglets receiving their diet in liquid form showed higher daily feed intakes and grew significantly faster throughout the entire trial period (P<0.01) representing an improvement in average daily gain of 37.7% compared with the dry fed animals. The feed conversion ratio of the dry fed pigs was better than the liquid fed group during the last 14 days but overall there was no difference between the treatments. Villus height and microvillus height and surface area were with the exception of the 50% position, significantly greater (P<0.001) at all locations in the liquid fed piglets. Examination of the mucosal surface showed more mechanical disruption and damage to the villus tips and microvilli in the dry fed pigs and the surface of the enterocytes lining the villi appeared to differ between treatments.

Table 1 Piglet performance from weaning to 28 days postweaning on liquid and dry diet.

Days	Average daily gain (g/day)					Daily feed intake (DM g/day)		Feed conversion ratio	
	Liquid	sem	Dry	sem		Liquid	Dry	Liquid	Dry
1-14	430.8	29.3	254.7	28.3	***	380.0	256.0	0.97	1.35
15-28	759.6	23.4	616.3	18.6	***	793.9	498.9	1.06	0.82
overall	585.0	34.2	424.9	35.8	**	586.9	377.5	1.01	1.08

Table 2 Effects of diet form on villus height, microvillus height and surface area in piglets 28 days postweaning.

Intestine	Villus height (µm)					Microvillus height (µm)					Microvillus surface area (μm^2)				
Position	Liquid	sem	Dry	sem		Liquid	sem	Dry	sem		Liquid	sem	Dry	sem	
2%	414	13.7	340	12.4	***	0.98	0.017	0.66	0.013	***	0.334	0.0094	0.220	0.0052	***
25%	368	10.3	294	11.1	***	1.11	0.038	0.89	0.026	***	0.411	0.0162	0.331	0.0129	***
50%	362	13.0	340	12.1	NS	1.23	0.020	1.26	0.032	NS	0.481	0.0103	0.470	0.0129	NS
75%	350	9.9	278	11.1	***	1.37	0.024	1.10	0.022	***	0.502	0.0140	0.371	0.0114	***

Values in both Tables are means ± sem. * P=<0.05 ** P<0.01 *** P<0.001

Conclusions Piglets readily accepted a non-fermented liquid feed which resulted in higher levels of intake and average daily gain without an overall deterioration in feed conversion ratio. The results suggest liquid feeding caused a marked increase in mucosal surface area for digestion and absorption in pigs up to twenty eight days after weaning that was reflected in their performance. Moreover, it is possible this difference in small intestine characteristics if caused by diet form would continue in older liquid fed pigs and further work is required to establish a functional relationship.

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Influence of diet acid binding capacity on gut morphology and digesta pH in piglets

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Introduction Newly weaned piglet diets are normally highly digestible, and as such are composed of ingredients with high acid-binding capacities (Bolduan *et al.*, 1988), which are potentially detrimental to the maintenance of a low gastric pH. The most active element effecting the phenomenon of acid-binding capacity (ABC) is calcium, and a high concentration in pig starter diets can significantly reduce post-weaning growth performance (Hardy, 1992). However, the mechanism by which ABC retards growth performance is largely unknown. Accordingly a preliminary study was designed to examine the effects of ABC on gut morphological characteristics such as villous height, width and crypt depth, together with digesta pH which is an independent factor influencing microflora colonisation.

Materials and Methods Twenty piglets weaned at 25 days of age were group fed *ad-libitum* one of two conventional diets based on commercial raw materials meeting energy and nutrient requirements of the weaned piglet. Diets were both isonitrogenous and isoenergetic, but diet 2 possessed a markedly lower acid binding capacity. This was achieved by decreasing the dietary content of limestone flour. The experimental period was for 14 days and 2 piglets from each group were slaughtered on day 0, 2, 4, 6 & 14. At slaughter, ileal digesta samples were taken and later analysed for pH. In addition, 6-8 cm length pieces of intestinal tissue were ligated at distances of proportionately 0.25, 0.50 and 0.75 along the gut from the gastric pylorus to the ileocaecal valve. The ligated intestinal tissue was subsequently fixed and embedded in paraffin wax. From each of these, sections were cut, mounted on slides, stained and examined under a light microscope. Measurements of villous height, width, and crypt depth were taken.

Results Analysis of data proceeded through establishing linear and non-linear contrasts with time. There was a significant Day x Diet (P=<0.001: <0.001; polynomial >2) interaction (see table 1). Piglets which had received the low ABC diet displayed more rapid recovery of villous height in the post-weaning period (P<0.001) and also maintained gut pH at a more acid level. Villous width followed a similar pattern; Day x Diet was significant (P<0.001), with a significant non-linear response (P=0.001; polynomial >2). Crypt depth also displayed a significant Day x Diet non-linear interaction (P<0.001; polynomial 2). Feed intake did not differ between the two treatment groups, and there was no significant effect of dietary treatment on piglet daily liveweight gain or digesta pH. However, digesta pH did increase between days 2 and 4 from an immediate post-weaning value of 7.0 (the more so in diet 2; high Ca), before returning to levels of approximately 7.2 after 14 days.

Table 1: Effect of dietary treatment on villous height

	Day					ANOVA					
Diet	2	4	6	14	**Mean**	Diet	P	Day	P	Diet*Day	P
1	408	355	348	471	**396**	5.3	<0.001	7.5	<0.001	10.6	<0.001
2	484	328	489	497	**450**				<0.001 (L)		0.122 (L)
Mean	**446**	**342**	**419**	**484**	**423**				<0.001 (Q)		<0.001 (Q)
									<0.001 (Dev)		<0.001 (Dev)

Conclusions The use of a low ABC diet for 14 days immediately post-weaning did improve gut morphological characteristics. The recovery of villous height occurred primarily between days 4 and 6 post-weaning. There were no differences in nutrient intake post-weaning therefore this cannot explain this improved recovery. The data therefore suggests that the structure of the small intestine can recover more rapidly when a low ABC diet is given after weaning; this will increase the absorptive surface area in the gut and could lead to reductions in the growth check which is often observed after weaning (although non-significant, growth rate on diet 2 was lower). The post weaning changes in digesta pH, albeit transitory and comparatively modest (and non-significant), may have important implications for gut microflora load.

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Enzyme linked immuno-absorbent assay (ELISA) to determine the effectiveness of anti-adhesive factors in blocking the binding of F4(K88)ac E coli to pig intestine

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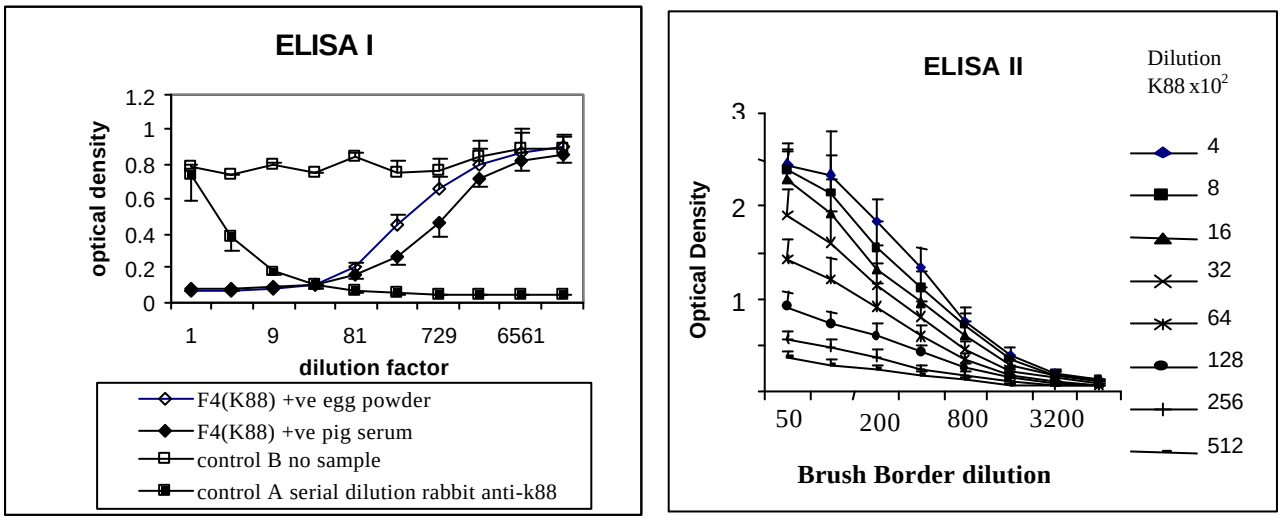
Introduction Concern over the use of prophylactic antibiotics and the potential of them being banned has increased interest in other methods of controlling disease. Specifically postweaning diarrhoea in the pig, often associated with E Coli (F4 K88ac) is a candidate for an alternative strategy. If in-feed additives can be demonstrated which effectively block the binding of the F4 (K88)ac pilli to the surface of the pig enterocytes then the severe form of the disease could be controlled without the use of antibiotics. The work reported here provides a screening method to determine whether potential in-feed additives can block the binding of purified F4(K88)ac to isolated brush border membranes taken from neonatal F4(K88)ac positive pigs.

Material and Methods Briefly the F4(K88)ac antigen was isolated by removing the fimbriae from a pure culture of F4(K88)ac E Coli, by heating at 65ºC for 30 minutes, homogenisation and then centrifugation at 26,500g for 10 minutes and filtration through a 0.45um filter to remove bacterial debris. Finally the protein was precipitated by adding 35% ammonium sulphate, centrifuged, re-suspended in physiological buffered saline and dialysed for 24hours. Purity of the F4(K88)ac was confirmed by fast performance liquid chromatography and eluted fractions screened using a rabbit ant--F4(K88)ac antiserum supplied by Central veterinary Laboratory, Weybridge. Brush borders were isolated from everted sausages (30cm) of small intestine taken from neonatal pigs, first by washing in PBS to remove debris and then in EDTA buffer to separate enterocytes from the gut wall. Separated enterocytes were ruptured by hypotonic EDTA and homogenised. The suspension was then filtered through glass wool to remove cell content debris and diluted to produce a stock solution containing $1x10^6$ brush borders per ml. Two separate ELISA were developed to establish both whether the binding of F4(K88)ac to isolated brush borders could be blocked and to elucidate the mechanism. Each consisting of steps separated by washing the plate 6 times with PBS tween. **ELISA I:** Step 1 coat plate with F4(K88)ac (1ug/ml), Step 2 blocked remaining empty sites on plate with 2% chicken serum, Step 3 rabbit anti-K88 antibody (1/2500 dilution); step 4 monoclonal anti-rabbit IgG peroxidase conjugate (1 in 30,000 dilution supplied by Sigma) and finally Step 5 OPD substrate (20mg/50ml citric acid buffer pH 5). **ELISA II:** Step 1 coat plate with sonicated brush border (1/800), Step 2 F4(K88)ac antigen (1ug/ml), Step 3 rabbit anti-K88 antibody (1/5000 dilution); Step 4 monoclonal anti-rabbit IgG peroxidase conjugate (1 in 30,000 dilution supplied by Sigma) and finally Step 5 OPD substrate (20mg/50ml citric acid buffer pH 5). In both ELISA the colour development is stopped with 50ul 3M H_2SO_4 after 30minutes when the peak OD reading (492nm) is approximately 0.8. Samples to be tested for anti-adhesive properties were incubated on the plates for 2.5 hours between steps 1 and 2 and then washed off. In ELISA I, pig anti-F4(K88)ac anti-sera and egg powder from poultry vaccinated against F4(K88)ac (Lohmann Animal Health) were included and serially diluted across the plate. If the sample bound to F4(K88)ac then colour development will be inhibited. Similarly if a sample binds to brush borders then inhibition of ELISA I will occur.

Results



Results of ELISA I shows that the assay is specific for F4(K88)ac, has low background and that the inclusion of either pig or egg anti F4(K88)ac blocks the binding of rabbit anti-F4(k88)ac . ELISA II also demonstrates low background but to date no material has been found which can bind to the brush borders and thus inhibit this assay.

Conclusion The ELISA described offer a method to determine whether the binding in vitro of F4(K88)ac to isolated brush borders can be inhibited and whether any inhibition is through binding either to F4(K88)ac or brush borders.

The effect of temperature and fermentation time on the survival of *Salmonella typhimurium* DT104:30 in liquid piglet feed fermented with *Pediococcus pentosaceus*.

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Introduction. Contamination of pig feed with enteric pathogens such as salmonellae has implications for the dissemination of salmonellosis in pigs and subsequent spread of these organisms through the food chain to humans. Liquid feed has the potential to be a vector for enteropathogens unless it is sterilised. However, if liquid feed is fermented with lactic acid bacteria (LAB), the production of lactic acid and consequent reduction in pH have an antimicrobial effect that can eliminate potential pathogens from the feed. The objective of this study was to determine whether the temperature at which fermented liquid feed (FLF) is fermented and maintained and the length of fermentation time affect the ability of Salmonellae to survive in the feed.

Methods. An experiment was conducted using a 2 x 3, factorial design. Factor 1 was the temperature of fermentation (20°C or 30°C) and factor 2 was the length of the fermentation period (48, 72 or 96 h). Three replicates were prepared for each fermentation period/temperature treatment by mixing sterile (irradiated with Co^{60}) piglet feed with sterile water (1 feed : 3 water) and inoculating with *ca*. 10^6 c.f.u. ml^{-1} *P. pentosaceus.* After fermentation samples were inoculated with *ca*. 10^6 c.f.u ml^{-1} of *S. typhimurium* DT104:30. The number of viable Salmonellae remaining was enumerated at 20min intervals, for 2 h post inoculation, for samples maintained at 30°C and at hourly intervals, for 8 h, for samples maintained at 20°C. Salmonellae were enumerated by performing serial dilutions (10 fold) in buffered peptone water (BPW) and plating appropriate dilutions onto blood agar + 2µg ml^{-1} crystal violet. Low numbers of surviving Salmonellae were detected by pre-enrichment and enrichment techniques (BSI 1990). For samples fermented at 30°C this procedure was carried out at hourly intervals for 3-8 h and for samples fermented at 20°C at 8, 24, 30 and 48h post inoculation with *S. typhimurium.* The lactic acid content of the fermented feeds was measured by high performance liquid chromatography and pH was measured using a pH meter. The decimal reduction time 'D value' for *S. typhimurium* DT104:30 was calculated from the following equation. $D_{x/y}$ = 1/ slope of the survival curve, where x = temperature and y = fermentation time. All data were analysed statistically by analysis of variance using Minitab.

Results. Analyses of D values for *S. typhimurium* DT104:30, lactic acid concentration and pH are presented in Table 1.

Table 1 D_{value} of DT104:30, lactic acid concentration and pH of FLF fermented for 48, 72 or 96 h at 20°C or 30°C.

Temperature °C	20			30			Temperature		Time		Interaction	
Fermentation time (h)	48	72	96	48	72	96	*P*	SED	*P*	SED	*P*	SED
D_{value} DT104:30 (min)	250	164^{b}	137^{b}	45	38^{a}	34^{a}	<0.001	7.9	<0.001	9.8	<0.001	13.8
[lactic acid] mmol L^{-1}	115	164^{a}	167^{a}	161^{a}	196^{b}	203^{b}	<0.001	4.1	<0.001	5.1	ns	7.1
pH	4.2	3.9^{a}	3.8^{a}	3.8^{a}	3.8^{a}	3.8^{a}	<0.001	0.01	<0.001	0.02	<0.001	0.02

^{a,b} means with the same superscript, for each parameter are not significantly different $P > 0.05$

In FLF at 20°C no *S. typhimurium* DT104:30 were detectable 48 h after inoculation regardless of the fermentation period. In contrast in FLF at 30°C no *S. typhimurium* DT104:30 were detected after 7 h in the 48h fermentation or after 6 h in the 72 and 96 h fermentations.

Discussion and Conclusions. The temperature at which fermented liquid feed is maintained has a profound effect on the survival time of Salmonellae. Lactic acid concentrations were higher in FLF maintained at 30°C than 20°C. However, lactic acid concentration alone was not responsible for the observed decrease in D_{20} compared with D_{30}. There was no significant difference in the lactic acid concentration of FLF fermented at 20°C for 72 h or 96 h and FLF fermented at 30°C for 48 h (all produced *circa* 160-170 mmol lactic acid). However, $D_{20/72}$ and $D_{20/96}$ were *circa* four times longer than $D_{30/48}$. This may be due to a number of factors. Firstly, *S. typhimurium* expresses cold-shock proteins at temperatures below 24°C (Phadtare *et al*. 1999), which may have a protective function. Secondly, one of the effects of organic acids is to inhibit nucleic acid synthesis (Cherrington *et al*. 1990). Therefore, under conditions of low cell turnover (i.e. lower temperatures) the antimicrobial effect of lactic acid may be reduced. In this study it was necessary to inoculate FLF with high numbers of *S. typhimurium* in order to calculate D values. However, the number of Salmonellae contaminating feed introduced to a liquid feed system are likely to considerably lower than used in this study, namely 10 c.f.u g^{-1} or less. Thus in practical situations, all Salmonellae could be eliminated from FLF, fermented at 30°C, for 72 h, in less than 1 hour.

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Transfer of vitamin E to piglet tissue, placenta, colostrum and milk from sows supplemented with vitamin E and vitamin C.

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Introduction. The effective level of dietary supplementation of vitamin E and vitamin C is difficult to define because it depends of several factors such as composition of the diet, feed consumption, rate of growth, animal production and living conditions, stress, crowding and environment. Research has demonstrated that supplemental vitamin E improved litter size, increased sow serum α-tocopherol content and enhanced health status (Mahan, 1994; Wuryastuti et al., 1993). Some reports have suggested that the low plasma and tissue levels of α-tocopherol in new-born pigs, suggests a low rate of vitamin E transfer across the placenta which is not influenced by dietary supplementation of the sow during pregnancy. The aim of this experiment was to determine the effect of vitamin E and vitamin C supplementation of sow diets on transfer of vitamin E to piglet tissues via placenta, colostrum and milk.

Materials and methods The experiment was carried out on a commercial farm in summer in Sonora, México. At the beginning of gestation 36 multiparous sows were allocated individually (6 per treatment) to following the treatments: 1. Control (commercial diet, vitamin E 30 mg/kg feed); 2. Control + vitamin C 1 g/day; 3. Control + vitamin C 10 g/day; 4. Control + vitamin E 200 mg/kg feed; 5. Control + vitamin E 400 mg/kg feed; 6. Control + vitamin E 200 mg/kg feed + vitamin C 1g/day. The vitamins were supplemented daily throughout. Colostrum samples and placenta were collected at farrowing (day 0) and milk at weaning on day 21. One piglet from each litter was killed by lethal barbiturate injection on days 0 and 21 and samples of liver taken. Piglet blood was collected at birth from the umbilical cord and at weaning (day 21) by cardiac puncture. The vitamin E analyses were carried out by HPLC. Data were analysed by one way ANOVA using Minitab®

Results The vitamin E content of piglet serum and liver was significantly higher ($p<0.001$) at birth and weaning in the groups supplemented with vitamin E. Similar effects of treatments were also seen in colostrum and milk. The vitamin E concentration increased in piglet serum and liver at weaning. The value for vitamin E in colostrum was significantly higher compared with milk. In placenta the concentrations of vitamin E were significantly higher ($P<0.05$) in sows supplemented with vitamin E at 400 mg/kg feed and in those supplemented with combined vitamins.

Table 1 Vitamin E content of sow and piglet tissues, colostrum and milk

	Treatments						s.e.m.	significance
Day 0	1	2	3	4	5	6		
Placenta (µg/g)	0.11^{ab}	0.08^{a}	0.09^{ab}	0.16^{ab}	0.22^{b}	0.21^{b}	0.014	*P<0.05*
Colostrum (µg/ml)	9.8^{a}	9.1^{a}	10.4^{a}	20.5^{b}	25.1^{b}	20.1^{b}	0.53	*P<0.001*
Serum (µg/ml)	0.35^{a}	0.32^{a}	0.35^{a}	0.42^{ab}	0.42^{b}	0.48^{b}	0.007	*P<0.001*
Liver (µg/g)	0.12^{a}	0.14^{a}	0.11^{a}	0.12^{ab}	0.19^{b}	0.16^{b}	0.006	*P<0.001*
Day 21								
Milk (µg/ml)	2.5^{c}	2.4^{c}	2.4^{c}	5.3^{a}	7.1^{b}	5.5^{a}	0.106	*P<0.001*
Serum (µg/ml)	1.97^{a}	2.14^{a}	2.17^{a}	5.04^{b}	4.52^{b}	4.29^{b}	0.102	*P<0.001*
Liver (µg/g)	0.59^{a}	0.63^{a}	0.62^{a}	1.31^{b}	1.58^{b}	1.13^{b}	0.036	*P<0.001*

Mean values within a row with different superscripts are significantly different

Conclusions There is little transfer of vitamin E from the maternal circulation to the placenta and to the piglet *in utero*. Piglet serum and tissue concentrations of vitamin E increase rapidly due to transfer via colostrum and milk. Supplementation of sows with vitamin E had its biggest effect on piglet liver and serum vitamin E concentration postpartum.

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The effect of pellet size on the voluntary food intake and performance of young pigs

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Introduction Immediately following weaning, voluntary food intake in the young pig is low and very variable. This can lead to reduced digestive efficiency and poor physical performance. One approach to try to stimulate intake is to manipulate the physical form of the diet. Previously it has been shown that, contrary to popular belief, young pigs are very adaptable with regards to diet presentation and in particular with respect to pellet size(Edge *et al.*, 2000). Pigs from 10 to 56 days of age would consume a 5.0mm pellet as readily as a 1.8mm pellet with no adverse effects on production variables.

Materials and Methods 187 Large White x Landrace pigs were used in the trial with approximately equal numbers of male and female animals. Piglets entered the trial at birth and remained on trial until 48 days of age. Sows and litters were housed in conventional farrowing crates with an enclosed heated creep area until the piglets were weaned at 28 days of age. After weaning piglets were moved into weaner accommodation and housed in groups of 8 pigs on fully slatted floors. Pigs were assigned to groups on the basis of previous treatment, sex, dam and weaning weight.
Treatments were offered in a 2x4 factorial design with all litters receiving either a 1.8mm or 9.0mm diameter pellet in the farrowing house as a supplementary creep feed, followed by each pen receiving either a 1.8mm, 2.4mm, 5.0mm or a 9.0mm pellet after weaning. All possible combinations were accounted for. All pellets for the same stage of growth were made to the same formulation with the only difference being pellet diameter. Food was offered on an *ad libitum* basis
Production data were collected at key stages of the trial; at the introduction of creep feed, at the point of weaning and at subsequent weekly intervals. Data were analysed using an analysis of covariance with weaning weight as the covariant.

Results All food was consumed in normal quantities by the piglets with no apparent differences between the treatments in terms of piglet health. The mean piglet weights, feed consumption and performance data are displayed in Table one. There were no significant differences between the treatments in piglet weights at the changeover from stage one to the stage two feed (mean 9.54kg, s.d.6.37) or at the end of the trial (mean 15.63, s.d. 11.44). There were no significant differences in food intake (FI), liveweight gain (LWG) or feed conversion ratio (F.C.R.) of the piglets at any stage of the trial.

Table 1 – Effect of pellet diameter on piglet performance

	Dietary Treatment (pellet diameter pre/post weaning) mms								
	1.8/1.8	1.8/2.4	1.8/5.0	1.8/9.0	9.0/1.8	9.0/2.4	9.0/5.0	9.0/9.0	s.e.m
W.Wt (kg)	7.99	7.95	8.08	7.44	8.52	8.39	8.19	8.30	0.12
LWG (kg)	8.20	8.48	7.47	7.14	8.28	7.67	8.59	7.72	0.51
F.I. (kg)	9.55	8.47	8.46	8.83	7.79	8.81	8.88	9.22	0.11
F.C.R.	1.30	1.09	1.30	1.40	1.07	1.31	1.12	1.34	0.03

The effects of preweaning treatment, postweaning treatment and their interactions were not significant ($p<0.05$)

Conclusions From the data generated by the trial it can be seen that young pigs will consume a wide range of pellet diameters as readily as each other, and are very adaptable with respect to changes in diet presentation. This confirms the findings of our earlier work on pellet size. Those pigs who were offered two different pellet sizes whilst on trial had similar growth rates and feed intakes to their contemporaries who received a single pellet size throughout. It appears therefore that pellet size *per se* cannot be used as a tool to increase the voluntary food intake of the young pig.

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Food intake and performance of newly-weaned pigs: effect of pairing with an experienced pig

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Introduction In intensive pig production systems piglets are weaned at between 21 and 28 days of age and it is common for piglets to take a few days to discover and accept solid food as a source of nutrients and this results in a loss of weight (the weaning growth lag). Therefore, if a means could be found to encourage the acceptance of solid food by piglets, benefits in terms of their growth and welfare would ensue. The transmission of information about foods from one animal to another has been demonstrated in the rat (Galef, 1993) and ruminants (Provenza and Balph, 1987) but there have been no studies with pigs at the time of weaning. Therefore, piglets were tested in pairs of experienced and inexperienced animals, with varying degrees of contact, with constant access to food over a period of seven days after weaning of the inexperienced animals.

Material and Methods The experiment used 6 litters of piglets (Large White x Landrace) which did not receive solid food until they were weaned. Two creep foods, intended for pigs of 3 or 10kg live weight, were used (three litters per food). Three pigs were weaned at 21 days of age and were housed together for 7 days and offered solid food. At 28 days of age all the remaining pigs in the litter were weaned. Four pairs of pigs were formed so that there were 3 experienced demonstrators paired with three inexperienced observers of a similar live weight and a pair of inexperienced pigs to act as negative controls. Of the demonstrator / observer pairs, in the first treatment the demonstrator and observer were separated by one pen but could see and hear each other through the wire mesh partitions of the pens. In the second treatment the demonstrator and observer were in adjacent pens and could see, hear and touch each other through the wire partitions. In the third treatment the demonstrator and observer were housed together in a double sized pen. In the fourth (control) treatment the two inexperienced pigs were housed together in a double sized pen. Solid wooden partitions were placed between the treatment pairs of pigs so that they could not see a member of another pair. Food intake and pig weight were recorded daily. Since individual food intakes could not be measured for the pairs of pigs housed together (Treatments 3 and 4), the total intake of each pair over the seven days was calculated and subjected to analysis of variance. Daily live weight gain, estimated by linear regression of live weight on day, was analysed for the pairs and separately for the experienced and inexperienced pigs. In all these statistical analyses the starting live weights of the pigs were used as covariates.

Results The pair of pigs on Treatment 4 ate significantly less that the pairs on Treatments 1 and 2 ($P<0.01$) and Treatment 3 ($P<0.001$). Treatment pair 2 ate less than pair 3 ($P<0.05$) (Table 1). The live weight gain of Treatment pair 3 was significantly greater than that of the pairs on Treatments 1 ($P<0.05$) and 4 ($P<0.01$) (Table 1). The significance of the difference between pairs on Treatments 2 and 3 was between $0.1>P>0.05$. In the case of individual pig daily live weight gain, there were no significant differences between treatments; experienced: treatment 1: 270, 2: 292, 3: 376g (sed 39.6) and inexperienced: treatment 1: 138, 2: 137, 3: 216, 4: 159g (sed 58.9).

Table 1. Mean daily food intake and daily live weight gain of the pairs of pigs on each treatment

	Food	Treatment					Food		Treatment	
		1	2	3	4	mean	sed	sig	sed	sig
Pair	1	557	482	622	366	507	99.8	NS	46.8	***
Food intake	2	382	403	497	219	375				
(g/d)	mean	470	442	559	292					
Pair	1	580	509	684	474	562	111.5	NS	78.6	*
Weight gain	2	243	356	509	140	312				
(g/d)	mean	412	432	597	307					

Conclusions Close contact between an experienced and an inexperienced pig (Treatment 3) resulted in enhanced food intake and live weight gain of the pair. The enhanced gain was attributable to small non-significant increases in gain for both pigs in the pair.

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Herbage intake of growing pigs in an outdoor organic production system

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Introduction Standards for organic pig production recommend that growing pigs are maintained on pasture. There is currently no information on the nutritional implications of such a system, since grazing intakes have not been recorded in pigs of this production stage. This study used n-alkane methodology previously validated in sows (Wilson *et al*., 1999) to measure the herbage intakes of individual pigs under such conditions.

Material and Methods Two groups of 6 Camborough 12 x Duroc pigs were used in the study. They were maintained in adjacent paddocks (30 x 10m) on a one year ley consisting of grass and white clover managed according to organic standards. Each paddock provided a hut for shelter, a feeder from which an organic concentrate feed was available *ad libitum* and a drinker providing freely available water. Pigs in paddock 1 had a mean liveweight of 61.7±5.25 kg, and those in paddock 2 of 50.1±4.51 kg. The pigs were individually trained to accept hand feeding of a small cake twice daily. Five pigs which reliably accepted and consumed this cake were then selected for the detailed study. From the start of the experimental period, these pigs received a daily dose of 125 mg C_{32} alkane absorbed into the two cakes, which continued to be fed twice daily for a 10 day period. The first 5 days were used as an adaptation period, and during the second 5 days all faeces voided by each individual pig between 08.00 and 16.30 each day were collected from the ground and frozen. On the first and last day of collection, herbage samples were cut to 1.5 cm above ground level at 5 randomly selected sites in each paddock. These were separated into grass and clover components prior to freezing. The alkane (chain length C_{25} to C_{33}) concentrations in freeze-dried samples of faeces, herbage and concentrate feed were determined which, together with the known C_{32} dose, allowed the estimation of the individual intakes of each feed component and OM digestibility using a least squares optimisation algorithm (Microsoft Excel Solver). It was necessary to correct the faecal alkane concentrations for incomplete recovery. A value of 75% was assumed (Wilson et al., 1999).

Results The individual intakes of concentrate, and grazed grass and clover are shown in Table 1. The mean calculated intake of concentrate OM did not differ significantly from the measured mean concentrate OM disappearance from the feeder of 1.93 kg/pig/day for each paddock over the 10 day study period. Intake of herbage showed generally low variability and corresponded to only 4% of daily OM intake. Only one pig showed evidence of selective grazing of the clover component of the sward.

Table 1 The calculated OM intakes from different dietary components and OM digestibility of the total diet in growing pigs grazing organically managed pasture and offered *ad libitum* concentrate

Intake (kg OM/pig/day)	Grass	Clover	Concentrate	OM digestibility (%)
Paddock 1:				
Pig 1	0.07	0.02	1.88	83.3
Pig 2	0.06	0.03	2.01	82.9
Pig 3	0.05	0.02	2.25	83.1
Paddock 2:				
Pig 4	0.07	0.09	2.12	84.8
Pig 5	0.07	0.02	2.70	87.9
Mean ±s.e.m	0.06±0.004	0.04±0.014	2.19±0.140	84.4±0.937

Conclusions When concentrate was available *ad libitum*, the herbage intake of growing pigs at pasture was only 4% of daily OM intake. There was no evidence for consistent species selection from a grass/white clover sward.

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A survey to investigate the influence of commercial human-animal interaction during rearing on the welfare and subsequent production of the dairy heifer

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Introduction Recent increases in mechanisation, a trend towards larger dairy units and current financial pressure on dairy farmers all combine to result in a reduction in labour and therefore time available to spend with the animals. increasing numbers of stock per attendant allowing them less time and opportunity to spend with their animals. If we view the reduction in human-animal interaction in routine husbandry tasks as positive because it frees up more quality time to spend with the stock; how will this best be spent to enhance the animal-worker bond, is it feasible in a practical situation and can we back this ideal up with monetary values and benefits to the different interested parties of the industry? This survey was designed to compliment some experimental work being carried out at Newcastle University (see companion paper). The objective was to gather information from dairy farmers on the heifer rearing systems currently in use; establish the different levels of human interaction the heifer experiences during rearing in commercial practice and explore whether this relates to other indices of welfare and production.

Materials and methods The survey was piloted and then distributed to 1000 dairy holdings across England and Wales taken at random from the sample set (published National Milk Records (NMR) for England and Wales 1998, - to give objective production data to complement the responses).

Results The survey met with a high level of support: 516 usable cases. The preliminary analysis, based on frequencies, gives an overview of heifer management during rearing on dairy farms across England and Wales and details of the respondents views and attitudes relating to dairy cow welfare along with information on their demographics. The table presents information on the frequency of human attention to heifers:

Table 1: Intensity of human attention as rearing progresses:

Valid percent	Birth - weaning	Weaning – 6 months	6 – 15 months	15 – 24 months
Less than daily	1	2.1	8.9	8.2
Daily	12.3	26.9	57.8	63.8
2x daily	67.6	58.5	28.2	21.3
>2x daily / >15mins daily	19.2	12.6	5.1	6.6

The trend is a decline in attention with age. 26% of respondents identified the calves as having received positive human interaction between birth – weaning ranging from hand feeding with a bucket, to talking to and stroking the calves or where it was expressed that the staff were encouraged to interact with the stock. This reduced to 19% from weaning to 6 months: including going into the pen and allowing the stock to interact. For the remaining 6 – 24 months this dropped to 14%. When asked to outline the close human contact or handling procedures the heifers underwent 30% listed neutral (neither positive or negative) experiences compared to 99% stating the heifers close human contact was a procedure of an aversive nature: 13% identifying three, 86% naming four or more.

Respondents were asked to comment on the frequencies of certain behaviours in the first few weeks of lactation. It can be concluded that in general heifers are harder to handle than older cows in the first few weeks of lactation, it would commonly take them a week to settle into the milking routine, where initially incidences of kicking dunging and urinating would occur. Only 6% said heifers did not add on any additional time to milking, 50% thought they added up to an additional five minutes. 24% of heifers enter the parlour ranging from hesitantly to with great difficulty, implying they find the parlour aversive in some way.

48% of respondents thought that previous human interaction led to more docile cows. For problem milkers 5% said it was either due to negative experiences as a heifer or the problem milkers were the heifers. 9% gave the reason that previous experiences with humans led to difficult behaviour and a further 20% gave the blanket reason of previous experience (which could include maltreatment from humans).

42% of respondents cull heifers on a regular basis with behaviour a key factor in the decision making.

Conclusions This highlights what problems can ensue where heifers do not take to the milking routine calmly and identifies the influential role stockpeople believe humans can play in shaping an animal's temperament. The extent of negative procedures is alarming as it is the proportion and quality of interactions in relation to the total that determine the animals'overall experience. This information will prove invaluable for setting the concurrent experimental work in context, and extrapolating the findings to the U.K.'s dairy industry. More in depth analysis will explore associations between management practices and aspects of welfare and production. The response to the survey highlights the importance farmers place on welfare.

The use of texture analysis to assess the structural strength of hoof horn of dairy cows

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Introduction The quality of hoof horn is related to 3 factors: architecture of the Stratum corneum (number of tubules and inter-tubular space), cellular factors (type of keratinization, orientation of keratin fibres) and inter-cellular factors (amount and chemical composition of the inter-cellular substance (Pellman *et al*., 1993). Kempson and Logue (1993) related poor hoof horn quality at 1 month before calving to the occurrence of moderate to severe solar haemorrhage at 10 to 20 weeks after calving. The objective of this experiment was to develop the use of texture analysis as a method to measure the structural strength of hoof sole tissue and assess the influence of sample width and bruising on structural strength.

Materials and methods The structural strength of the hoof sole tissue was studied in 36 dairy cows with an average milk yield of 8500 l/305 days and that were in the second to fifth lactation. All cows received the same diet consisting of a total mixed ration (TMR), based on grass and maize silage (composition 40.0 g/kg DM, 11.3 MJ/kg of ME, 17.3 g/kg CP, 402.30 g/kg NDF). In addition, cows received a maximum of 8 kg of concentrated feed (22 % CP) in the parlour. The cows were loose housed in the same area conforming to FAWC (1997) consisting of 42 cubicles fitted with cow mats and sawdust. Cows were milked and passages scrapped twice daily. Samples of hoof sole tissue were collected, with a hoof knife, from all claws at 30 (s.d 7), 60 (s.d. 4) and 180 (s.d. 8) days post partum. The first outer layer (1 mm) of horn of the sole and white line of the distal part of the hoof was discarded and a sliver of 0.1 to 1.8 mm thickness was kept for analysis. Samples were collected in a sealed plastic bag and stored in a refrigerator at a temperature of 3 - 5 °C until analysis the following day. Samples were analysed for puncture resistance using a P/2N needle probe on a TA.XT2i texture analyser (Stable Micro Systems). A total of 15 to 20 tests were completed on the different areas of each claw. Data collected from sole and white line areas were recorded separately. Each sample was scored for level of haemorrhaging, using a scale of 0 to 5 (0- no haemorrhaging and 5- severe haemorrhaging) and the width of the tested area was recorded simultaneously. The data was analysed (Minitab 12.0) by ANOVA using GLM using cow and haemorrhage score as fixed effects and width and days post partum as a covariate. The effect of width, haemorrhage and days post partum on puncture force was tested by regression analysis.

Results The mean force required to puncture sole horn tissue of differing haemorrhage score are presented in Table 1. There was no significant difference in force required to puncture sole and white line tissue. The level of haemorrhage and width of the sample both had significant effects ($p<0.001$) on the force required to puncture the sample. There was a positive linear relationship between puncture force and sample width ($y = 616.69 + 644.99\ x$, $R^2 = 31.09$ %). Thicker samples required a greater force to be punctured. The level of sole bruising had a negative linear relationship with the puncture force, demonstrating a lower structural integrity of these samples. The puncture force was affected by the number of days post partum, with the force required to puncture the samples decreasing as the lactation progressed ($p<0.001$).

Table 1 Effect of haemorrhage (H) score on mean puncture force of sole horn tissue

H.score	0	1	2	3	4	5
Force	888.6	870.1	822.0	790.2	620.2	508.3
No.	1130	546	401	304	74	23
s.e.m.	7.8	10.4	12.5	15.1	33.1	46.0

Conclusions The results of the present study demonstrate that the occurrence of bruising on the sole horn tissue leads to a loss of the structural strength. In addition, as the number of day post partum increased the structural strength reduced and the sole tissue was more susceptible to puncture. This reduced structural strength may contribute to lameness.

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Relationship between the scoring of hoof lesions and lameness in dairy cows

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Introduction Claw horn lesions are the most common cause of lameness in dairy cows and the development of lesions is related to the days in lactation. The lameness caused by this lesions is influenced by different factors (Offer et al., 2000). The objective of this experiment was to study the relationship of severity of lameness and severity of scoring for hoof horn lesions.

Materials and methods 36 dairy cows with an average milk yield of 8500 l/305 days, that were in the second to fifth lactation, were assessed for hoof horn lesions and lameness. All cows received the same diet consisting of a total mixed ration (TMR), based on grass and maize silage (composition 40.0 g/kg DM, 11.3 MJ/kg of ME, 17.3 g/kg CP, 402.30 g/kg NDF). In addition, cows received a maximum of 8 kg of concentrated feed (22 % CP) in the parlour. The cows were loose housed in the same area conforming to FAWC (1997) consisting of 42 cubicles fitted with cow mats and sawdust. Cows were milked and passages scrapped twice daily. All cows in the experiment were scored for lameness twice weekly. The scoring system used had 5 points (0-not lame and 5-severely lame). The cows had their feet assessed for sole ulcer, sole haemorrhage and heel erosion, before calving (0 – prior to supplementation) and at 30 (4-50), 60 (51-75) and 180 (145-220) days post partum. The lesions on each foot were scored according to Leach et al. (1998). Regression analysis was performed to compare the scoring of hoof horn lesions with lameness and study the effect of days of lactation (Minitab 12.0).

Results Locomotion scores recorded at the same time as the hoof lesion score and recorded 2 months before, were compared with the scoring for hoof lesion. Only scores for the hind left outer hoof recorded at 120 days showed a significant linear relationship ($p<0.01$) to locomotion scoring (Fig. 1). No relationship was found on the 3 recording periods between locomotion score and bruising score for any other hooves and total score of the cow. Cows with low locomotion score had low scores for hoof lesion and cows with high locomotion scores had variable scores. One cow with high locomotion score and low lesion score had an interdigital granuloma. Two other cows had sole ulcers and high scoring for lesions and locomotion at day 60 and low scores for lesions at day 180, but still high scores for locomotion. A significant positive linear relationship ($p<0.05$) was found between days of lactation and scoring for bruising (Fig. 2).

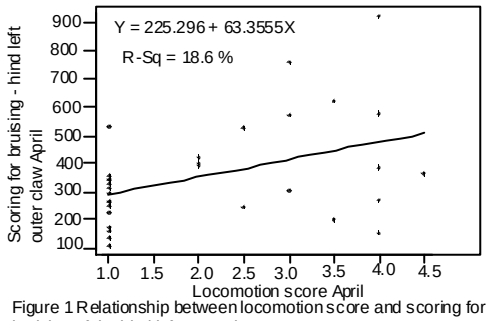

Figure 1 Relationship between locomotion score and scoring for bruising of the hind left outer claw

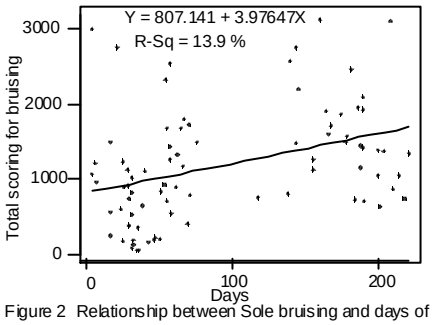

Figure 2 Relationship between Sole bruising and days of lactation

Conclusions The relationship between days in lactation and scoring for hoof horn lesion was reported before by Leach et al. (1998). Cows with locomotion score of 1 presented low scoring for hoof horn lesion and cows with locomotion score over 3 (lame cows) presented variable scores. This data demonstrates that there is considerable variability in the level of hoof horn lesion in lame cows, indicating that other factors and not hoof horn lesions alone are involved with the occurrence of high locomotion scores.

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Passive infrared detection (PID) of activity in groups of broiler chickens growing at different rates

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Introduction Activity of fast growing broiler chickens is known to decrease at two to three weeks of age, whereas slower growing hybrids are not only more active, but remain so throughout the growth period (Reiter and Bessei, 1998). The present experiment aimed to monitor diurnal patterns and time courses of activity in groups of commercial broilers fed one of two feed types differing in energy content. Activity was assessed using passive infrared detectors (PIDs), which sense movement of an object with a temperature different from that of the background.

Material and Methods Eight groups of 225 female Ross 208 broiler chickens were kept in straw-bedded rooms (18.9m^2) from 1 to 57 days of age with a lighting schedule (hr:min) of 17:30 light, 0:50 dusk and 5:40 darkness. The groups were fed one of two feeds (H=15.0 vs. L=13.4 MJ ME/kg DM). One PID unit was mounted on the back wall of each of the eight rooms 2 m above the ground at an angle of 45° together with a video camera. Activity data, accumulated each minute during 24-hours, were collected when the chickens were 29, 36, 37, 43, 44, 50 and 51 days old. Data were analysed in SAS using general linear mixed models. Age of chickens (n=7), light category (n=3; light, dusk and darkness) and feed type (n=2) were fitted as fixed effects with all interactions and room was fitted as random effect in all models to account for repeated measures. A large effect of light category was expected, and age, feed type and their interaction were subsequently fitted within each light category.

Results Activity measures were significantly affected by the light category ($F_{2,138}$=256.2; P<0.001). Activity levels during darkness were on average 0.40 of those measured during light, and activity during dusk was 0.85 of that during light. The onset of the light period caused a brief six-fold increase in activity (Figure 1). Feed type had no significant effects on activity at any point. The temperature in the litter differed between feeds (H vs. L: 30.8 vs. 26.9 °C; $F_{1,31}$=40.0; P<0.001) and increased by 34% over the period of measurements ($F_{3,31}$=35.4; P<0.001). During the same period, live weight increased by 170%, and on day 57 was (±s.e.) 2676±24g and 2499±27g for chickens fed H and L, respectively ($F_{1,6}$=23.8; P<0.01). Activity during the dark period, but not during dusk or light periods, showed an increase with age of the chickens, assumed to be due to the growth of the birds ($F_{6,35}$=98.8; P<0.001; Figure 2).

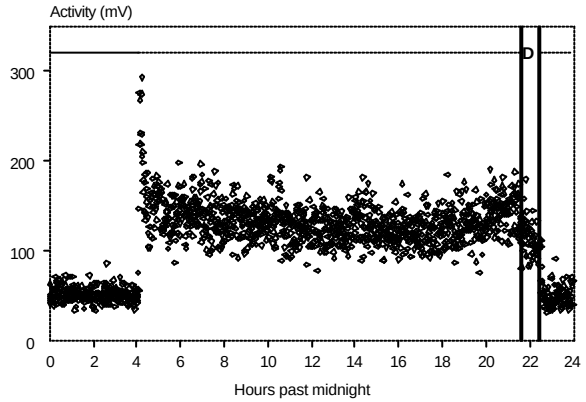

Figure 1 Mean activity data (mV; one reading per minute) across all PID units plotted against time of day. Along the top are indicated periods of light (---), dusk (D) and darkness (–).

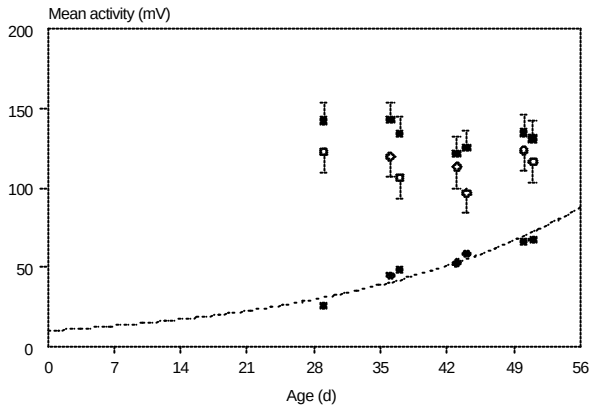

Figure 2 Mean (±SD) activity (mV) during darkness (●), dusk (○) and light (■) plotted against age. The stippled curve ($Y=\exp^{0.034X}$) indicates the increase, which is due to growth.

Conclusion The diurnal pattern of activity monitored by the PID units was that expected in groups of broilers kept under a lighting schedule, with the activity of the chickens substantially reduced during the period of darkness. However, activity data from the dark periods revealed a systematic increase, which appeared to reflect the growth of the chickens. Despite significant differences in growth rate between chickens on the two feed types, this was not reflected in the PID data. It is most likely that the difference was too small for the sensors to pick up, or it may have been masked by the concomitant increase in litter temperature, which also differed between the two feeds. No increase with age was found during the periods of light or dusk. This could be a consequence of the growth-related effect being obscured during periods of high activity, i.e. during light as opposed to the dark. It may also be that the increase in PID measures due to growth was counterbalanced by a concomitant decrease in activity with age.

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Hut space requirements for outdoor sows

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Introduction Whilst the lying space requirements for housed pigs have been scientifically defined (Petherick, 1983) it may not be appropriate to extrapolate these directly to the hut space requirement in the outdoor situation, where understocking carries a potential thermal penalty. However, it is important that hut space is adequate for all sows to obtain shelter when desired. The objective of this study was therefore to determine the preferred hut space requirements for outdoor sows, with the rationale that an inadequate hut space allowance would increase the number of animals forced to rest outside in a suboptimal thermal environment.

Materials and Methods Three treatments were compared which gave different hut stocking densities by varying group size at a constant hut space allowance. Adjacent paddocks on the same commercial outdoor unit contained 9, 10 or 12 sows, giving a space allowance within the single galvanised tin arc (4.85 x 3.0m) of 1.6m^2, 1.5m^2 and 1.2m^2 per pig, respectively. Treatments were replicated concurrently over time. The number of sows lying inside and outside the arc in each paddock was observed twice weekly, during the resting period in late evening, over a 7 month period (April to October). A total of 37 different groups of sows were recorded, with a mean observation period of 6 weeks per group starting from the introduction of the newly mixed group into the paddock approximately 9 weeks after service. Genotype, feeding and management conditions were standard for all treatments and typical of UK outdoor production. Ambient temperature and arc temperature were also recorded at the time of each observation. Data were analysed by both ANOVA and multiple regression techniques using models incorporating the influence of group, month, time since group formation, ambient temperature and treatment on the number of pigs observed outside the hut.

Results Ambient temperature had the greatest influence on the number of sows observed outside (R^2=5.3, $P < 0.001$) indicating that outdoor hut use was most strongly related to climate (Fig. 1). The number of pigs outside was not influenced by time since group formation.

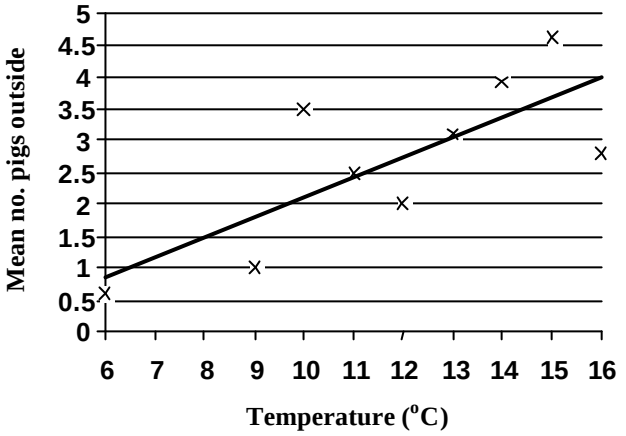

Figure 1
Mean no. of pigs outside at each daily recorded temperature

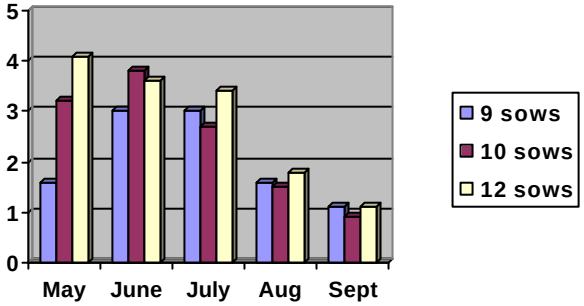

Figure 2
Mean number of pigs outside per month (s.e.m. 0.5)

Group size did not have a significant effect on the number of pigs remaining outside, despite a tendency for more pigs to be outside in the larger groups (2.4, 2.5, 3.0 s.e.m. 0.2, $P = 0.058$). This was primarily seen during May (the coldest month), when 18%, 32% and 34% of pigs were outside for group sizes of 9, 10 and 12 respectively ($P<0.05$, Fig. 2).

Conclusions A hut space allowance of 1.5 to 1.6m^2 per sow, which equates to 10 or 9 pigs per 14.55m^2 arc respectively, is provisionally recommended for outdoor sows. Further replication of this study throughout the winter months is required.

Acknowledgements We thank the staff of the outdoor unit for data collection.

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The effects of farrowing system design on welfare of sows and piglets of different genotypes

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Introduction Although often described as "welfare-friendly", the greater freedom of movement and choice of environments offered by communal farrowing systems does have potential welfare risks, primarily for the piglets. The maternal qualities of the sow will have a greater influence on the survival and growth of piglets in this communal farrowing system, than in a conventional farrowing crate and may be influenced by genotype. The objectives of this study were to compare the welfare of sows and piglets housed in farrowing crates and a communal farrowing system and to investigate whether sow genotype influences the quantity and quality of maternal behaviour and subsequent litter performance.

Materials and Methods A total of 37 Manor Meishan and 62 Camborough gilts (both PIC, Abingdon UK) were assigned to one of four treatments: MC - Manor Meishan gilts in conventional farrowing crates, CC - Camborough gilts in conventional farrowing crates, MP - Manor Meishan gilts in communal farrowing pen system; CP - Camborough gilts in communal farrowing pen system. Gilts arrived into the gilt service area where they were all served naturally during their second exhibited oestrus. After holding to service, they were moved to a free-access stall dry sow house, in groups of 3-5, until entry to the farrowing house, 5 days prior to farrowing date. The farrowing systems were housed in different rooms of the same 5-room building. Crate rooms housed six conventional farrowing crates. Pen rooms contained five individual strawed pens with a piglet creep and sloping walls with a piglet escape area underneath. The gilts accessed a communal passageway and a covered, outdoor dunging area. The piglets were contained within the home pen until the youngest litter in the room was 10 days old. Weaning of the whole group occurred when the youngest litter reached 23±2 days of age. The gilts were moved to the sow service area and then they were returned to the dry sow accommodation until about 5 days before the next farrowing date, when they were re-introduced into the same farrowing system to which they had been originally assigned. Of the original 99 gilts, 52 remained on the experiment for the 2nd parity. The numbers of animals reduced due to space constraints, with many of the Meishan gilts running simultaneously with the 2nd parity Camborough sows. Various measures of production, behaviour and physiology were taken over both parities. A two-factor ANOVA technique was used to analyse the data using genotype and farrowing system as factors, with gilt/sow nested within group. However, because the genotypes did not run simultaneously, the results of across-genotype comparisons should be treated with caution. Transformations were used to normalise data where appropriate.

Results Overall there were no differences in piglet growth rates or liveborn piglet mortality between the two farrowing systems during either parity. There was no difference in percentage liveborn piglet mortality between genotypes during the first parity, but during the second parity, Meishan sows had significantly lower mortality than the Camborough sows (6.0% vs 12.9%, $p<0.01$). Sows in pens had a higher score of aggression towards the stockperson than sows in crates (Parity 1 = 1.84 vs 1.40, $p<0.001$, Parity 2 = 2.20 vs 1.56, $p<0.001$). As gilts, Meishans were more aggressive than Camboroughs (1.91 vs 1.43, $p<0.001$) but this trend reversed during the second parity (1.69 vs 2.01, $p<0.05$). Overall, cortisol concentrations were higher in Meishan gilts but this appears to be physiologically normal, rather than stress-induced. Gilts in crates tended to have higher cortisol responses to weaning (+45.9nmol/l vs +4.7nmol/l, $p<0.1$) than penned gilts.

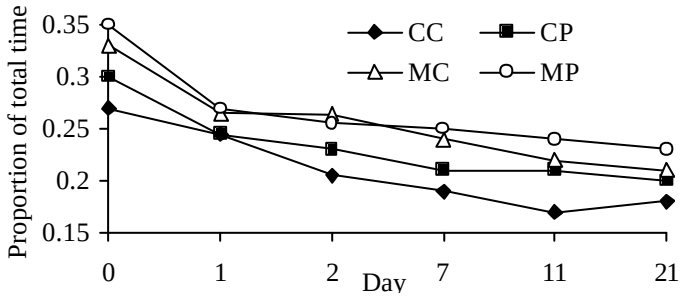

Figure 1: Time spent nursing piglets in relation to day within system, for gilts in the four different treatments

Gilts in crates spent more time in 'restless' postures and less time nursing their litters than gilts in pens (see Figure 1). Meishan gilts spent less time in 'restless' postures than Camborough gilts and spent more time nursing their litters. They also changed posture less and carried out more nest-building behaviours, perhaps indicative of increased maternal investment. During a human approach test, Meishan gilts had lower heart rate responses to human contact, vocalised less and performed less locomotor behaviour.

Conclusions Production within the communal pen system did not differ significantly from that within the conventional crate system. Meishan gilts differed from Camborough gilts in their behavioural time budgets over farrowing and lactation, performing more behaviour that appeared to show a greater maternal investment. Manor Meishan sows had low liveborn mortality and appeared to be well-suited to open farrowing systems, although performance as gilts was disappointing. This highlights the potential of different genotypes in the success of alternative systems to the farrowing crate.

Acknowledgement This study was funded by MAFF (AW0106).

An investigation into the effect of different protein and energy intakes on the tail chewing behaviour of growing pigs

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Introduction It has been widely reported that the pigs responsible for tail biting under both commercial and experimental conditions, are those which show poor growth rates. This may be because of an inability to obtain food because of social factors or poor pen design, or an inability to utilise food because of health or metabolic disorders. A major reduction in protein:energy ratio in the diet has been shown to increase attraction to blood in an experimental tail chewing model (Fraser, 1987). The aim of this study was to clarify whether the increased attraction to blood is a consequence of a specific metabolic state resulting from a protein deficiency (with effects on neurotransmitter pathways, Harper and Peters, 1989), or whether it occurs under conditions of a reduced growth rate irrespective of a protein deficiency.

Materials and methods 24 pigs were selected for testing at 61.9kg (S.D. = 3.9kg) from 8 litters, each providing either 3 male or 3 female siblings. The pigs were housed individually, had free access to water and were fed a controlled amount of feed once daily from the start of the experiment until slaughter. On each Monday, Wednesday and Friday for 6 weeks, the pigs were presented with 2 tail models on which they could chew. The tail models were made of sash cord that had been soaked overnight in either whole pig's blood or de-ionised water. The animals' chewing behaviour was directly observed and recorded every 6 seconds, for 24 minutes. Chewing was only scored if the tail was inside the pig's mouth. The chewing scores recorded in week 1 were used to allocate pigs between three experimental treatments, such that the amount of chewing performed by each group in the pre-treatment week was approximately the same (Fraser, 1987). Control pigs were fed at a level close to appetite (2.85 * maintenance energy requirement) on a diet containing 13.3 MJ DE kg^{-1}, 189g CP kg^{-1}). Treatment 2 (LCP) received the same daily energy intake from a low protein diet (12.9 MJ DE kg^{-1}, 980g CP kg^{-1}). Treatment 3 (LE) received the same daily protein intake as control pigs, but only 72% of the DE intake using a high protein diet (13.6 MJ DE kg^{-1}, 280g CP kg^{-1}). Pigs were weighed weekly and the food allowance for each group was recalculated to ensure they were being fed as closely as possible to their voluntary intake without any refusals. At the end of the experiment, blood was collected from all pigs at slaughter and analysed for total and free plasma tryptophan and serum NEFA. Data on total tail chewing score and preference score (chewing of the blood tail/total tail chewing) were analysed using a repeated measures analysis of variance.

Results Litter of origin was found to have no significant effect on the preference or chewing scores of the pigs. Individual pigs varied significantly in their preference for chewing the blood covered tail model ($P<0.05$), however, diet was found to have no effect on the total chewing or preference scores of the pigs. There was a significant effect of week on the preference ($P<0.01$, Figure 1) and total chewing scores ($P<0.001$, Figure 2) of the pigs. There were no interactive effects between diet and week on the preference or chewing scores of the pigs. Over the 6-week experimental period, the pigs on the control diet had a mean weight gain of 773g d^{-1} (SD = 72g d^{-1}), those on the LCP diet had a mean weight gain of 501g d^{-1} (SD = 96g d^{-1}) and those on the LE diet had a mean weight gain of 482g d^{-1} (SD = 95g d^{-1}).

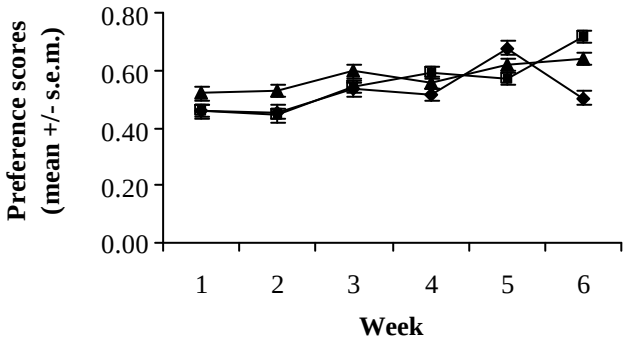

Figure 1 Dietary effect on preference scores

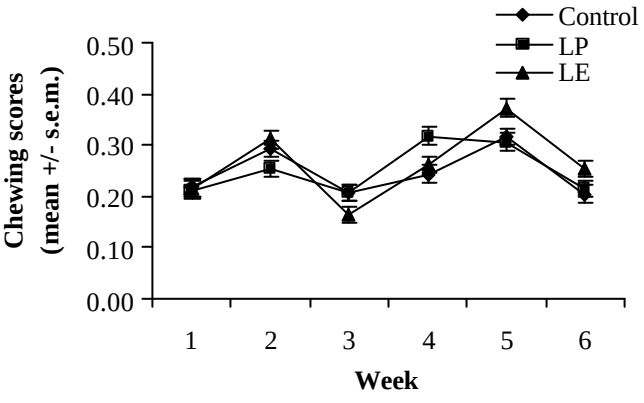

Figure 2 Dietary effect on chewing scores

Conclusions In contrast to Fraser (1987), this experiment failed to reveal any differences resulting from dietary modification in the pigs' total chewing score or preference for chewing a blood covered tail model. Reasons for this difference require further investigation if this experimental model is to be of value.

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

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Harper, A.E. and Peters, J.C. 1989. Protein intake, brain amino acid and serotonin concentrations and protein self-selection. Journal of Nutrition **119**: 677-689

The effect of varying lengths of chopped straw bedding on the behaviour of growing pigs

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Introduction Legislation in the United Kingdom states that all pigs should have access to straw or other material or object suitable to satisfy their behavioural needs (Welfare of Livestock Regulations, 1994). However, the use of straw bedding has not been universally adopted as its use is incompatible with housing systems which contain perforated flooring, and 76% of pig producers in the UK currently employ fully- or part-slatted finishing housing systems. The aim of this study was to investigate whether different lengths of chopped straw would achieve enhancements in pig welfare, by exploring the possibility that small quantities of chopped straw, in preference to unchopped straw, could constitute adequate provision in part- and fully-slatted systems, thus avoiding the blockage of perforated flooring.

Materials and methods Twenty-four groups of 10 growing pigs were exposed to one of four treatments in a randomised block design (N: no straw bedding, F: full length straw, H: half chopped straw, and C: full chopped straw). Both pen-mate- and straw-directed behaviours were recorded using *time* and *ad libitum* sampling in weeks one, four, seven and ten of the trial. The time sampling of behaviour involved observing the four focal animals within each group concurrently for 60 minutes. Within this observation period, and at exactly five minute intervals, the behavioural element which each focal animal was expressing was noted such that 12 samples were collected per animal. These observations were conducted either between 1000-1100h, or 1400-1500h, and the overall distribution of observation times was balanced within each treatment. The *ad libitum* sampling of behaviour involved watching a focal animal within a group between 1300h and 1540h for a six minute period and noting whenever it expressed specific behavioural elements. The data were analysed using repeated measures ANOVA.

Results General levels of straw directed-activity were found to be significantly affected by straw treatments in the *ad libitum* sampled data-sets (mean levels of activity were 90.2, 80.7 and 19.3 behavioural events per six minute period for F, H and C groups respectively; SED=15.18; $P<0.001$). Activity levels also decreased over time (mean values were 92.9, 73.8, 43.8 and 43.2 behavioural observations per six minute period for weeks one, four, 7 and 10 respectively; SED=10.76; $P<0.001$). The level of tail-biting was significantly affected by the straw treatments (mean proportions of active observations were 0.0000, 0.0001, 0.0007 and 0.0013 for N, F, H and C respectively; SED=0.00043; $P<0.005$). Interactions between treatment and week are useful indicators as to when the effects of time (such as those associated with behavioural development) differ significantly between treatments. There was a significant interaction for aggressive behaviour where N groups of pigs were most aggressive during week one (Figure 1a), and for nosing where N groups interacted with other pigs at a consistently higher level than their H, F and C counterparts (Figure 1b).

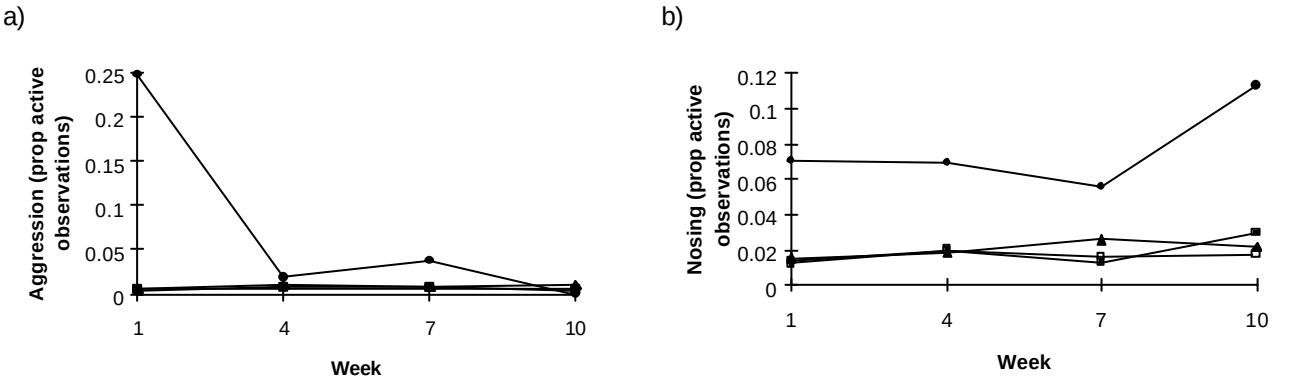

Figure 1: The interaction between treatment and week on: a) the level of aggression (SED=0.0053), and b) the level of nosing other pigs (SED=0.0079). Values represent means for N (●), F ( ), H (▲) and C (■) respectively.

Conclusions The use of chopped straw in growing/finishing housing systems may be beneficial in reducing the occurrence of certain adverse behaviours, however, its use in part- or fully-slatted housing systems is inadvisable whilst there is a possibility that levels of tail-biting may be increased. Furthermore, straw which has been finely chopped is not able to accommodate many of the behaviours which pigs direct towards longer lengths of straw, and the degree to which this reduces the efficacy of the substrate in improving welfare remains to be determined.

Acknowledgements This work was funded by MAFF.

Reference Welfare of livestock regulations. SI No. 2126. (1994). HMSO, London.

The effects of chain and feeder position on lying and dunging behaviour of finishing pigs in the presence and absence of straw

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Introduction The use of fully slatted flooring and absence of bedding for finishing pigs are being questioned for welfare reasons at Council of Europe level and have already been made illegal in some EU member states. Part slatted flooring offers an alternative, but their success depends to a large extend on the correct use of functional pen areas by the pigs: dunging should take place on the slats, lying on the solid floor. Pen lay-out has a large effect on the degree of pen fouling. The effects of straw provision on the solid floor area are less well documented. The present study tests the hypothesis that pen fouling in a converted standard Dutch pig pen is not affected by straw use, but by the position of the single space feeder as well as the position of a 40 cm chain (toy).

Materials and Methods A total of 384 finishing pigs (weight: 24.9kg, s.d.: 5.1) were allocated in 48 groups of 8 pigs to a 2x2x2 factorially designed experiment. The treatment factors were: feeder location (front or back of pen), chain location (front or back) and straw (either 35g pig^{-1} day^{-1} or no straw at all). Each combination of factors was repeated six times. The groups were split into two consecutive batches of 24 each, using four experimental rooms with similar ventilationsystems. The lay-out of the pens is shown in Figure 1. They were typical Dutch pens with an arched solid floor. The small slatted area nearest to the feeder passage was closed in all pens to allow the use of straw. Straw was kept out of the large slatted area by a 15 cm high wooden straw barrier. Pen fouling was scored twice weekly throughout the 16 week finishing period by estimating the percentage of soiled solid area. At the same time the number of pigs lying was scored. Lying behaviour was also scored indirectly around week seven of the finishing period by scan sampling 2x24 hour video taped recordings of each pen.

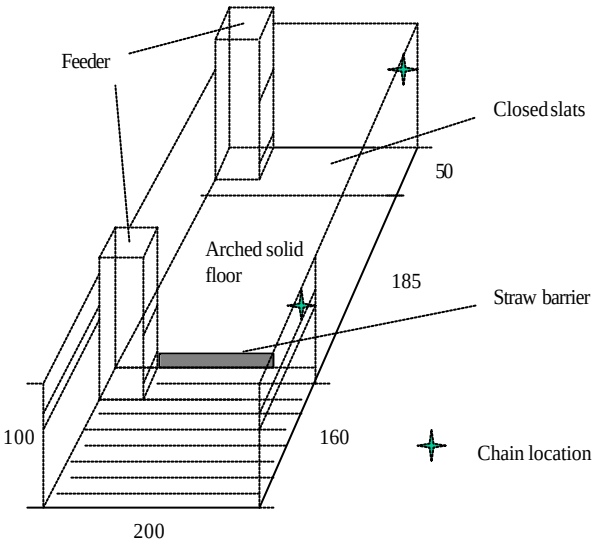

Figure 1 Pen lay-out. Feeders and chains were either placed at the front or at the back

Results
The results suggest straw use affects the number of pigs which lie during direct observation periods (with the observer walking through the feeder passage) but not during video recorded observations (Table 1). Pen fouling appears to be affected by feeder position and straw use, but not by the position of the chain (Table 1). The best results in terms of pen cleanliness were obtained by positioning the feeder at the front of the pen and not using straw .

Table 1 Average percentage of lying animals per pen per treatment (S = straw; F = feeder; C = chain) with direct observations and indirect (videotaped) observations, and average percentage of pen soiling.

S	Yes				No					P-Values [1]						
F	Front		Back		Front		Back									
C	Front	Back	Front	Back	Front	Back	Front	Back	IQR[2]	S	F	C	S*F	S*C	F*C	S*F*C
Lying (direct) [3]	68.8	69.1	69.1	69.4	59.7	58.2	56.7	60.2	48.8-77.2	*	n.s.	n.s.	-	-	-	-
Lying (indirect) [3]	88.5	85.2	86.4	89.4	88.3	86.8	85.9	87.0	84.9-90.1	n.s.	n.s.	n.s.	-	-	-	-
Pen soiling [4]	1.5	3.2	5.7	3.7	1.1	0.6	1.6	1.7	0.9-4.4	***	*	n.s.	n.s.	n.s.	n.s.	n.s.

[1] Significance: n.s = not significant; * = p< 0.05; ** = p< 0.01; *** = p < 0.001
[2] Inter Quartile Range.
[3] Data could not be normalised. The statistical test used was Wilcoxon/Kruskal-Wallis.
[4] Data had to be transformed prior to analysis. Means are back-transformed values. IQR was based on original data.

Conclusions The observations on lying behaviour may suggest that straw helps to reduce 'nervousness' or 'alertness' in pigs. However, using straw in the present simple conversion of a typical Dutch finishing pen will result in increased pen fouling, even though a feeder at the front of the pen may to a degree mitigate this effect.

The effect of mixing piglets at different ages pre-weaning on pre-weaning behaviour

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Introduction In commercial practice, the mixing of pigs often occurs on more than one occasion. This can be very stressful for the pig, as new dominance hierarchies have to be formed at each mixing. Mixing piglets pre-weaning has been used as a method of removing the stress of mixing from the point of weaning (Allen *et al*., 2000; North & Stewart, 2000) but the effects of mixing at different ages on pre-weaning behaviour needs to be determined. The aim of this study was to determine if mixing piglets pre-weaning affects their sucking and social behaviour.

Material and methods Thirty-six high-health PIC Camborough 15 (Large White x (Landrace x Duroc)) sows and their piglets were kept in conventional farrowing crates. These were randomly allocated to three treatment groups, each containing three sows and their piglets, in a randomised block design with four replicates. The piglets were mixed at different times in relation to weaning (day 28) according to treatment: mixing at 21, 14 and 7 days prior to weaning (T1, T2 & T3 respectively). Piglets were mixed by removing boards between the farrowing crates allowing access to three pens whilst the sows remained confined in their crates. Any fostering was carried out within 24 hours of birth and normal management practices were followed. Creep feed was made available to all piglets from 14 days of age. Behavioural observations were carried out for three hours prior to mixing (day –1), six hours on the day of mixing and three hours on day 3 post-mixing. Suckling observations included a) number of sucklings and successful sucklings, b) duration of suckling, c) proportion of cross-suckers, d) initiator and terminator of suckling, e) synchronisation of suckling within the treatment. All agonistic interactions were recorded including a) duration and time of fight and b) littermate or non-littermate interactions. Behavioural data were statistically analysed by Chi-squared and mean number, duration and interval of sucklings and their relative changes were analysed by ANOVA.

Results The relative change in the number and duration of suckling were not significantly affected by the age at mixing, nor was the relative change in suckling interval pre- to post-mixing (Table 1). The proportion of synchronised sucklings, cross-sucking pigs, the number of initiations and the number of terminations of sucklings by the sow were not significantly affected by mixing at any age pre-weaning. Agonistic interactions were not significantly different on any observation days. The number of fights over teats as a proportion of total fights was significantly different in the second hour after mixing with piglets on treatment 3 fighting more over teats than piglets on treatments 1 and 2 (0.831 *vs.* 0.492 *vs*. 0.486 respectively, χ^2=9.701, P<0.05).

Table 1 *Pre-mixing suckling behaviour and their relative changes post-mixing*.

Treatment	T1	T2	T3	s.e.d	Significance
Day–1 Number of sucklings	4.3	4.2	4.0	0.30	NS
Change in number of suckling(%)					
Day –1 vs. Day 0	6.7	10.5	4.0	9.75	NS
Day –1 vs. Day 3	8.9	1.8	-7.4	8.19	NS
Day –1 Duration (secs)	361	296	297	42.8	NS
Change in duration of suckling(%)					
Day –1 vs. Day 0	-22.1	-12.5	-14.6	9.56	NS
Day –1 vs. Day 3	-8.3	-3.3	2.8	12.80	NS
Day-1 Suckling Interval (min)	49.3	37.7	36.9	5.59	NS
Change in suckling interval(%)					
Day –1 vs. Day 0	8.5	2.7	-1.1	11.61	NS
Day –1 vs. Day 3	19.0	-7.2	2.9	19.86	NS

Conclusions Mixing piglets pre-weaning at any age caused no significant change in suckling behaviour from pre-mixing to post-mixing. There was no significant effect of mixing on agonistic interactions, although mixing 7 days prior to weaning caused an increase in fights over teats compared to mixing earlier in lactation. Previous work (Allen *et al*., 2000) found that mixing pre-weaning improved production and welfare of the piglets and this study gives further evidence that mixing pre-weaning can be carried out without disrupting behavioural patterns.

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Allen, M.J., Stewart, A.H., Mackenzie, A.M. 2000. The effect of mixing piglets at different ages during lactation on post-weaning performance. *BSAS Occasional Meeting 'The Weaner Pig', Nottingham, Sept 2000,* pp14-15.

The influence of mineral blocks on the behaviour of newly weaned pigs

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Introduction Newly weaned pigs frequently show high levels of oral-nasal behaviour directed towards penmates which may cause injury if taking the form of ear and tail biting. It has been suggested that such behaviours may be motivated by both behavioural and nutritional influences. This experiment was carried out to investigate whether the provision of nutrient blocks supplying minerals, trace elements and herbs could reduce penmate-directed behaviours in weaned piglets.

Material and Methods A split plot design was used to compare the behaviour of groups of weaned pigs with or without provision of mineral blocks (Wright Herbal Pig Block, Frank Wright Ltd). Each week, 300 Large White x Duroc pigs were weaned at 3.5 weeks of age into 2 adjacent pens (150 pigs/pen) in the same controlled-environment, fully-slatted room. Each pen provided 54 m^2 of floor space, 3 automatic liquid feeders used in weeks 1 and 2, 3 *ad libitum* dry feeders used from week 2 onwards, 3 turkey drinkers and 2 wall-mounted nipple drinkers. A heat lamp provided supplementary heat in a localised area of the pen for the first 2 weeks after weaning. One pen in the pair was supplied with 5 mineral blocks, each one comprising a 7 inch cube weighing 10 kg. Within each group, 12 animals were selected at weaning to be focal pigs for detailed study. These comprised 4 pigs of heavy weight (8.6$\pm$0.1kg), 4 of median weight (7.1$\pm$0.1kg) and 4 of low weight (5.7$\pm$0.1kg) relative to the group as a whole. Behaviour of these focal pigs was recorded by time sampling at 12 minute intervals for two 90 minute periods on one day each week, using an ethogram of 19 mutually exclusive behaviours. Four replicate pens were each studied for a period of three weeks from weaning. Data were analysed by ANOVA using a model incorporating replicate, treatment and weight class.

Results Summarised results of piglet behaviour are shown in Table 1. Piglets provided with blocks spent 3.4% of observations in block directed behaviour and significantly more time in feeding and drinking. They showed a significantly lower frequency of receiving ear biting, but no differences in other non-injurious social behaviours. Fighting was recorded more frequently when blocks were present in the pen. There were significant differences between replicates in the frequency of locomotory ($P<0.001$), eating ($P<0.001$) and drinking ($P<0.01$) behaviour, but not in exploratory or harmful social behaviours. Weight class significantly influenced only drinking behaviour, with median pigs spending fewer observations drinking than those of heavy or light weight (3.1, 1.6, 2.9 % of time, sem 0.31, $p<0.05$); the tendency for light weight pigs to show a higher frequency of ear biting and being ear bitten was not significant (both $P<0.10$). Neither treatment nor weight class significantly affected liveweight gain of the focal pigs in the three weeks after weaning.

Table 1 The percentage of observations spent in different behaviours by pigs in the first three weeks after weaning in the presence or absence of mineral blocks.

% of time	BLOCK	NO BLOCK	s.e.m.	Sig
INACTIVE				
- Lying	43.4	48.5	1.61	P<0.05
- Standing	8.3	11.0	0.60	P<0.01
LOCOMOTORY				
- Walking	2.9	2.3	0.25	ns
SOCIAL				
- Non-Aggressive	3.5	2.8	0.32	ns
- Playing	0.3	0.1	0.07	ns
INGESTIVE				
- Eating	12.1	9.2	0.72	P<0.01
- Drinking	3.1	2.0	0.25	P<0.01
HARMFUL SOCIAL				
- Ear biting	1.6	1.9	0.22	ns
- Ear bitten	1.8	2.5	0.24	p<0.05
- Belly nosing	1.1	1.0	0.27	ns
- Belly nosed	0.9	0.6	0.18	ns
- Tail biting	0.2	0.4	0.10	ns
- Fighting	1.3	0.5	0.16	p<0.001

Conclusions The use of mineral blocks may reduce predisposition to perform some types of harmful social behaviour and promote ingestive behaviour, but might also provide a focus for competition.

Acknowledgements We thank Mr M Forbes and the staff of the East Denside pig unit.

Effect of replacing soyabean meal with maize distillers grains on feed intake and milk yield of lactating dairy cows

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Introduction Maize distillers grains (MDG) is a high quality by-product feed containing 317 g crude protein (CP)/kg DM and 13.5 MJ metabolisable energy/kg DM, and as such is a valuable traceable feed resource. An earlier study conducted at the Centre for Dairy Research (Sutton *et al*. 2000) with cows in late lactation using a total mixed ration (TMR) based on maize silage, compared the protein value of MDG with that of soyabean meal (SB). The study showed that MDG could be used to replace SB on a total nitrogen (TN) basis without effecting feed intake or nutrient digestion in the rumen, or flow of non-ammonia nitrogen to the duodenum. The aim of the current study was to determine the effect of replacing SB with MDG on a TN basis, on DM intake and milk production in high yielding lactating dairy cows.

Materials and methods Sixteen multiparous Holstein-Friesian cows (DIM 42-56) were used in a 4 x 4 latin square designed trial. The trial contained four, four-week periods in which the first three weeks of each period were used for adaptation. Data for statistical analysis was collected in the fourth week. At the start of the study cows were blocked according to milk yield and allocated to four treatments (T1-T4). All cows received a TMR which contained 351 and 175 g/kg DM of maize and grass silage, respectively. The composition of maize and grass silage for corrected dry matter (CDM), CP, neutral detergent fibre, starch, total fermentation acids, ammonia-nitrogen (g/kg total nitrogen), pH and estimated ME values were 386 and 278 g/kg, 79 and 115, 333 and 532, 324 and 0, 101 and 172 g/kg DM, 63 and 110 g/kg TN, 3.9 and 3.9, 11.5 and 11.1 MJ/kg DM, respectively. In addition the TMRs contained cracked wheat, SB, MDG, molassed sugar beet feed, rapeseed meal, megalac, urea and minerals, which in T1-T4 represented on a DM basis 186, 102, 0, 92, 70, 14, 0, and 9; 182, 69, 57, 90, 55, 9, 3 and 9; 173, 34, 115, 86, 49, 4, 5, and 9; 155, 0, 173, 75, 58, 0, 4, 9 g/kg, respectively. The ratio of MDR:SB nitrogen in T1-T4 was 0, 0.33, 0.67 and 1.0 respectively. The TMR was fed once daily at 9.00h in individual mangers. The nutrient composition of the TMRs for the four treatments is shown in Table1.

Table 1 Nutrient composition of TMRs

g/kg CDM	T1	T2	T3	T4
Ratio MDG : Soya N	0	0.33	0.67	1
Crude protein	178	177	167	172
Neutral detergent fibre	319	321	337	351
Oil-B	40	44	41	44
Starch	238	237	243	223
Estimated ME (MJ/kg DM)	12.2	12.3	12.1	12.2

Results Treatment means and standard error of the difference (SED) between two treatment means for intake and milk production parameters are shown in Table 2. There were no significant treatment effects on any of the parameters measured.

Table 2 Treatment mean values for feed intake and milk production

Treatment	T1	T2	T3	T4	
Ratio MDG : Soya N	0	0.33	0.67	1	SED
Total DM intake (kg/day)	21.6	21.3	21.5	21.6	0.60
Milk yield (kg/day)	29.8	30.0	30.2	29.4	0.62
Milk fat (g/kg)	50.2	49.9	49.3	49.5	1.16
Milk protein (g/kg)	33.5	33.2	33.5	33.4	0.37
Milk lactose (g/kg)	46.5	46.3	46.3	46.6	0.23
Milk fat (g/day)	1496	1497	1490	1457	46.6
Milk protein (g/day)	994	989	1007	976	22.9
Milk lactose (g/day)	1389	1388	1401	1369	29.6

Conclusions The main conclusion of this study is that maize distillers grains which is a high quality by-product can be used in a TMR based on maize and grass silage to replace soyabean meal without compromising DM intake, milk yield or composition.

Acknowledgement This work was funded by Trident Feeds.

Reference Sutton, J. D., Humphries, D. J. and Witt, M. 2000. Replacement of soyabean meal by maize distillers grains for lactating dairy cows. *Proceedings of the British Society of Animal Science*, 92.

The effect of level of concentrate feeding on the response of lactating dairy cows to dietary inclusion of fodder beet

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Introduction Traditional options to increase the energy intakes of lactating dairy cows include raising the level of concentrate feeding and/or improving the quality of grass silage. However, each of these options have limited potential to increase total ME intake (Ferris *et al.,* 2000). The inclusion of an additional dietary component, such as whole crop cereal silage or fodder beet constitutes an alternative approach to increase energy intake (Phipps *et al.,* 1995). An experiment was designed to examine the potential of fodder beet to increase the energy intake and performance of lactating dairy cows across a range of levels of concentrate feeding.

Materials and methods Ten treatments were examined in a 3 period (period length 4 weeks) partially balanced change-over design trial involving 40 Holstein-Friesian dairy cows of mixed parity. Animals were a mean of 31 days calved at the start of the trial, and formed two blocks, each of 20 animals. Treatments were arranged in a 2 x 5 factorial design. Factors examined comprised grass silage alone or grass silage mixed with fodder beet (70:30 DM ratio), and five levels of concentrate supplementation (3.4, 6.0, 8.5, 11.1 and 13.6 kg fresh weight/d). The concentrate offered had a crude protein concentration of 242 g/kg DM, while the silage offered had DM, crude protein and D-value (determined using sheep) of 237 g/kg, 169 g/kg DM and 699 g/kg respectively. The diets were offered *ad libitum* in the form of a complete diet, and individual feed intakes recorded. The digestibility of each of the diets was determined using two lactating dairy cows per treatment. The ME concentrations of the rations offered were calculated, assuming a methane energy loss equivalent to 0.06 gross energy intake. The data were statistically analysed using ANOVA.

Results In the absence of significant interactions only main treatment effects are shown in Table 1. Increasing concentrate feed level resulted in a linear increase in total DM intake, ME intake and milk yield, and in milk protein and energy concentrations ($P \leq 0.05$). Milk protein yield also increased in a linear manner ($P<0.001$) while yields of fat and energy showed a curvilinear increase ($P<0.05$). The inclusion of fodder beet in the diet increased total DM intake, ME intake and milk yield. Significant increases were also obtained in both the concentration of protein and the yields of the measured milk constituents.

Inclusion of fodder beet in the diet increased the digestibility of energy (0.752 and 0.772, sem 0.003, $P<0.001$). While increasing the level of concentrate feeding and introducing fodder beet into the diet each increased both ME intake and milk yield, comparing the responses in milk energy output to increase in ME intake indicates ratios of 0.39 and 0.10 MJ milk/MJ for concentrates and fodder beet respectively.

Table 1 Treatment effects on feed intakes, milk production and milk composition

	Concentrate level (kg/d)						Significance		Beet factor			
	3.4	6.0	8.5	11.1	13.6	sem	Lin	Quad	No beet	Beet	sem	Sig
Feed intakes												
Silage (kg DM/d)	8.3	7.8	7.4	6.4	6.0	0.34	***	ns	7.5	6.9	0.19	*
Fodder beet (kg DM/d)	2.1	1.7	1.7	1.3	1.3	0.11	***	ns	0	3.2	0.06	***
Concentrates (kg DM/d)	3.0	5.2	7.5	9.8	12.0	0.07	***	ns	7.5	7.5	0.03	*
Total DM intake (kg/d)	13.3	14.8	16.6	17.5	19.3	0.44	***	ns	15.0	17.6	0.23	***
ME intake (MJ/d)	147.9	166.4	190.3	201.2	221.7	5.00	***	ns	167.8	203.2	2.58	***
Milk yield (kg/d)	20.0	23.6	26.3	27.5	28.5	0.64	***	*	24.8	25.6	0.27	*
Milk composition												
Butterfat (g/kg)	40.6	41.2	40.2	41.1	41.0	0.73	ns	ns	40.5	41.1	0.28	ns
Protein (g/kg)	30.4	31.6	33.1	34.0	35.3	0.25	***	ns	32.4	33.4	0.09	***
Energy (MJ/kg)	3.09	3.21	3.19	3.2	3.26	0.045	*	ns	3.14	3.22	0.029	ns
Yield of milk components												
Butterfat (g/d)	819	972	1047	1117	1148	29.1	***	*	998	1044	12.5	**
Protein (g/d)	614	741	862	925	996	19.9	***	ns	804	851	24.2	***
Energy (MJ/d)	62.6	74.8	82.8	87.9	91.6	2.00	***	*	78.2	81.6	0.88	**

Conclusion The inclusion of fodder beet produced an important increase in ME intake of lactating dairy cows, and the absence of interaction with level of concentrates shows that the response to fodder beet was not affected by the level of concentrate feeding. However, the milk yield response to additional ME from fodder beet was considerably lower than the equivalent response with concentrates. No reason has been identified for the difference in response.

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Factors influencing individual predicted total dry matter intake of dairy cattle on farms

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Introduction The aim was to estimate the influence of genetic merit (£PIN95) and level of concentrate feeding (Cgrp) on predicted total dry matter intake (tDMI) of individual cows, using records collected from commercial farms. The method described by Wicks & Leaver (2000) was used to estimate individual daily dry matter intakes from seven farms, totalling 4282 monthly records over a two-year period. The method was based on milk production records supplemented by body condition scores and height at withers, which were used to calculated the ME requirements of individual animals. All the records were collected, from autumn and winter (July to March) calving cows during the housed period (August to March).

Materials and Methods A general linear model was used to analyse the tDMI and estimate the influence of £PIN95 and Cgrp on the total dry matter intake of dairy cattle. The model included the fixed effects of; year (Y) and season (S) of calving, parity (P), stage of lactation (St), and the interaction between stage and parity (St*P). Other parameters in the model included daily concentrate intake (kg/d) (C), Profit Index (£PIN95), concentrate feeding group (Cgrp) (Table 1), the interaction between parity and concentrate feeding group (P*Cgrp) and the interaction between £PIN95 and Cgrp (£PIN95*Cgrp). Cgrp was used to indicate level of feeding on individual farms, and due to the correlation between £PIN95 and individual concentrate intake, C was included as a co-variate.

Table 1 Definition and range of concentrate intake by concentrate feeding group (Cgrp)

Cgrp	diet ME MJ/kg DMI	Fresh Weight (kg/d) Range	Mean	Stdev
Low	11.0	< 7	5.93	0.856
Medium	11.7	7 to 9	7.86	0.380
High	11.4	>9	9.96	0.632

$$tDMI = Y + S + P + St + St*P + C + £PIN95 + Cgrp + P*Cgrp + £PIN95*Cgrp$$

Results The model accounted for 51.5% of the variation in predicted total dry matter intake of dairy cattle, and all factors in the model were significant ($P<0.05$). The influence of £PIN95 on tDMI was positive, with a mean increase in tDMI of 0.037kg/d per £1 of £PIN95. The co-efficient of £PIN95 for tDMI declined as the level of feeding increased (0.050, 0.038 and 0.022 for low, medium and high concentrate feeding groups, respectively). However, the confounding effect of forage quality and level of concentrate input meant that the high concentrate group were fed a diet with lower ME concentration than the medium concentrate group. The mean tDMI of cows was 18.2 kg/d, but varied between years (Table 2). The difference in tDMI between the three parity groups reduced as lactation progressed. Figure 1 shows (interaction P*Cgrp) that level of concentrate feeding had no effect on tDMI for first parity animals, while the high and medium Cgrp levels had beneficial effects on tDMI for cows ≥2. On average each 1kg fresh concentrate/d was estimated to increase tDMI by 0.25kg/d.

Table 2 Least square means of tDMI by year, season and parity

Factor	Level	TDMI (kg/d)	SEM
Year	97	18.6	0.09
	98	17.7	0.09
Season*	Winter	18.3	0.12
	Autumn	18.0	0.09
Parity	1	16.3	0.14
	2	18.3	0.12
	≥3	19.7	0.11

*Winter defined as December to March calving
Autumn defined as July to November calving

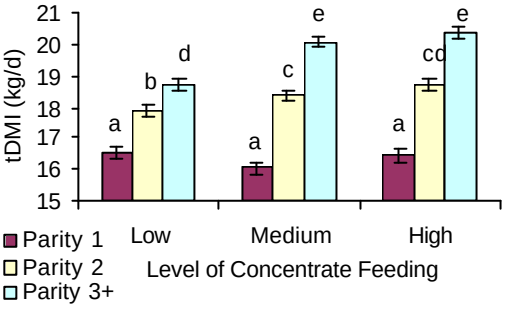

Figure 1 Least squares means of tDMI, by level of concentrate feeding, and parity

Conclusions The methodology proved satisfactory for estimating total DMI of dairy cows on farms, and the general linear model showed significant influences of £PIN95 and Cgrp on tDMI. The mean predicted total DM intake of 18.2kg/d is lower than that recorded from experimental work. This highlights the problems associated with translating research findings under controlled experimental conditions to farm conditions. Predicted total DMI increased with £PIN95. First lactation animals did not increase tDMI in response to level of concentrate feeding.

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Reference Wicks, H.C.F. & Leaver, J.D. 2000. Development of a model to predict dry matter intake of individual dairy cattle on commercial farms. *Proceedings of British Society of Animal Science*, p.141.

Effects of the ratio of effective rumen degradable protein to fermentable metabolizable energy on voluntary intake and milk yields of dairy cows

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Introduction Diets with low ratios of effective rumen degradable protein (ERDP) to fermentable metabolizable energy (FME) are often offered to dairy cows in Portugal, because they are based on maize silage and protein sources are very expensive. It seems likely that this will restrict microbial protein synthesis and voluntary intake and, consequently, lead to reduced milk yields. The objective of this study was to examine the production response of dairy cows offered diets differing in ERDP/FME ratio.

Materials and methods Six multiparous and three primiparous Holstein cows were used. The cows had an initial mean lactation stage of 149 days (s.d. 13.0) and a mean milk yield of 29 kg/d (s.d. 4.6). The animals were assigned to three blocks based on lactation number and lactation performance. Each block was a 3x3 Latin Square design, with 4-week periods. Three iso-energetic diets with 8, 10 and 12 g ERDP/MJ FME were formulated, using the RUMNUT 4.0 program, based on maize silage, ryegrass hay and concentrates (Table 1). The digestible undegradable protein (DUP) requirements were, in all cases, satisfied. The diets were offered to the animals as a complete feed for *ad libitum* intake at 08:30h and at 15:00h. Blood samples were collected three hours after the morning-feed on 2 consecutive days of the last week of each experimental period and analysed for urea (Bauer, 1982). Feed intake and milk production results from the final week of each period were used in the analysis of variance (Systat 5.0). One cow had a displaced abomasum and was excluded from the analysis.

Table 1 Formulation of the concentrates (g/kg as-mixed)

	ERDP/FME (g/MJ)		
	8	10	12
Wheat	200	170	230
Maize gluten feed	141	318	55
Maize gluten meal	70		
Citrus pulp	330	55	44
Rapeseed meal	40	180	250
Soyabean meal	75	50	125
Sunflower meal	75	130	190
Molasses	30	30	30
Fat prills	6	30	40
Dicalcium phosphate	11		
Calcium carbonate		15	14
Salt	5	5	5
Sodium bicarbonate	8.5	8.5	8.5
Magnesium oxide	6.5	6.5	6.5
Mineral-vitamin mixture	2	2	2

Results The maize silage was of good quality (DM: 327 g/kg; starch: 300 g/kg DM; NDF: 462 g/kg DM and pH 3.55). The effect of ERDP/FME ratio on voluntary intake, milk yield and composition and on blood urea are given in Table 2. ERDP/FME ratio had a significant effect ($P<0.05$) on voluntary intake, milk yield and blood urea. Milk fat and protein contents did not differ significantly between diets. The diet with an ERDP/FME ratio of 10 g/MJ promoted higher DM intakes and milk yields. The primiparous cows had significantly ($P<0.001$) lower DM intakes and milk yields than multiparous cows, regardless of diet. As expected, blood urea concentration increased significantly ($P<0.001$) in response to the increase in ERDP/FME ratio.

Table 2 Effect of ERDP/FME ratio on voluntary intake, milk yield and composition and blood urea

	Diet (g ERDP/MJ FME)			s.e.m.	P
	8	10	12		
Total diet dry matter intake (kg/day)	19.3	21.3	20.7	0.28	<0.05
Milk yield (kg/day)	27.6	30.1	29.2	0.46	<0.05
Milk fat (g/kg)	37.2	34.7	38.8	1.40	NS
Milk protein (g/kg)	33.4	32.7	33.4	0.43	NS
Blood urea (mg dl^{-1})	23.3	30.0	36.5	1.06	<0.001

Conclusions The results of the present study demonstrate that diets with ERDP/FME ratios lower than the Agricultural and Food Research Council (AFRC, 1993) reference values restrict DM intake and milk yield. Conversely, supplying ERDP in excess of the amount necessary to match the amount of FME supplied by the diet and utilisable by the rumen microbes, did not increase voluntary intake or milk yield and increased blood urea, leading to ERDP waste and environmental pollution.

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Lactational performance and body weight change in cows fed the fungal treated wheat straw

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Introduction In Iran, the availability of high quality forages is restricted. The use of wheat straw, which is accessible in a huge amount, is limited due to its low nutritional value. Recently, biological method using white-rot fungi to improve the nutritional value of straw have been reported (Zadrazil 1997). However, the data available have been obtained from *in vitro* works. Additionally, little information is available on the utilization of fungal treated straw fed to dairy cow. Therefore, this experiment was carried out to assess the effect of fungal treated wheat straw on the performance of lactating cows.

Materials and methods Wheat straw was soaked in tab water for 24 h. After steaming the straw for one h, it was inoculated with spawn of *Pleurotus 41*, packed in the polyethylene bags (12 kg of fresh weight straw per bag). Subsequently, the bags have left for 50 days to be fermented. Individually housed eight primiparous Holstein cows, in late lactation period, yielding 10.3 ±1.3 kg/d of 4FCM were allocated in two groups using a complete randomized design. The two diets, which contained either 30% of untreated or fungal treated wheat straw were formulated according to NRC (1989) and offered *ad lib* as TMR. *In vivo* digestibility of the diets was measured using acid insoluble ash technique. The dry matter intake, milk production and composition were weekly recorded. The cows were weighed at the beginning and at the end of the trial, which lasted one month.

Results Treating the straw biologically resulted in a significant increase in the digestibility of DM and OM at the level of ($P<0.05$) and ($P<0.01$) respectively. Dry matter intake (DMI), organic matter intake (OMI) and digestible organic matter intake (DOMI) were significantly ($P<0.001$) improved in the cows fed the treated straw.

Table 1 DM and OM digestibility and the animal performance.

	Untreated straw	Treated straw	SEM	Significance
DM digestibility	52.3^{b}	58.8^{a}	1.3	*
OM digestibility	51.3^{b}	58.8^{a}	1.1	**
DMI (kg/d)	10.6^{b}	12.2^{a}	0.59	***
OMI (kg/d)	9.9^{b}	11.4^{a}	0.52	***
DOMI (kg/d)	5.1^{b}	6.7^{a}	0.17	***
Milk production (kg/d)	7.5^{b}	9.0^{a}	0.7	*
4FCM production (kg/d)	7.1^{b}	8.2^{a}	0.5	*
Milk composition:				
Milk Fat (g/kg)	35.6^{a}	34.2^{a}	2.3	NS
Milk Protein (g/kg)	35.7^{a}	32.3^{b}	1.5	*
Body weight gain (g/d)	272^{b}	743^{a}	5.0	***
Feed efficiency:				
4FCM/DMI (kg/kg)	0.67^{a}	0.68^{a}	0.07	NS
BWG/DMI (g/kg)	26.2^{b}	61.2^{a}	4.0	*

4FCM = 4% fat corrected milk; BWG = body weight gain; NS = non-significant; SEM = standard error of means.

Feeding treated straw had led to increase the production of milk significantly ($P<0.05$). Milk fat content did not differ between cows fed either the treated or untreated straw. However, the protein concentration was significantly ($P<0.05$) higher in the diet contained untreated straw. The body weight gain was statistically ($P<0.001$) higher for cows fed treated straw in comparison to those offered control diet. The type of the diet did not affect the 4FCM yield per kg of DMI. The amount of BWG per kg of DMI was significantly ($P<0.001$) higher for the cows fed treated straw.

Conclusion The cows, which fed fungal treated straw diet, had higher DMI, OMI and DOMI. The 4FCM yield of cows fed treated straw was increased in average of 13%. Daily body weight gain was improved in the animals fed the treated straw in comparison to other diet. The improvement in the performance of the dairy cows fed the treated straw could be a reflect of the increase in the digestibility of this diet.

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Production of lactating dairy cows fed diets with lucerne or red clover silage with or without supplemental maize silage

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Introduction Lucerne (Medicago sativa) is a major, high protein forage fed to dairy cattle. However, during ensiling, much of the CP in lucerne silage (LS) is broken down to nonprotein N (NPN); high levels of NPN in LS depress protein utilisation in lactating dairy cows. Red clover (Trifolium pratense) is a high quality legume forage that can be grown in Britain and Northern Europe. Polyphenol oxidase, an enzyme system in red clover, converts plant phenols into quinones that react rapidly with forage proteins in the silo and result in red clover silage (RCS) having less NPN than LS. Earlier (Broderick et al., 2000), we reported that replacing LS with RCS lowered milk yield but improved feed efficiency and apparent digestibility. Our objective was to compare the production of dairy cows fed equal amounts DM as LS or RCS, with or without maize silage (MS) and supplemental protein added to the diet.

Materials and Methods Lucerne and red clover were field wilted to about 35% DM, chopped and ensiled in bunker silos (LS) or plastic bags (RCS). The LS averaged (DM basis) 21.7% crude protein (CP), 45% neutral detergent fibre (NDF), 36% acid detergent fibre (ADF), and 64% NPN (% of total N); RCS averaged 19.1% CP, 43% NDF, 33% ADF, and 40% NPN. Four diets were fed as TMR containing (DM basis): 1) 60% LS, 2) 60% RCS, 3) 48% LS plus 12% MS, and 4) 48% RCS plus 12% MS. Diets 1-3 contained 2.9% soyabean meal plus 36% rolled high moisture maize (HMM); diet 4 contained 5.6% soyabean meal plus 33% HMM. Diet 1 contained 18.4% CP; diets 2-4 averaged 16.5% CP. Twenty-four Holstein cows were blocked into six groups by days-in-milk; cows within blocks were assigned randomly to balanced 4X4 Latin squares. Diets were fed for 4-week periods before switching (total 16 weeks); intake, yield, and milk composition data were collected during weeks 3 and 4 of each period. Apparent digestibilities were estimated using indigestible ADF as an internal marker in TMR composites and fecal grab samples. Procedures of SAS were used to analyse data as a replicated 4X4 Latin square with a model including diet, square, cow-within- square, period, and period-by-diet. Where diet was significant ($P < 0.05$), mean separation was by LSD at alpha = 5%.

Results The LS contained more CP than RCS but RCS contained less NDF and ADF; as expected, RCS had less NPN (only 62% of LS). Although intake was lower on 60% RCS than on both diets containing LS, yields of milk, fat-corrected milk (FCM), protein, and SNF were equal on these three diets (Table 1). Replacing 20% of RCS with MS, and supplementing with soyabean meal, increased DM intake (vs. the 60% RCS diet) and resulted in the highest yields of milk, protein, and SNF. Milk fat content was lower on RCS than on LS but fat yield was not influenced by diet ($P = 0.32$). Feed efficiency (milk/DM intake) and N efficiency (milk N/N intake) were greater on RCS than on LS. Apparent digestibility of organic matter, NDF, and ADF were highest on 60% RCS, intermediate on RCS + MS, and lowest on the two LS diets. These results indicated that utilisation of energy and CP in RCS exceeded that in LS.

Table 1. Effect of feeding forage as lucerne silage (LS) or red clover silage (RCS), with or without maize silage (MS), on the production of lactating dairy cows.

Item	LS	RCS	LS + MS	RCS + MS	SEM	$P > F^1$
DM intake, kg/d	23.5a	21.8c	23.8a	22.8b	0.2	< 0.01
Milk, kg/d	30.4b	30.4b	30.3b	31.7a	0.3	0.02
3.5% FCM, kg/d	31.6	31.1	32.0	32.4	0.5	0.27
Fat, %	4.30a	4.06b	4.33a	4.08b	0.07	< 0.01
Fat, kg/d	1.15	1.11	1.16	1.15	0.02	0.32
Protein, %	3.33	3.30	3.39	3.36	0.03	0.29
Protein, kg/d	0.89b	0.90b	0.91b	0.95a	0.01	< 0.01
SNF, %	8.81	8.87	8.89	8.95	0.08	0.64
SNF, kg/d	2.35b	2.43b	2.39b	2.54a	0.03	< 0.01
Milk/DM intake	1.30b	1.40a	1.28b	1.40a	0.01	< 0.01
Milk N/N intake	0.204c	0.249a	0.228b	0.256a	0.003	< 0.01
Apparent digestibility, %						
Organic matter	66.7c	73.3a	67.4c	70.0b	0.6	< 0.01
NDF	44.1c	55.3a	44.3c	49.8b	0.7	< 0.01
ADF	44.2c	54.2a	43.4c	49.1b	0.7	< 0.01

a,b,cMeans within the same row without common superscripts differ ($P < 0.05$).

1Probability of a significant effect of diet.

Conclusions When LS or RCS was fed at 60% of dietary DM, cows ate less DM on RCS but had equal yields of milk, FCM, protein, and SNF. Adding enough soyabean meal to a RCS + MS diet (MS providing 20% of the forage) to make CP equal to that in a LS + MS diet increased yields of milk, protein, and SNF. Replacing LS with RCS increased

digestibility and both feed and N efficiency. Utilisation of energy and CP in RCS exceeded that in LS.

Reference Broderick, G. A., Walgenbach, R. P, and Sterrenburg, E. 2000. Performance of lactating dairy cows fed alfalfa or red clover silage as the sole forage. *Journal of Dairy Science* **83:** 1543-1551.

Evaluation of legume silages offered to Holstein-Friesian cows with small amounts of concentrates

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Introduction The high intake characteristics of red clover silage has been recognised for many years (e.g. Thomas *et al*., 1985). Our previous study (Dewhurst *et al*., 2000) demonstrated the considerable intake and milk production potential of clover silages made using a new approach based on preparation of big-bales from wilted material with the use of biological inoculant additives. The objective of this study was to verify the positive results obtained with red and white clover silages using material taken from a further series of cuts taken in a subsequent year and to evaluate the legume silages with low levels of concentrate feeding.

Materials and methods This study used the stands of pure red clover (cv. Milvus), white clover (cv. Aran) and ryegrass (mixture of cvs. AberElan, AberComo and Augusta) used previously. Fertiliser applications followed the pattern of the previous year. White clover was harvested over 3 cuts finishing on 13 October 1999, whilst four cuts were attempted for grass (completed by 12 October 1999) and red clover. Three cuts of red clover were completed by 24 August 1999 and the regrowth was grazed with sheep because the ground had become waterlogged.
Crops were mowed using a disc mower fitted with rubber rollers, left in the swath until shortly before baling and wilted aiming for a DM content of 30% (maximum 48 hours). Crops were baled using a round baler with a biological additive (Ecosyl) applied at 1.5 litres per tonne of crop. Mixtures of the three/four cuts, in proportion to production, were used in feeding experiments in order to make the results representative of the season's production.
Twenty-one Holstein-Friesian dairy cows in early- to mid-lactation were used in a 3-period incomplete changeover design experiment with 7 treatments, based on 4 forages (grass silage (G), red clover silage (RC), white clover silage (WC) and a 50/50 mixture (DM basis) of grass silage and red clover silage (GRC)), with 2 levels of concentrates (4 or 8 kg/day). The standard concentrate had the following analysis: starch: 231 g/kg DM; neutral detergent fibre (NDF): 246 g/kg DM; crude protein (CP): 250 g/kg DM). The cows had *ad libitum* access to the forages through roughage intake control feeders. Feed intake, milk yield and milk composition were recorded continuously and values from the final week of each 4-week period were used for the statistical analysis. Results were analysed using REML (Genstat 5; Lawes Agricultural Trust, 1998) with a fixed model of 'diet' and a random model of 'period + cow'.

Results Chemical analysis of G, RC and WC silages gave the following values: for oven-DM: 284, 221 and 284 g/kg; for CP: 151, 190 and 230 g/kg DM; for NDF: 571, 428 and 323 g/kg DM; and for pH: 4.22, 4.38 and 3.98 respectively. Table 1 shows the effects of treatments on intake and production.

Table 1 Effects of legume silages on feed intake and milk production

	Treatment:							s.e.d.	Sig
	G4	G8	GRC4	GRC8	RC4	RC8	WC8		
Silage DM intake (kg/d)	12.8	11.5	13.4	12.5	15.8	14.5	14.6	0.57	P<0.001
Total DM intake (kg/d)	16.2	18.3	16.7	19.3	19.2	21.3	21.3	0.58	P<0.001
Milk (kg/day)	23.5	27.5	23.7	28.6	25.6	30.2	33.2	0.83	P<0.001
Milk fat (g/kg)	37.3	41.0	36.7	37.9	39.1	37.4	35.2	1.86	NS†
Milk protein (g/kg)	29.8	30.4	29.8	31.1	29.4	29.7	31.7	0.49	P<0.001
Milk lactose (g/kg)	46.2	45.9	45.6	46.0	46.2	46.1	46.0	0.49	NS

†P<0.05 for comparison of WC with all other forages

Considering the treatments based on grass and red clover silages, there were no significant interactions between forage and concentrate level in their effects on both forage intake and milk yield.

Discussion Feed intake and milk production responses were very similar to those observed in the previous year (Dewhurst *et al*., 2000), despite the increased cutting frequency for grass and red clover. Clover silages have high intake characteristics, which are partially offset by lower digestibility in the case of red clover. Whilst yields of white clover grown as a pure stand were disappointingly low (less than half of yields from grass), these results show that encouraging the presence of white clover in swards will add intake and milk production potential to grass silages.

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Late summer concentrate supplementation of dairy cows at grass

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Introduction Concentrate supplementation at grass enables the higher genetic merit cow to perform closer to her production potential (McGilloway and Mayne, 1996). Responses to concentrate supplementation are usually greater in late summer and autumn as availability and quality of grazing declines. The objective of this study was to quantify milk production responses when early lactation, high yielding dairy cows at grass, were supplemented with high levels of concentrates, over the late summer period.

Materials and methods Forty-eight Holstein-Friesian cows were allocated to one of three treatments and offered either 6, 9, or 12kg concentrate per day for a 5 week period, beginning 13th August 1999. Cows were on average 66 days in milk and yielding 36.8kg milk per day at the start of the experiment. Animals were continuously grazed on swards of predominantly perennial ryegrass at SAC Crichton Royal Farm, Dumfries. Grazing area was adjusted according to sward surface height with a target height of 9cm (Barthram, 1986). Cows fed 6kg concentrate per day had concentrate split between *am* and *pm* milkings. At higher feeding levels concentrate was offered in three equal feeds with an additional feed at 11*am*, when animals were removed from grazing for approximately 30 minutes. Milk yield was recorded daily and milk samples taken weekly from two consecutive milkings. Cow live weight and condition score were recorded weekly. Simulated grazing samples were hand plucked weekly and concentrate samples taken weekly for analysis. Grazing behaviour of individual cows was observed for a 24 hour period beginning at 09:00hrs. Cows were observed for 15 seconds in every 10 minutes during daylight and every 15 minutes in darkness. Results were analysed using ANOVA with milk yield and composition at allocation used for covariate analysis.

Results Herbage had an average analysis of 223g/kg dry matter (DM), 176g/kg DM crude protein (CP), 85g/kg DM water soluble carbohydrate and 10.2MJ/kg DM metabolisable energy (ME). Concentrate offered had an average analysis of 853g/kg DM, 216g/kg DM CP, 191g/kg DM neutral detergent fibre, 286g/kg DM starch, 13.6MJ/kg DM ME. Milk yield (Table 1) increased as concentrate feeding level increased ($p<0.05$). An increase in milk yield of 0.86kg per kg concentrate, was observed between 6 and 9kg treatments. Milk yield response above 9kg concentrate was smaller at 0.71kg extra milk per kg concentrate. Milk composition was unaffected by concentrate feeding level, although there was a tendency for milk protein concentration to increase as concentrate supplementation level increased ($p=0.059$). This resulted in a greater milk protein yield when concentrate level was increased from 6kg to 9kg ($p<0.05$). Grazing time (Table 2) was reduced by 1.5 hours and 1.9 hours as concentrate was increased from 6-9 and 9-12 kg/day respectively, although disruption of grazing for groups fed 9 and 12 kg by an 11 am concentrate feed must be considered.

Table 1 Effect of concentrate level on animal performance

	Concentrate level kg/d fresh weight (FW)				
	6kg	9kg	12kg	s.e.d.	Sig.
Milk yield kg/d	28.8[a]	31.4[b]	33.6[c]	1.048	***
Milk composition g/kg					
Fat	37	33.9	33.3	0.161	NS
Protein	31.8	32.5	32.7	0.061	NS
Yield of constituents g/d					
Fat	1046	1059	1080	54.2	NS
Protein	849[a]	1022[b]	1063[b]	37.0	***
Live weight change kg/d	0.26[a]	0.14[a]	0.69[b]	0.15	**

Table 2 Grazing and ruminating behaviour 2-3 September 1999 (hours/cow per day)

	Concentrate treatment kg/day				
	6	9	12	s.e.d.	Sig.
Grazing	11.1[a]	9.6[b]	7.9[c]	0.35	***
Ruminating	6.7[a]	8.7[b]	8[c]	0.32	***

Means with different superscripts differ $p<0.05$
Sig. *$p<0.05$,**$p<0.01$,***$p<0.001$, NS $p>0.05$

Conclusions The greatest milk yield response occurred when concentrate feeding was increased from 6 to 9kg/day. Milk production responses at higher levels of concentrate supplementation may have been compromised by the higher substitution of herbage for concentrate, as suggested by a reduction in grazing time. In economic terms, optimum level of concentrate feeding will be dependent on cost of concentrate relative to milk price.

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Extended grazing of dry cows

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Introduction Our earlier studies with dry cows have highlighted a number of factors that affect performance in the subsequent lactation, including forage intake in the peri-parturient period, body condition at calving and supply of Metabolisable Protein (MP) (Moorby *et al*., 1996; Dewhurst *et al*., 2000). There is now increased interest in making more use of grazed grass, as a cheap feed, particularly through extending grazing into the late-autumn and winter. Dry cows are suited to extended grazing because they do not need to be brought in for milking each day. The high voluntary intakes of grass may be beneficial for dry cows, though the low MP supply from autumn-grass might be a disadvantage. This experiment evaluated the effects of extended grazing, compared with housing and silage-feeding of dry cows.

Materials and methods Forty multiparous Holstein-Friesian cows which had previously been trained to use roughage intake control (RIC) feeders (Insentec B.V., The Netherlands) and which calved in October and November 1999 were used. Cows were dried off 60 days prior to anticipated calving dates, given long-acting antibiotics, and grazed on a bare sward for 4 days. Half of the cows were housed and offered grass silage *ad libitum* through RIC, whilst the others grazed until calving. The grazing area received its final application of fertiliser in late-August (50 kg/ha N; no P or K) and the grazing area was extended to maintain grass supply into late season (set stocked to maintain a sward height in the range 5-10 cm). Cows were moved to calving boxes just prior to calving and suckled their calf on the day of calving. All cows were housed after calving and received 8 kg/day of standard concentrate (NDF and crude protein: 241 and 228 g/kg DM respectively) and *ad libitum* grass silage until the end of lactation week 10. All cows were weighed and body condition score (BCS) was recorded (0-5) scale at the tail site each week. Results were analysed by analysis of variance (REML or repeated measures analysis of variance, as appropriate), with a treatment structure of 'dry period diet'. Intake of grazed grass was estimated, using *n*-alkanes, with 4 and 7 cows in the middle of their dry period on 28 September and 20 October respectively.

Results The mean concentrations (g/kg DM) of crude protein, NDF and digestible organic matter (*in vitro*) for grass and grass silage were 227 and 186, 472 and 525, and 595 and 620 respectively. The grass silage was of only moderate quality, with a pH of 3.96, lactic acid content of 56 g/kg DM and ME-tick of 10.9 MJ/kg DM. Mean silage DM intake (kg/day) in the dry period was 11.6 (s.d.=1.83). Mean sward height was 7.1 (s.d.=2.27) cm and mean grass DM intakes (kg/day) were 9.5 (s.d.=2.00) and 8.1 (s.d.=2.04) in September and October respectively.

Table 1 Effects of dry period treatments on weight and body condition score changes

	Grass	Grass silage	s.e.d.	Significance
Weight change over the dry period* (kg)	-14.5	+3.3	7.25	P<0.05
Weight change over first 10 weeks of lactation (kg)	+10.6	+0.7	8.19	NS
BCS change over the dry period*	-0.06	+0.19	0.085	P<0.01
BCS change over first 10 weeks of lactation	+0.08	+0.04	0.097	NS

*changes recorded from the start of the dry period until the first week after calving

There were no significant effects of dry period treatments on performance in the first 10 weeks of the subsequent lactation. Silage DM intake (kg/day), milk yield (kg/day), milk fat (g/kg) and milk protein (g/kg) were 10.65 and 10.11 (s.e.d.=0.334), 26.5 and 27.1 (s.e.d.=0.44), 39.5 and 41.0 (s.e.d.=1.35) and 32.2 and 31.9 (s.e.d.=0.54) for grazed and silage-fed cows respectively.

Discussion There was little effect of dry period treatment on milk yield and milk quality in the first 10 weeks of the subsequent lactation, despite the fact that the extended grazing dry cows were in quite low condition (BCS 2) at calving. This was in contrast to the negative consequences of restricting BCS gain by incorporating straw into silage-based dry cow diets in earlier work (Dewhurst *et al*., 2000). Milk yields from both groups were disappointingly low, probably because the grass silage was of only moderate quality. Health problems, particularly milk fever, might limit the application of extended grazing for dry cows, though there were no significant health problems with these cows.

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Close-up dry period protein supplementation influences milk, fat and protein yields of multiparous Holstein dairy cows in the first half of their next lactation

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Introduction Considerable effort has recently been directed to better defining protein requirements of dry dairy cows. Early efforts (Moorby *et al*., 1996) suggested substantial increases in milk and milk protein yield of multiparous cows to a small amount of a high undegradable dietary protein (UDP) supplement in the late dry period. Recent studies have not consistently supported these findings. The objective of this experiment was to define the impact of supplementation of a high UDP supplement in the late dry period of multiparous dairy cows on production of milk and its components.

Material and methods A close-up dry period ration, based on maize silage (0.17 of dry matter, DM), lucerne hay cubes (0.24), oat hay (0.25), barley (0.16) and maize grains (0.16), was limit fed at 12.1 kg DM/d. Diets were: D_0, no supplement; D_1, with 1.1 kg/d of a UDP supplement; and D_2, with 2.3 kg/d of the supplement, comprising rumen-protected rapeseed meal (0.6), dried distillers grains (0.2), blood meal (0.1), feather meal (0.05), and maize gluten meal (0.05). Final crude protein (CP) contents of the diets (P) were 118, 148 and 178 g/kg DM. Milk, protein and fat yields of 121 multiparous Holstein cows, each offered one of the dry period diets for up to 16 d (n = 47, 40 and 34 for D_0, D_1 and D_2), were measured monthly for the first 150 d of lactation. Following calving, all cows received the same complete diet formulated to contain 177 g CP/kg DM and 320 g neutral detergent fibre/kg DM. Cows were allocated to one of four groups based upon time close-up (T) for statistical analysis (1-4, 5-8, 9-12 and 13-16 d). Parity effects, beyond the primiparous/multiparous parity split, were not considered. Yields of milk, protein and fat for each cow during the first 150 d of lactation were used to calculate a mean for each treatment group. Data were analysed by multiple regression with a maximum model of: y = constant + P + P^2 + T + T^2 + T^3 + T^4 + P×T + P×T^2 + P×T^3 + P×T^4 + P^2×T + P^2×T^2 + P^2×T^3 + P^2×T^4, with removal of terms until the best fit regression was achieved.

Results Milk, milk fat and milk protein yield were influenced, albeit in different ways, by increasing the level of diet UDP supplementation and increasing the time that cows received the supplement. Equations of the modelled responses are given in Table 1, with statistical analyses in Table 2. Milk yield was greatest for cows offered diet D_0 for shorter periods of time or D_1 or D_2 for longer periods of time. Milk protein yield tended to be greatest for cows offered diet D_1 for longer periods of time. Both milk and milk protein yields appeared to be depressed when cows on any diet were fed the protein supplement for approximately 5 to 7 d, although the extent of the depression in milk was greater in cows offered more protein supplement. Similarly, milk fat yield tended to be lower for cows supplemented for intermediate periods of time.

Table 1 Best-fit multiple regression equations of modelled responses of milk (kg/d), protein (g/d) and fat (g/d) yields (P = diet protein, T = time in close-up group)

Milk	= 7.54 + 2.714 P + 30.6 T - 2.419 T^2 - 2.979 P×T + 0.1764 P×T^3 + 0.00572 P×T^3 + 0.0522 P^2×T - 0.0004 P^2×T^3
Protein	= 1400.8 + 525 T – 52.4 T^2 – 72.5 P×T + 7.26 P×T^2 + 2.36 P^2×T – 0.2386 P^2×T^2
Fat	= 1517.3 – 16.9 T^2 – 2.94 P×T + 2.5 P×T^2 – 0.0779 P^2×T^2

Table 2 Significance and model parameters of multiple regressions (P = diet protein, T = time in close-up group)

	Constant	P	P^2	T	T^2	T^3	T^4	P×T	P×T^2	P×T^3	P×T^4	P^2×T	P^2×T^2	P^2×T^3	P^2×T^4	SE	R^2	$P^\dagger$
Milk	NS	*	-	*	*	-	-	*	*	*	-	+	-	*	-	1.05	0.76	+
Protein	***	-	-	+	+	-	-	+	+	-	-	NS	+	-	-	40.5	0.48	NS
Fat	***	-	-	-	NS	-	-	+	NS	-	-	-	NS	-	-	82.4	0.22	NS

[†]Regression significance; -, excluded from model; NS, not significant but in model; +, P<0.1; *, P<0.05; ***, P<0.001

Conclusion The amount of protein supplement fed close to calving, and the length of time that animals received it, both influenced milk and milk component production. While these results are broadly consistent with an earlier study (Robinson *et al*., 2000), it is evident that relationships between close-up period protein supplementation and production in the next lactation are complex. Experiments are required to define the characteristics of dry cows that influence their potential to respond, and actual response, to dietary protein if prediction of lactation responses are to be accurate.

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Late gestation protein supplementation influences milk, fat and protein yields of primiparous Holstein dairy cows in the first half of their first lactation

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Introduction Considerable effort has recently been directed towards better defining protein requirements of dairy cows approaching parturition. Little effort has been directed towards cows entering first lactation, although Van Saun *et al*., (1993) suggested modest increases in milk protein production to a supplement of high undegradable dietary protein (UDP) in the late dry period. The objective of this study was to define the impact of supplementation of a high UDP protein supplement in the late gestation of cows entering first lactation on production of milk and its components.

Material and methods A close-up dry period ration, based on maize silage (0.17 of dry matter, DM), lucerne hay cubes (0.24), oat hay (0.25), barley (0.16) and maize grains (0.16), was limit fed at 12.1 kg DM/d. Diets were: D_0, no supplement; D_1, with 1.1 kg/d of a UDP supplement; and D_2, with 2.3 kg/d of the supplement, comprising rumen-protected rape seed meal (0.6), dried distillers grains (0.2), blood meal (0.1), feather meal (0.05), and maize gluten meal (0.05). Final crude protein (CP) contents of the diets (P) were 118, 148 and 178 g/kg DM. Milk, protein and fat yields of 192 primiparous Holstein cows, each offered one of the dry period diets for up to 16 d (n = 54, 73 and 65 for D_0, D_1 and D_2), were measured monthly for the first 150 d of lactation. Following calving, all cows received the same complete diet formulated to contain 177 g CP/kg DM and 320 g neutral detergent fibre/kg DM. Cows were allocated to one of four groups based upon time close-up (T) for statistical analysis (1-4, 5-8, 9-12, 13-16 and 17-20 d). Yields of milk, protein and fat for each cow during the first 150 d of lactation were used to calculate a mean for each treatment group. Data were analysed by multiple regression with a maximum model of: y = constant + $P + P^2 + T + T^2 + T^3 + T^4 + P{\times}T + P{\times}T^2 + P{\times}T^3 + P{\times}T^4 + P^2{\times}T + P^2{\times}T^2 + P^2{\times}T^3 + P^2{\times}T^4$, removing terms until the best fit regression was achieved.

Results Milk, milk fat and milk protein yield were influenced, albeit in different ways, by increasing the level of diet UDP supplementation and increasing the time that cows received the supplement. Equations of the modelled responses are given in Table 1, with statistical analyses in Table 2. Milk yield was greatest for animals offered diet D_0 for longer periods of time and for animals offered diet D_3 for intermediate periods of time, with evidence that cows offered the highest level of protein supplement for the longest period of time had a reduced level of milk production. Milk protein yield showed a similar pattern, without the tendency to higher yields from cows offered D_0 for longer times. Both milk and milk protein yields appeared to be depressed when cows on any treatment were exposed to the close-up diet for approximately 5 to 7 d.

Table 1 Best-fit multiple regression equations of modelled responses of milk (kg/d), protein (g/d) and fat (g/d) yields (P = diet protein, T = time in close-up group)

Milk	$= 36.042 + 0.854\ T^2 - 0.0508\ T^3 - 0.1272\ P{\times}T^2 + 0.00756\ P{\times}T^3 - 0.00506\ P^2{\times}T + 0.00515\ P^2{\times}T^2 - 0.000289\ P^2{\times}T^3$
Protein	$= 1223.6 - 102.4\ T + 35.92\ T^2 - 2.723\ T^3 + 0.0545\ T^4 - 1.782\ P{\times}T^2 + 0.1047\ P{\times}T^3 + 0.0372\ P^2{\times}T^2 - 0.0001265\ P^2{\times}T^4$
Fat	$= -1440 + 409\ P - 15.4\ P^2 + 533\ T + 70\ T^2 - 10.49\ T^3 + 0.2789\ T^4 - 117.5\ P{\times}T + 0.6657\ P{\times}T^3 - 0.01776\ P{\times}T^4 + 5.19\ P^2{\times}T - 0.2737\ P^2{\times}T^2$

Table 2 Significance and model parameters of multiple regressions (P = diet protein, T = time in close-up group)

	Constant	P	P^2	T	T^2	T^3	T^4	$P{\times}T$	$P{\times}T^2$	$P{\times}T^3$	$P{\times}T^4$	$P^2{\times}T$	$P^2{\times}T^2$	$P^2{\times}T^3$	$P^2{\times}T^4$	SE	R^2	$P^\dagger$
Milk	***	-	-	-	*	*	-	-	*	*	-	*	*	*	-	0.73	0.67	*
Protein	***	-	-	*	**	**	**	-	**	**	-	-	**	-	**	13.8	0.76	*
Fat	NS	+	*	*	*	**	**	*	-	**	**	*	**	-	-	24.0	0.84	+

[†]Regression significance; -, excluded; NS, not significant but in model; +, $P<0.1$; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$

Conclusion Level of feeding of a protein supplement near calving, and the length of time that cows received it, both influenced milk and milk component yield. While these results are broadly consistent with an earlier study (Robinson *et al*., 2000), it is evident that the optimal combination of level of protein supplement offered, and time of offer, is a complex response that requires further research. Nevertheless, results demonstrate for the first time that performance in the next lactation may be reduced if excess protein supplement is fed to close-up heifers entering first lactation.

Acknowledgements We are grateful to M. Arana, L. Castelanelli, R. Hinders, T. Graham, the staff of Castelanelli Brothers Dairy, Lodi, California, and H. Goodby for supplying the Alberta Gold™ Hi-Bypass Canola Pellets.

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The effects of feeding different starch sources and concentrations on milk production of high yielding Holstein cows

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Introduction Mansbridge (1995) reported that replacing ground wheat with a mix of ground wheat and maize grain increased milk protein concentration, which led the authors to speculate that increased inclusion of maize grain increased rumen by-pass starch. Indeed, de Visser *et al* (1990) reported that feeding less rapidly degradable starches has led to increased milk protein concentration.

The objective of this study was to examine the effects of starch concentration and source on feed intake, milk yield and milk composition of dairy cows.

Materials and methods In a 2 x 3 factorial designed experiment, 72 high yielding Holstein cows were allocated to the experiment, with cows averaging 55 days post calving at the start. Diets were fed as total mixed rations (TMR) containing grass silage (275g/kg DM; CP 149g/kg DM; 12.2 MJ/kg DM), sugarbeet pulp, extracted rapeseed meal, extracted soyabean meal, calcium soap, vitamins and minerals and either slowly degraded (Hereward) or quickly degraded (Consort) ground wheat with or without ground maize grain. Treatment diets were applied so that there were two starch concentrations (either 93 g/kg diet DM or 187 g/kg diet DM) and three starch sources (quickly degraded ground wheat; slowly degraded ground wheat or a mixture of ground maize grain and quickly degraded ground wheat). Two weeks prior to the start of the study (week -2) when the cows were fed a common ration, intake and milk yield were recorded and milk samples were collected for determination of milk fat and protein concentration. A changeover week enabled the cows' diets to be changed to the study diets gradually. Repeated measures analysis of intake and milk yield were recorded in weeks 1 to 8 and in weeks 2, 4, 6 and 8 for milk quality. Data were analysed using analysis of variance using week -2 data as the covariate.

Results There were no effects of starch source or concentration on intake and there were no effects of starch source on milk yield. However, there was an effect of time and starch concentration ($P<0.001$) on milk yield, with yields declining faster with the high starch treatments (Figure 1). Milk fat concentration was unaffected by starch source or concentration, but the high starch concentration significantly increased milk protein content (Table 1). There was no effect of starch source on milk composition.

Figure 1: *The interaction between time and starch concentration on milk yield*

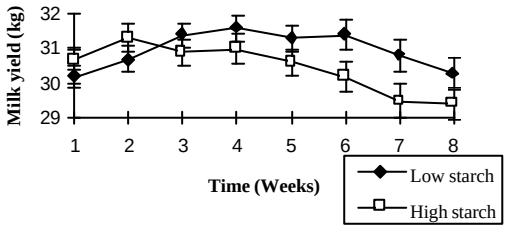

Table 1: *Overall effects of starch concentration on milk composition*

Starch concentration	Milk fat (g/kg)	S.e.	Milk protein (g/kg)	S.e.
Low	44.7	0.90	32.5	0.21
High	44.1	0.97	34.0	0.23
P	ns		***	

Conclusions The high starch concentration resulted in increased milk protein, but a decline in milk yield over time. No adverse effects on milk fat were recorded. A proportion of the dietary starch from maize grain did not affect any of the production parameters. Feeding ground Consort wheat at the high starch concentration (equivalent to an intake of 6.2 kg wheat as fed/cow/day) did not result in dry matter intake reduction when fed as a TMR to early lactation cows as part of a grass silage based ration.

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The effects of adding oil to liquid feed supplements on feed intakes and milk production of high yielding Holstein cows

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Introduction The feed intake of high yielding, early lactation Holstein cows is often inadequate to meet the animal's requirements for energy and a more concentrated energy source is essential. Oil has a high energy content, so feeds containing oil can help increase the overall concentration of energy in the diet. However, high oil inclusions can lower milk fat and protein level.

The objective of this study was to examine the effects of the addition of increasing levels of oil to a molasses blend (4 kg/cow/day) on feed intake, milk yield and milk composition of dairy cows.

Materials and methods In a 4 x 4 Latin square, 16 second parity Holstein cows were allocated to treatments, with cows averaging 30 days post calving at the start. Diets were fed as total mixed rations containing grass silage (277g/kg DM; CP 215g/kg DM; 11.6 MJ/kg DM), maize silage (351g/kg DM; CP 93g/kg DM; 10.9 MJ/kg DM), maize gluten, soyabean meal, rapeseed meal, ground wheat, chopped straw, limestone flour, vitamins and minerals and molasses with or without oil addition. Treatments applied were as follows:- Treatment A (basal diet containing molasses without oil supplement); Treatment B (basal diet containing molasses with 5.5 g vegetable oil/kg DM); Treatment C (basal diet containing molasses with 6.9 g fish oil/kg DM); Treatment D (basal diet containing molasses with 14.2 g fish oil/kg DM). Diets were fed in 4 week periods. Feed intake and milk yield were recorded in weeks 3 and 4 of each period and milk quality in week 4. Data were analysed using analysis of variance for a multiple Latin square design. Where significant treatment differences were recorded, a Dunnett's test was performed to test for differences between the treatments and the control.

Results When high yielding, early lactation Holstein dairy cows were fed molasses with 14.2 g fish oil/kg DM milk yield tended to increase (P=0.06), dry matter intake decreased, milk fat decreased and milk protein remained the same when compared to cows fed molasses with no oil inclusion. Feeding molasses containing either 5.5 g vegetable oil/kg DM or 6.9 g fish oil/kg DM had no significant effects on dry matter intake, milk yield or milk composition (Table 1). Feeding 4 kg/cow/day (double typical on-farm rate) of a molasses blend did not cause diarrhoea in any of the cows.

Table 1: *The effects of adding oil to liquid feed supplements on feed intakes and milk production*

Treatment	Dry matter intake (kg DM/d)	Milk yield (kg/d)	Milk fat (kg/d)	Protein (kg/d)	Lactose (kg/day)
A	22.1	35.9	1.42	1.11	1.69
B	21.9	36.9	1.28	1.11	1.74
C	21.6	36.7	1.29	1.09	1.72
D	21.2	37.5	1.17	1.12	1.76
s.e.	0.19	0.41	0.150	0.035	0.022
P	**	ns	*	ns	ns
Contrasts					
A v B	ns	-	ns	-	-
A v C	ns	-	ns	-	-
A v D	**	-	**	-	-

Conclusions The trend towards increasing milk yield with increasing inclusion of oil supplementation is consistent with the work of Keady and Mayne (1998) who reported that increasing the level of fish oil from zero inclusion to 300 g/cow/day led to increased milk yield from 22.5 to 25.2 kg/day and decreased the concentration of milk fat. However, milk protein was reduced in their study, but unaffected in this study. Molasses containing 14.2 g fish oil/kg DM has potential as a supplement for early lactation cows to increase the energy density of the ration and may have a role in quota manipulation by reducing milk fat whilst maintaining milk protein yields. Inclusion of vegetable oil in the molasses supplement did not effect dry matter intake or milk quality.

Acknowledgments This work was funded by Intermol SVG.

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The relationship between milk composition and volatile fatty acids in the rumen in cattle

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Introduction It is well recognised that the fat and protein concentration in the milk of dairy cows is influenced by volatile fatty acids (VFAs) produced in the rumen. There has however been little information available on models to predict milk composition from rumen VFAs in the literature. The objective of the present study was to develop empirical relationships to predict milk fat and protein concentration using rumen VFA data.

Material and methods From 1991 to 1998, a total of 11 grass silages were examined in 44 treatments over 8 feeding studies with lactating dairy cows at this Institute. All silages were well preserved with a mean DM concentration of 248 (s.d. 77.0) g/kg, pH of 4.0 (s.d. 0.33) and ammonia-N/total-N of 0.108 (s.d. 0.0443). The silage DM proportion in total diets ranged from 0.39 to 1.00 with a mean of 0.64 (s.d. 0.151). The same diets were also offered respectively to rumen-fistulated dry cows or beef steers for measurement of the rumen fermentation variables, including acetic (Ac), propionic (Pr), butyric (Bu) and valeric (Va) acids. In 6 of the 8 studies (25 treatments), the rumen samples were taken hourly for 24 hours at the end of each feeding period. The rumen samples in the remaining 2 studies were obtained by the suction strainer technique (Raun and Burroughs, 1962) via a stomach tube, at intervals of 0, 4, 7 and 10 hours following morning feeding on the final day of each period. The dairy cattle used were Holstein Friesian cows. The treatment mean data were used to examine the relationship between milk composition and rumen VFAs in a number of linear and multiple regression models. The unit for fat, protein and lactose concentration in milk is g/kg and for each VFA is a proportion of total VFAs (mmol/mmol).

Results The minimum, maximum, mean and s.d. data on milk and rumen fermentation used for the present prediction are presented in Table 1. There was no significant relationship between milk lactose concentration and any VFA variable. The relationships for prediction of milk fat concentration was also poor, with only a significant and positive relationship with Bu ($p<0.05$). Milk protein concentration was significantly related to Ac ($p<0.001$), Bu ($p<0.001$), Va ($p<0.05$) and Ac/Pr ($p<0.05$), respectively, with positive relationships with Bu and Va and negative with Ac and Ac/Pr. Relationships between milk fat/protein and each of VFAs were all significant ($p<0.001$). The relationships were positive with Ac, Ac/Pr, (Ac+Bu)/Pr and (Ac+Bu+Va)/Pr and negative with Pr, Bu and Va.

Table 1. The data on rumen fermentation and milk (Treatment means, n=44)

	Minimum	Maximum	Mean	s.d.
Rumen variables (mmol/mmol)				
Acetic	0.536	0.684	0.643	0.0377
Propionic	0.160	0.240	0.192	0.0185
Butyric	0.102	0.176	0.134	0.0187
Valeric	0.006	0.069	0.032	0.0131
Milk of dairy cows (kg/d or g/kg)				
Yield	11.2	35.1	21.8	5.80
Fat	27.3	48.1	40.1	3.72
Protein	25.7	36.4	30.7	3.24
Lactose	40.6	52.1	49.4	3.06

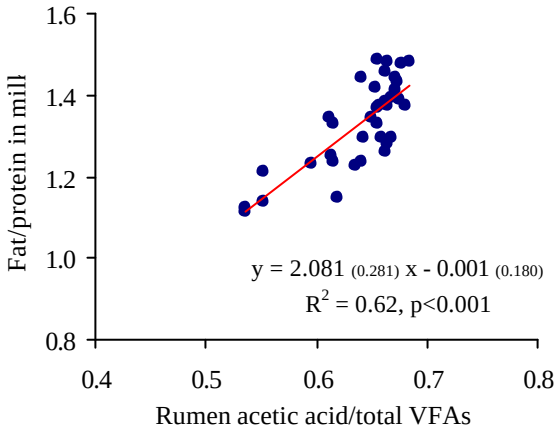

Fig. 1. Fat/protein *v.* acetic acid/VFAs

Stepwise regression techniques were used to examine the relationships between milk composition and all VFA variables. The following three equations were considered the most appropriate for prediction purposes and were all significant ($p<0.001$). Eq. (3) is also presented in Figure 1. The constant was not significant in any equation, while each predictor in each equation was statistically significant ($p<0.05$ or less).

$$\text{Protein} = 41.8_{(12.7)}\,\text{Pr/Ac} + 120.3_{(19.4)}\,\text{Bu} - 116.5_{(44.8)}\,\text{Va} + 5.79_{(3.57)} \qquad R^2 = 0.65 \qquad (1)$$

$$\text{Protein} = 88.1_{(27.2)}\,\text{Pr} + 144.5_{(20.7)}\,\text{Bu} - 92.1_{(39.3)}\,\text{Va} - 2.55_{(5.75)} \qquad R^2 = 0.65 \qquad (2)$$

$$\text{Fat/Protein} = 2.081_{(0.281)}\,\text{Ac} - 0.001_{(0.180)} \qquad R^2 = 0.62 \qquad (3)$$

Conclusion Three equations were established to predict fat and protein concentration in milk using rumen VFAs.

Acknowledgement The present study was funded by DARD and LINK Sustainable Livestock Production project: Feed into Milk.

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Replacing grazing with a maize silage-based indoor diet for lactating dairy cows in autumn

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Introduction Reducing the time available for grazing from 20h to 5h daily, and replacing this with access to a maize silage/soyabean meal diet indoors had no significant effect on milk yield in spring (Hernandez-Mendo and Leaver, 1999). Grazing conditions in autumn present additional problems of shorter daylength and accumulated herbage contamination. The objectives were to examine the production and behavioural responses of grazing dairy cows to reducing access to grazing and increasing access to a maize silage/soyabean meal diet offered indoors.

Materials and methods The study was carried out from 27 September to 8 November 1999. Twenty-four multiparous Holstein Friesian cows were allocated to 6 treatments in a factorial design, where time available for grazing (TAG) was 19, 10, and 5 hours and time available for maize silage plus soyabean meal (TAMS) indoors for 1, 10 and 15 hours, respectively, each examined at 0 and 6 kg/day of concentrates. The sward was maintained at a height (rising plate meter) of 6-8 cm throughout. When indoors, cows received maize silage *ad libitum* + soyabean meal (DM ratio 0.8:0.2). The concentrate (16% CP in DM) contained barley + soya bean meal. Milk yield (MY), MY decline (MYD), milk composition, live weight gain (LWG), grazing time (GT), and maize/soya eating time and DM intake were recorded. Herbage DM intake was estimated indirectly from the ME requirements of cows. Results were analysed in a 3 systems x 2 concentrate level factorial design using the GENSTAT 5 package. Initial MY, LW and milk composition were used as covariates to adjust for initial differences between groups.

Results The main effects of the treatments are shown in Table 1.There were no significant differences between the three TAG+TAMS treatments in MY, MYD, milk fat and protein content or total DMI. Grazing time (GT), maize silage plus soya (MS) eating time, herbage intake and MS intake were significantly affected by the system. Intakes of MS were much higher than in a previous experiment carried out in the spring. Rates of DM intake for 5+15, 10+10, and 19+1 treatments were for MS, 5.3, 6.6, and 7.4 kg/h, and for herbage 0.2, 0.5, and 0.9 kg/h respectively. Increased concentrate level significantly increased MY and milk protein concentration. GT, MS eating time, herbage intake and MS intake were significantly depressed by feeding concentrates. There were significant interactions between system and concentrate level in GT and MS eating time. Concentrates had a greater depressing effect on GT, but a less depressing effect on MS eating time on the 10 and 19 h TAG, than on the 5 h TAG.

Table 1 Production and feeding behaviour of dairy cows offered different grazing and indoor feeding systems and two concentrate levels

TAG + TAMS (h)	MY (kg/d)	MYD (kg/d)	Fat (g/kg)	Protein (g/kg)	LWG (kg/d)	GT (min/d)	MS eating time (min/d)	Herbage DM intake (kg/d)	MS DM intake (kg/d)	Total DMI (kg/d)
5 + 15	25.0	0.04	41.6	34.7	0.47	191	161	0.5	14.1	17.2
10 + 10	26.1	0.08	40.4	35.4	0.68	382	106	3.4	11.7	17.7
19 + 1	25.3	0.03	39.5	34.1	0.50	485	50	7.5	6.2	16.3
s.e.d.	0.99	0.033	1.60	0.87	0.191	19.1	5.9	0.81	0.39	0.87
significance	NS	NS	NS	NS	NS	***	***	***	***	NS
Conc (kg/d)										
0	24.4	0.04	41.5	33.9	0.47	386	118	4.3	12.3	16.6
6	26.6	0.05	40.2	35.4	0.63	320	93	3.3	8.9	17.5
s.e.d.	0.81	0.027	1.29	0.65	0.156	15.6	4.8	0.66	0.32 ***	0.71
significance	*	NS	NS	*	NS	***	***	NS		NS

Conclusions At a sward height of 6 to 8cm there were no production benefits from replacing grazing with time available for eating a maize silage/soyabean meal diet indoors. The reduced grazing time in 5+15 and 10+10h indoor treatments, was replaced by increased maize/soya eating time and intake. Rate of intake was very high on the MS diet. The response to concentrates was low averaging 0.17kg milk/kg concentrate DM. The results indicate that under good grazing conditions in autumn there is no advantage in replacing grazing with indoor feeding of a forage-based diet.

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Development of empirical models to describe the response in lactating dairy cattle to changes in nutrient intake

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Introduction Empirical models relate nutrient inputs and outputs statistically, without considering the intervening metabolism of the animal (France and Thornley, 1984). Current rationing systems (e.g. ARC, 1980; Jarrige, 1988) are based on empirical models of energy and protein utilisation within the animal and do not enable prediction of responses to changes in nutrient inputs (Beever *et al*., 1991). Rook *et al*. (1990) had limited success in attempting to develop empirical regression models to describe the effect of nutrient input on milk constituent yields in dairy cows. The aim of the current study was therefore to use data from the literature to develop more appropriate empirical models that would allow the prediction of response in milk output and its constituent, to changes in energy intake.

Materials and Methods Treatment mean data from twenty experiments published in the scientific literature covering the past 25 years (n=116 observations) were used to develop the models. The published studies provided information on the effect of a minimum of 3 planes of nutrition on milk yield (MY), milk fat yield (MF), milk protein yield (MP) and milk energy (EY). When EY was not given, it was calculated from the given MY and concentration of milk fat, protein and lactose. The metabolisable energy intake (MEI) of each treatment group was either as presented or calculated from determined (*in vivo*) ME concentration of the forage and calculated ME concentration of the concentrate offered. In addition and where appropriate, MEI (MJ ME/day) was calculated in some studies from (1.) digestible energy intake minus urine energy and predicted methane energy (2.) net energy for lactation or (3.) the digestible organic matter present in the dry matter. In each instance, the MEI was corrected for feeding level.

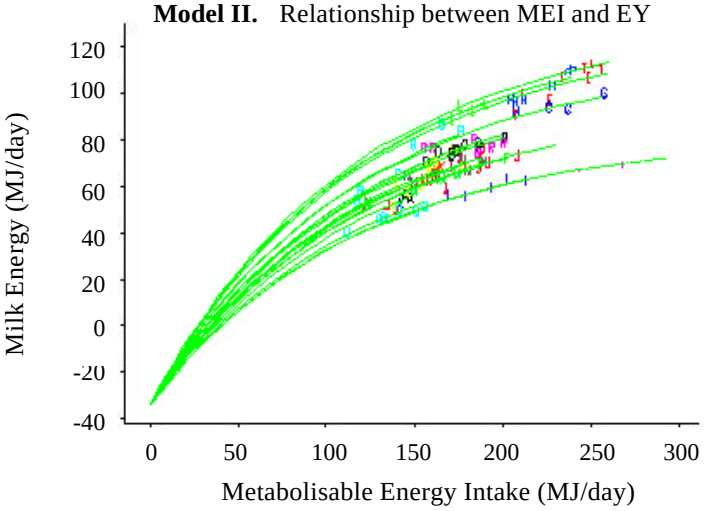
The asymptotic relationship $y=a_i+b_i*r^x$ was used to describe the response for study *i*, where a is the maximum value (upper asymptote) on the y-axis, b is the difference between a and the y-value at x=0, r (<1) is an indication of curvature of the relationship and x is the value for MEI. The models were constrained in two ways: (I) to pass through a common x-value on the y-axis i.e. x=k at y=0 giving the model $y=b_i*(r^x-r^k)$ and (II) to pass through a common y-value on the x-axis i.e. y=c at x=0 giving the model $y=c+b_i*(r^x-1)$.

Results The range in MEI in the data was 112-297 MJ ME/day. The corresponding ranges for MY, MF and MP were 13-39 kg/day, 479-1544 g/day and 359-1313 g/day respectively. Models I and II fitted the data in a similar fashion as shown by the R^2 values (Table 1).

Table 1. Goodness of fit of Models applied

Model I	**R^2**	**k-value**
Milk yield (kg/day)	0.965	21.1
Milk fat yield (g/day)	0.941	71.2
Milk protein yield (g/day)	0.960	29.5
Milk Energy (MJ/day)	0.959	46.7
Model II	**R^2**	**c-value**
Milk yield (kg/day)	0.965	-2.98
Milk fat yield (g/day)	0.941	-1918.0
Milk protein yield (g/day)	0.961	-191.8
Milk Energy (MJ/day)	0.959	-33.9

Model II. Relationship between MEI and EY



Conclusions Results suggest that the variation accounted for in models I (y=0) and II (x=0) are very similar and high. The best fit will be determined with model validation.

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The development of a system based on near infrared spectroscopy to predict the intake of grass silage as the sole feed by the dairy cow

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Introduction Grass silage remains the major forage component of winter dairy rations, yet its intake characteristics are particularly variable. Major research projects at SAC and ARINI over the past few years have produced two large datasets on *ad libitum* intake of grass silage and shown that improved silage characterisation methods (NIRS and electrometric titration) can substantially improve the accuracy of prediction of silage intake as the sole forage (Offer *et al*., 1994; Steen *et al*., 1995). The objective of this research programme was to integrate these two datasets to provide a common prediction system which would be appropriate for adoption across the United Kingdom

Materials and methods The 136 silages from ARINI were fed as the sole feed to beef cattle and the 34 silages from SAC were fed to dairy cows producing 27 kg milk and receiving 7 kg concentrate. The aim was to develop a method of predicting silage intake under defined conditions (zero supplement, 8 kg/d milk yield – the *FEED INTO MILK (FIM) Standard Intake*) as the basis for calculating actual intake in different circumstances. Reconciliation of the data from ARINI and SAC was carried out by deriving appropriate relationships from independent sources to correct both the beef cattle and dairy cow intakes to the FIM Standard Intake. The first step was to establish a relationship between intakes by dairy cows and beef cattle at zero supplement level . The data for this were taken from studies at ARINI in which 13 silages were offered as the sole feed to both beef cattle and dairy cows (producing 8 kg/d milk). The relationship was best described using the equation:$y = 23.3 + 0.874\ x$, $R^2=0.74$, $P<0.001$,where y = dairy cow intake as sole feed (g DM/kg $^{0.75}$) and x= beef cattle intake as sole feed (g DM/kg $^{0.75}$). This equation was used to correct the beef cattle intake data to the *FIM standard intake.* The second step was to correct the SAC dairy cow intakes to the same standard. The correction for the effect of supplementary concentrate level was derived from a separate study with 8 silages, each offered to dairy cows at 4 levels of feeding ranging from 0 – 12 kg concentrate/day. The relationship obtained from this study was: $SDMI_c = 1.191\ SDMI_0 – (0.191 SDMI \times 1.013^c)$, where: $SDMI_c$ = silage intake at concentrate intake c (g DM/kg $^{0.75}$); $SDMI_0$ = silage intake a zero concentrate intake (g DM/kg $^{0.75}$); C = concentrate intake (g DM/kg $^{0.75}$). Correction of the SAC dairy cow intake data for the effects of milk yield was carried out using the factor of 0.1486 (g DM/kg $^{0.75}$ per kg milk yield) which was obtained from a review of relevant literature This factor is close to that suggested by Vladiveloo and Holmes (1979).. Once all intakes had been corrected to the *FIM Standard Intake,* NIRS models were developed from undried spectra. obtained by scanning each silage in triplicate on a Foss NIRSystems 6500 at 2 nm intervals across the wavelength range 400 – 2500 nm. A range of mathematical treatments was applied to the spectra in developing the model and the best model was developed using a first derivatized, SNVD 1,4,4,1 maths treatment. The model was validated on an independent set of data (n= 32 silages) from ARINI for which dairy cow intakes, and the silage samples, were available. Predicted vs actual values were compared using the mean square prediction error method described by Rook *et al*. (1990).

Results

The NIRS calibration statistics for the integrated model produced were SEC 6.02, R^2 0.66, SECV 6.15 and R^2_{CV} 0.65. The results of the validation exercise (Table 1) showed good agreement between actual and predicted.

Table 1 Results of validation exercise

	Integrated system
R^2	0.761
Mean prediction error	0.064
Proportion of MSPE	
Bias	0.170
Line	0.010
Random	0.820

Conclusions A integrated ARINI/SAC model, based on NIRS scanning undried grass silage, has been developed for the prediction of intake of grass silage by the dairy cow when offered as the sole feed. This system is currently being adopted across the United Kingdom.

Acknowledgements The present study was funded by DARD (NI) and the LINK Livestock Production programme: Feed into Milk.

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The determination of meal criteria for cows: the use of mixed distribution models

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Introduction Analysis of short-term feeding behaviour may improve our understanding of food intake regulation and diet choice. Feeding behaviour of animals consists of feeding events separated by non-feeding intervals. Feeding events are often observed to be clustered into bouts that may be called meals. Determining a meal criterion (the longest non-feeding interval which is accepted as part of a meal) allows feeding events to be grouped into meals. Tolkamp & Kyriazakis (1999) presented a model that described three populations of $\log_e$-transformed intervals in the form of three normal distributions (Gaussians). These populations represent intervals within meals, with or without drinking, and intervals between meals. This model predicted that the probability of a meal starting, first increased, then decreased, with time since the last meal. This is in contrast to the satiety concept, which predicts that the probability of an animal starting a meal will increase with time since the last meal. This study aims at developing a model that best describes, biologically and statistically, the distribution of non-feeding intervals, thus leading to a more accurate meal criteria.

Materials and Methods This study is based upon data collected from 16 lactating dairy cows. Cows received food that was a mixture of 70% grass silage and 30% concentrate on a fresh weight basis, via 12 computerised food dispensers. This resulted in 79575 intervals between visits to the feeder being recorded. All combinations of, three-population, Gaussian $[(1/(s\sqrt{(2\pi)}))\exp(-(t-m)^2/2s^2)]$ and Weibull $[(ct^{(c-1)}/a^c)\exp(-(t/a)^c)]$ distribution models were investigated (where t = $\log_e$ transformed interval lengths, in seconds and s, m, c, a are model parameters). Models were fitted with a maximum likelihood procedure (Tolkamp & Kyriazakis, 1999) with the quality of fit to the observations assessed by comparison of the likelihood values for the models. Meal criteria were estimated, from the parameters of the models, as the point at which the distributions describing the 2nd and 3rd populations crossed. Observed starting probabilities (the probability that a cow will start feeding within the next 15 min after a non-feeding interval of 15 min) were calculated. Predicted starting probabilities were calculated from the parameters of models.

Results A model with Gaussian distributions describing the first two populations, and a Weibull distribution describing the between meal intervals (Figure 1a) was found to be statistically better than the existing three-Gaussian model when fitted to the observations pooled across individual cows. This resulted in a predicted meal criterion of 28.9 min compared to 49.5 min for the three-Gaussian model. However, the effect on the number of meals per day was limited (5.85 vs 5.59). This was a consequence of the infrequency of intervals with a length of 20 to 50 min, equating to 7 to 8 $\log_e$ units (Figure 1a). Therefore, as the meal criterion varied within this range few intervals were re-assigned from the between, to within, meal populations. The model with a Weibull distribution describing the between meal population was also statistically better than the three-Gaussian model when fitted to individual cows. This resulted in estimated meal criterion that ranged from 17.4 to 39.5 min. The inclusion of a Weibull distribution to describe between meal intervals allowed predictions of starting probabilities that, over the range of interval lengths observed, were in better agreement with the satiety concept than predictions from the Gaussian model (Figure 1b). The Weibull distribution was also seen, in contrast to the Gaussian, to result in a starting probability that traced the observations well.

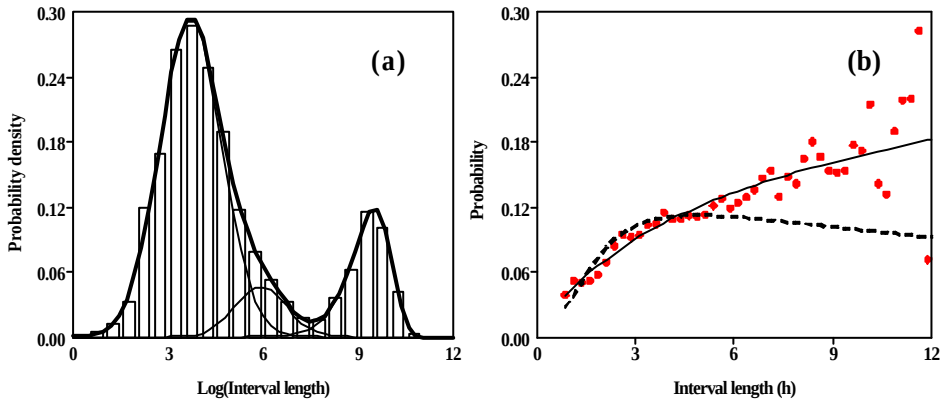

Figure 1 (a) The probability density function (line) for the proposed model (see text) fitted to the pooled observations (open bars). **(b)** The probability that animals will start feeding in the next 15 min; as predicted (solid line) by the proposed model (see text) and the three-Gaussian model (broken line), compared to the pooled observations (dots).

Conclusion A three-population model comprising of two Gaussians and a Weibull distribution, to describe the three populations of intervals, is proposed. This model describes the observations in a way that is both statistically superior to previous models, and also in better agreement with the predictions of the satiety concept, over the whole range of observed interval lengths. This allows meal criteria of cows to be determined with increased reliability.

Acknowledgements This work was funded by BBSRC, BOCM Pauls, and SERAD.

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Relationship between fish oil intake by dairy cows and the yield of eicosapentaenoic acid and docosahexaenoic acid in their milk

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Introduction The dietary essential fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are predominantly found in fish oil, but fish consumption in the UK is low. Increasing the yield of EPA and DHA in cows' milk would increase human intakes of EPA and DHA, and this can be achieved by including fish oil in cows' diets. However, because EPA and DHA are susceptible to rumen biohydrogenation, their transfer efficiency into milk is low. *In vitro* observations by Gulati *et al*. (1999) suggested that if the concentration of fish oil in the rumen exceeded 1 mg/ml, EPA and DHA were not hydrogenated. The objectives of this study were therefore to determine the relationships between fish oil intake by dairy cows, and the probable concentrations of fish oil in the cows' rumen, with the yield of EPA and DHA in their milk.

Materials and methods The data from six papers were collated (Cant *et al*., 1997; Chilliard and Doreau, 1997; Mansbridge *et al*., 1998; Offer *et al*., 1999; Wright *et al*., 1999; Keady *et al*., 2000). In these experiments, fish oil intake (FOI, g/d) was recorded and the yield of EPA and DHA in the milk was measured. Rumen volume (l) and concentrate fractional outflow rate (/h) from the rumen was estimated (Lescoat and Sauvant, 1998). From these data, the concentrations of fish oil in the rumen (CFOR) was calculated from CFOR=FOI/(24 x concentrate fractional outflow rate x Rumen volume). It was assumed that fish oil left the rumen with the concentrate fraction of the diet, and that none of it was hydrolysed in the rumen. Across experiments, fish oil intake (FOI, g/d) and CFOR (g/l) were regressed with EPA and DHA yield in the milk (EPAY and DHAY, g/d). Broken stick analysis was used to determine the relationship between EPAY and either FOI or CFOR.

Results No relationship was observed between DHAY and either FOI or CFOR. However, after a threshold FOI or CFOR had been reached, EPAY increased linearly. These data were best described by broken stick analysis, and the results of this analysis are summarised in Table 1. For both FOI and FCOR, the point of intersection was at an EPAY of 1.1 g/d

Table 1 *Relationship between EPA yield in the milk, and the intake of fish oil or estimated concentration of fish oil in the rumen*

Predictor	Slope of line (±St. Dev.)		Point of intersection	R^2
	1	2		
Fish oil intake (g/d)	0.001±0.0006	0.117±0.0505	428	0.807
Concentration of fish oil in rumen (g/l)	0.136±0.0561	3.194±1.3801	5	0.809

Conclusions EPAY increased linearly when cows were fed more than 428 g/d fish oil. At these high intakes, EPA may not be subject to rumen biohydrogenation. However, to achieve this, the required CFOR was five times that which was required *in vitro*. This may be because *in vitro* systems underestimate the extent of biohydrogenation. Biohydrogenation may also explain the lack of relationship between DHAY and either FOI or CFOR. If EPAY and DHAY are to be reliably increased, some form of protection from rumen biohydrogenation will be required.

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Influence of protein level of supplement on diet selection by dairy cows given a choice of grass or maize silages, and on intake of forages when offered separately

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Introduction In a previous study of forage choice (Syed and Leaver, 2000), cows selected a high proportion of grass relative to maize silage (0.87:0.13). Milk production level of cows and protein level in supplement were not influential in the proportion of forage selected. An understanding of forage preferences by cows should lead to the production of forages with improved intake characteristics. This experiment examined how intake of grass and maize silages offered as a choice compared with intake when offered separately with low or high protein supplements .

Material and methods Six Holstein-Friesian cows (mean milk yield 32.6 kg/day s.e.m. 1.39) were used in a 6 treatment, 4 period incomplete Latin Square design to study the following treatments: Choice of grass silage or maize silage; grass silage alone; or maize silage alone; offered with either low protein (6kg rolled barley + 1 kg soybean meal/day) or high protein (3.5 kg rolled barley + 3.5 kg soybean meal/day) supplements. The silages were fed at 8-00h daily and were offered *ad libitum.* The experiment lasted for 8-weeks, each period being 9 days introduction and 5 days measurement. The grass and maize silages contained respectively 342 and 280 g DM/kg, 710 and 732g DOM/kg DM, (estimated ME 11.5 and 10.9 MJ/kg DM), 192 and 83 g CP/kg DM, pH 3.98 and 3.68 respectively. The feeding behaviour was analyzed on the basis of a minimum of 20 minutes being regarded as an inter meal interval. The incomplete Latin Square with six animals and four periods was analysed using the GENSTAT 5 package.

Results The results are presented in Table 1. Forage DM intake and total DM intake were significantly different ($p<0.001$) for silage treatments but were not significantly different for protein supplement. The ratio of grass to maize silage eaten by the choice treatment was 0.63: 0.37 (with DM intake of 9.9 and 5.6 kg/day, s.e.m. 0.70 and 0.42). The CP contents of the total diet averaged 151 and 186 g CP/kg DM for the low and high protein supplement diets respectively. Rate of intake (g DM/min) was significantly higher ($p<0.05$) for maize silage compared with the other diets but rate of intake was not significantly different for protein level. Number of meals/day was not significantly different between treatments. Average size of meal (g DM) ($p<0.05$), average meal duration (min) ($P<0.001$) and total eating time/day (min) ($P<0.01$) were significantly lower for maize silage only, but were not significantly different for protein supplement. There were no significant interactions between diet and protein supplement. The largest meals were taken after new forage was offered in the morning, and on the choice treatment cows invariably ate both grass and maize silages at each meal.

Table 1 Intake and feeding behaviour of lactating dairy cattle offered a choice of grass silage or maize silage, or offered separately with high and low protein supplements

	Silage			Protein Supplement		Silage		Protein Supplement	
	Choice	Grass alone	Maize alone	HP	LP	s.e.d.	sig	s.e.d.	sig
Forage DMI (kg/day)	15.5	15.5	12.3	14.7	14.1	0.79	***	0.65	NS
Total DMI (kg/day)	21.5	21.5	18.3	20.7	20.1	0.79	***	0.65	NS
Rate of Intake (g DM/min)	87	85	106	88.5	97	7.1	*	5.8	NS
No of meals/day	7.9	8.0	8.3	8.0	8.1	0.58	NS	0.47	NS
Average size of meal (g)	1980	1910	1540	1800	1820	154	*	126	NS
Average meal duration (min)	24	24	15	21	20	1.9	***	1.6	NS
Total eating time/day	177	185	120	164	157	10.1	***	8.2	NS

*= ($P< 0.05$), **= ($p<0.01$), ***= ($p<0.001$), NS=non-significant

Conclusions When given a choice, cows selected a high proportion of grass relative to maize silage (0.63:0.37). Consumption of grass silage fed alone was 1.26 that of maize silage fed alone. Maize silage was eaten at a higher rate of intake but in shorter and smaller meals than grass silage. Level of protein in the supplement was not influential on forage choice. In this experiment the cow showed preference for the silage with the highest digestibility and protein content.

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Reference Syed, J.S. and Leaver, J.D. 2000. Influence of milk production level of cow and protein content of supplement on selection of maize and grass silages by dairy cattle given a choice of forages. *Proceedings of the British Society of Animal Science* p.145.

Nutritional strategies to maximise forage intake in high yielding dairy cows.

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Introduction Cow genetics, forage supply and quality, and feeding systems are relatively fixed in the short term. Therefore, it is important to establish the effect on intake and performance of a range of nutritional strategies. The purpose of the current study was to determine the effects on milk production and composition of nutritional strategies designed to increase forage intake by altering the composition of the concentrate supplement, and reducing the level of concentrate feeding.

Materials and methods Forty-eight multi-parous Holstein cows were randomly allocated to four treatments in a continuous design study in which week 3 of lactation was used as a covariate and the experimental period was weeks 4-13 of lactation. All cows received *ad libitum* a forage mixture containing maize and grass silage in a 3:2 DM ratio. The maize and grass silage fed averaged 357 and 232 g volatile corrected dry matter (DM)/kg, 86 and 124 g crude protein (CP)/kg DM and 10.7 and 10.8 estimated metabolisable energy (ME)/kg DM respectively. The concentrate supplements (Table 1) were commercial products formulated to be of moderate energy and protein as a control (T1), high energy and protein (T2) and high protein (T3), while T4 consisted of a high energy and protein blend. The energy density of supplements T2 and T4 was increased by inclusion of a rumen by-pass fat source and a heat-treated rapeseed expeller meal respectively. Concentrates were offered at the rate of 12 (T1) or 9 kg (T2-T4) fresh weight/day. Diets were not formulated to provide balanced energy and protein replacement comparisons. Rather they were selected to provide examples of nutritional strategies that might be adopted when concentrate feed rate and composition are adjusted in order to maximise forage intake.

Table 1. The composition of the concentrate diets

	T1	T2	T3	T4
DM(g/kg)	868	870	887	880
Crude protein (g/kg DM)	235	283	336	303
Neutral detergent fibre (g/kg DM)	305	216	272	227
Starch (g/kg DM)	166	182	67	207
Oil (g/kg DM)	46	56	46	41
ME (MJ/kg DM)	11.7	13.4	12.1	13.4

Results Forage intakes were higher ($P<0.05$) and total DM intakes lower ($P<0.01$) when concentrate feeding rates were reduced (Table 2). However, the higher nutrient content of the treatment concentrates offset the reduction in DMI such that estimated ME intakes and milk yields were not affected. Although there were no overall effects of diet on milk composition and component yield (Table 2), milk fat content and yield were numerically lower when less concentrate was fed and milk protein content was lower for T2 than for T3 ($P<0.05$). There were no treatment effects on live-weight gain (0.09 kg/d) or final condition score (1.8).

Table 2. Summary of results for feed intake, milk yield and composition

	T1	T2	T3	T4	s.e.d.
Forage (kg DM /d)	12.1	13.2*	13.5*	13.3*	0.40
Concentrate (kg DM/d)	10.6	7.9	7.9	7.9	
Total (kg DM/d)	22.7	21.1**	21.4**	21.2**	0.40
Estimated ME (MJ/d)	253	248	241	249	
Crude protein (kg/d)	3.71	3.56	4.01	3.74	
Milk yield (kg/d)	35.8	36.2	34.7	35.0	0.98
Fat (g/kg)	48.5	45.9	46.9	45.5	1.87
Protein (g/kg)	31.6	30.8	31.8	31.3	0.45
Lactose (g/kg)	46.4	46.5	46.7	46.8	0.23
Fat (g/d)	1729	1664	1623	1600	76.7
Protein (g/d)	1123	1113	1103	1093	28.3
Lactose (g/kg)	1656	1683	1622	1635	47.2

* denotes statistical significance at $P < 0.05$, ** denotes statistical significance at $P<0.01$

Conclusions Reducing concentrate intake increased forage intake. The results of the present study do not show any significant overall effect of the concentrate formulations used on milk yield or composition. However, milk proteins for T2 showed a tendency to be lower than for T1 ($P<0.13$). The association between rumen bypass fat supplementation and reduced milk protein content has been reported previously (DePeters and Cant, 1992), and may be a consideration on farms where milk protein content is a problem.

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Live weight, condition score and *Longissimus dorsi* responses to energy and protein supplies during the dry period in dairy cows

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Introduction Body fat and protein reserves at calving can affect milk production and composition (Garnsworthy, 1988; Moorby *et al*., 1996). Milk producers frequently feed their dry cows with a low quality diet to prevent them from becoming too fat before calving. However, the cow must nourish the foetus and develop mammary secretory tissues, which can be a problem if she is offered a low protein diet. This experiment was designed to test the interaction between energy and protein supplies during the dry period on changes in live weight (LW), condition score (CS) and muscle *Longissimus dorsi* depth (LD). Subsequent milk production and composition data are reported in a separate summary (Jaurena *et al*., 2001).

Material and methods Forty Holstein-Friesian dairy cows were balanced for parity and allocated to one of four diets (10 cows per treatment) with a factorial arrangement of energy (high/low) and protein (high/low). The experimental diets were: low-protein, low-fat (Ll): second cut grass silage only; low-protein, high-fat (Lh): the same silage with 10% Megalac™ (mixed on a dry matter (DM) basis); high-protein, low-fat (Hl): silage with 5% prairie meal (PM); high-protein, high-fat (Hh): silage with 5% PM and 10% Megalac™. All the diets were individually offered *ad libitum* and dry matter intake (DMI) was recorded daily for the last 6 weeks of the dry period. Animal LW and CS (0-5 scale), and LD depth (measured by ultrasound at the 6th lumbar process) were measured weekly from 6 weeks before calving. Udder development was also determined at these times by making four external measurements (circumference, length, width, and height between a fixed mark made in the rear abdominal attachment, and the rear teat baseline). Calf birth weights were recorded within 24 hours of calving. Live weight gains (LWG) were estimated by linear regression of data over the dry period. Results were analysed by analysis of variance, blocking by parity (2 vs >2), and with a factorial treatment structure of dry period diet energy × protein.

Results The composition of diets Ll, Lh, Hl, Hh respectively were: freeze DM: 246, 246, 255 and 272 g/kg; crude protein: 156, 142, 162 and 170 g/kg DM; acid detergent fibre: 287, 266, 275 and 244 g/kg DM. Dry matter intake increased when silage was enriched with both fat and protein (Table 1). Cows on the two high protein diets had significantly increased LWG and therefore increased maximum LW. There was no effect of diet energy (fat) content on these variables, and despite large treatment differences in LWG due to diet protein, there were no treatment differences in maximum CS. Calf birth weight was similarly significantly increased by diet protein content, and differences in the depth of muscle *L. dorsi* at one week before calving suggests that part of the extra protein supplied also led to improvements in the dam's body protein reserves. External udder measurements were not significantly different between treatments, with grand means over the last two weeks of the dry period of circumference (130.0 cm), length (42.2 cm), width (35.1 cm) and height (35.9 cm).

Table 1 Mean dry period DM intakes (DMI), maximum live weight and condition scores, live weight gains, *L. dorsi* depths and udder volumes at 1 week before calving, and calf birth weights of cows offered diets Hh, Hl, Lh, Ll

	Precalving diets					Significant
	Ll	Lh	Hl	Hh	SEM	factors[†]
DMI (kg DM/d)	11.2	10.4	11.5	12.6	0.41	P×E (*)
Maximum live weight (kg)	672	663	723	709	10.8	P (**)
Live weight gain (g/d)	975	748	1261	1313	108.7	P (**)
Maximum condition score	2.4	2.3	2.6	2.5	0.12	ns
Longissimus dorsi (mm)	43	43	46	45	0.8	P (*)
Calf birth weight (kg)	43.5	43.8	50.2	45.3	1.30	P (*)

[†]Statistically significant factors, * P<0.05, ** P<0.01, ns not significant; where E, energy; P, protein.

Conclusion Dry period diets with an increased protein content increased cow LWG, although this can be partially explained by increased conceptus development, which together with udder development may hide the true maternal body weight changes. Increased *L. dorsi* depths of animals offered the high protein diets suggests some repletion of protein body reserves.

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Lactational responses to energy and protein supplies during the dry period in dairy cows

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Introduction Nutritional manipulation during the dry period can alter subsequent animal responses to feeding in terms of milk yield and composition. Previous research has shown interesting differences in milk production and composition due to energy or protein supply in the dry period (Moorby *et al.*, 1996). The objective of this experiment was to test the interaction between energy and protein supplies during the dry period on subsequent milk production and composition. Effects on live weight gains and condition scores are reported in a separate summary (Jaurena *et al.*, 2001).

Material and methods Forty Holstein-Friesian dairy cows were balanced according to parity, and were allocated to one of four treatments (10 cows per treatment) in a factorial arrangement of energy (high/low) and protein (high/low). The experimental diets were: Low-protein, low-fat (Ll): second cut grass silage only; low-protein, high-fat (Lh): the same silage with 10% Megalac™ (mixed on a dry matter (DM) basis); high-protein, low-fat (Hl): silage with 5% prairie meal (PM); high-protein, high-fat (Hh): silage with 5% prairie meal and 10% Megalac™. All diets were individually offered *ad libitum* for the last 6 weeks of the dry period. After calving, all animals were individually offered *ad libitum* access to the same diet of ryegrass silage plus 8 kg/d of a commercial dairy concentrate (13.5 MJ metabolisable energy/kg DM; 240 g crude protein/kg DM). Milk production and composition were recorded weekly for the first 20 weeks of lactation. Results were analysed by analysis of variance, blocking by parity (2 vs >2), and with a factorial treatment structure of diet energy × protein using a covariate of previous lactation yield or composition data.

Table 1 Mean dry matter intakes (DMI), milk, protein, fat and lactose yields for the first 20 weeks of lactation of dairy cows offered diets Hh, Hl, Lh, Ll during the last 6 weeks of the dry period

	Dry period diets				
	Ll	Lh	Hl	Hh	SEM
DMI (kg DM/d)	16.7	16.3	16.7	16.7	0.40
Milk yield (kg/d)	27.5	27.5	25.2	27.9	1.09
Fat (g/d)	1099	1062	999	1120	41.5
Protein (g/d)	860	834	791	878	28.0[†]
Lactose (g/d)	1293	1287	1167	1287	56.2

[†] *P* of energy × protein interaction = 0.058

Table 2 Four-week mean milk yields (kg/d) to week 20 of lactation of dairy cows offered diets Hh, Hl, Lh, Ll during the last 6 weeks of the dry period

	Dry period diets				
Weeks of lactation	Ll	Lh	Hl	Hh	SEM
1-4	30.2	30.7	27.4	31.7	1.39
5-8	29.3	29.3	27.6	30.7	1.16
9-12	26.9	27.0	25.2	27.2	1.05
13-16	26.1	25.4	23.5	25.5	1.11
17-20	25.0	24.7	22.1	24.4	1.18

Results The composition of dry period diets Ll, Lh, Hl, Hh respectively were: freeze DM: 246, 246, 255 and 272 g/kg; crude protein: 156, 142, 162 and 170 g/kg DM; acid detergent fibre: 287, 266, 275 and 244 g/kg DM. Mean DM intakes (weeks 1 to 20) and milk composition (fat 39.7 g/kg; protein 31.2 g/kg; lactose 46.5 g/kg) were not affected by dry period diet treatments (Table 1). Mean milk yields of cows previously offered diet Hl were some 2 kg/d lower than the rest, although non-statistically significant, and this was due to much lower milk yields during the first 4 weeks of lactation (Table 2). This same group of cows had the lowest protein yields, differing significantly from cows previously offered diet Hh ($P < 0.05$), leading to a marginal interaction between dietary energy and protein supplied during the dry period. Milk fat and lactose yields were not significantly affected by dry period treatment.

Conclusion Supplementing grass silage with a protein supplement alone during the dry period resulted in a marginal reduction in milk and protein production, although when offered in combination with a fat supplement, production was increased. These effects were achieved without changes in the subsequent DM intakes of the animals, and were probably mediated through differences in the animals' body reserves at calving (Jaurena *et al.*, 2001).

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The utilization of a commercial rapeseed meal product (RaPass) as a protein supplement for lactating dairy cows

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Introduction Rapeseed meal is a common protein supplement in ruminant diets that is characterized by high rumen protein degradability (Bertilsson *et al*., 1994; Vanhatalo *et al*., 1995). Appropriate treatment can however reduce ruminal protein degradability and increase the efficiency of protein utilization. RaPass (UM Feeds Marketing, Burton on Trent, Staffs, UK) is a commercial rapeseed meal product that made using the Maillard reaction. This is the non-enzymatic browning reaction between proteins and reducing sugars that protects protein from rumen degradation. Release of the protein at abomasal pH allows the peptide chains to be digested at an efficiency similar to that of untreated rapeseed meal (Moss *et al* 2000). Cows fed rapeseed meal that was treated to increase the rumen undegradable protein (RUP) content have been reported to perform better than those fed untreated rapeseed meal (Bertilsson *et al*., 1994). This study evaluated the potential of using RaPass as a protein supplement in dairy cow rations.

Materials and Methods Forty-two Holstein Friesian cows that were on average in their 12th week of lactation were allocated on the basis of parity, calving date, previous lactation yield; pre trial milk and composition yield to one of two dietary treatments (RaPass and Control). The cows in each group had *ad libitum* access to a total mixed ration (TMR) containing (DM basis) grass silage, maize silage, molasses, wheat, mineral mix (51.7:14.5:4.29:13:0.9). The TMR for cows in the RaPass group also contained 8% RaPass and 7.7% of a commercial protein (350 g CP/kg DM) supplement that contained Soya (1.34 and 1.30 kg/cow respectively),, while that of the Control group contained 15.5 % (2.60 kg/cow) of the same protein supplement but no RaPass. In addition, all cows received 6 kg/d of a commercial concentrate (200 g CP/kg DM) and *ad libitum* access to water. Both groups were on the Control diet for two weeks pre trial and at the start of week four a rumen inert energy supplement (Megalac; Volac International Limited, Royston, Herts) was introduced into both rations in equal amounts (0.3 kg/cow). Group feed intake and milk yield were recorded daily. Milk samples were taken weekly at two consecutive milkings and bulked in proportion to yield to give one sample per cow per sampling week. The milk samples were analysed for butterfat and protein by National Milk Records, Yeovil, using a 'Milko-Scan' (Foss Electric (UK) Ltd, York). Analysis of covariance using the two pre-trial weeks as covariates was carried out using General Linear Model – Univariate of the SPSS (version 9.0 for Windows).

Results The cows that received RaPass had a lower group DM intake than those in the Control group. In contrast, the RaPass fed cows produced on average a higher (P<0.05) milk yield than those fed the Soya based protein supplement alone (Table 1). However, the difference in weekly milk yield (Figure 1) was only numerically higher (P>0.05) in the RaPass group than in the Control group. The 4 % fat corrected milk yield, milk fat and milk protein yields as well as the protein content of the milk were not significantly different between the RaPass and Control groups. The milk fat content was however higher in the Control group than in the RaPass group.

Table 1: Dry matter intake (kg/d), milk yield (kg/d) and milk composition of dairy cows

	RaPass	Control	Prob.
Total DM intake	22.5 ± 0.20	25.1 ± 0.27	-
Milk yield	33.0 ± 0.51	31.0 ± 0.51	P<0.05
4% Fat CMY	34.1 ± 1.11	35.6 ± 1.12	NS
Composition (g/kg)			
Milk fat	42.5 ± 0.90	45.0 ± 0.90	P<0.05
Milk protein	33.3 ± 0.20	33.4 ± 0.20	NS
Yield (g/d)			
Milk fat	1364 ± 44.4	1425 ± 44.6	NS
Milk protein	1095 ± 17.0	1051 ± 17.0	NS

CMY: corrected milk

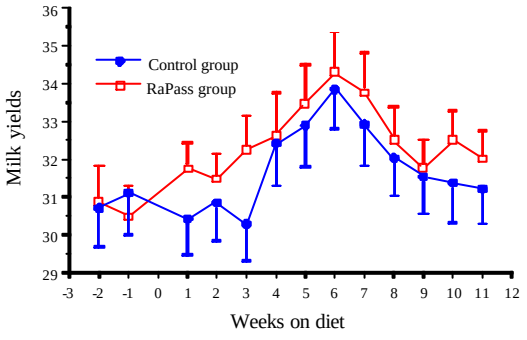

Figure 1. Milk production pattern of cows

Conclusions RaPass can be used as a protein supplement to partly replace Soya in dairy cows ration without reduction in milk production and quality. Increased average milk yield with lower feed intake may be due to the increased supply of RUP in RaPass fed cows.

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The influence of body condition and level of feeding on the heat production of nonpregnant, nonlactating dairy cows

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Introduction Chowdhury and Ørskov (1994) observed that heat production in sheep was reduced by approximately 10% below that at fasting when the animal was offered one third of the predicted maintenance requirement through intragastric nutrition. These authors also suggested that this indicated a glucose deficient state in the fasted animal, and that as a result the heat production at fasting was artificially raised. In agreement with Ørskov and MacLeod (1990), Chowdhury and Ørskov (1994) further stated that when measuring heat production to estimate basal metabolism animals should have their heat production measured when being offered one third of maintenance rather than fasting, although this was not tested by experiment. Birnie *et al*., (2000) reported that fasting heat production was influenced by the condition score of the cow. The objectives of this experiment were firstly to investigate the influence of maintenance and sub-maintenance levels of feeding on the observed heat production in nonpregnant, nonlactating dairy cows offered a more normal diet and secondly to further examine the influence of cow condition score on the fasting heat production.

Materials and methods Twelve nonpregnant, nonlactating Holstein Friesain dairy cows were used in this experiment. The cows were chosen to reflect a range of condition scores. Dried grass pellets were offered throughout the experiment. Four diet levels were offered to each cow – maintenance (M), 2/3 maintenance (2/3M), 1/3 maintenance (1/3M) and fast (FAST). Maintenance energy requirement was estimated to be 0.58 MJ ME/kg $^{0.75}$. The twelve cows were run in three groups of four over three periods, one group in each period. Each group of four was run as two pairs, with one pair offered descending diet levels and the other pair offered ascending diet levels. All cows were offered dried grass pellets at maintenance level for a minimum of 21 days prior to commencement of the actual experiment. Diet digestibility balances were carried out at the maintenance level of feeding only and respiratory exchange was measured for each cow in each period using open circuit indirect respiration calorimeters. Water was freely available to the cows throughout the experiment. Cows were weighed to determine liveweight and body condition score every third day during the experimental period. Data were analysed by ANOVA using GENSTAT 5 after removal of period and cow effects. Linear regression analysis was also undertaken to determine the relationship between the heat production of the cow and metabolizable energy intake, thereby providing an estimate of the efficiency of use of metabolizable energy for maintenance (k_m).

Results This experiment has shown (Table 1) that feeding at 1/3 of maintenance does not reduce the heat production of the cow below that observed at fasting. Rather the results demonstrate a linear increase in heat production in proportion to the amount of food consumed by the animal.

Table 1 Condition score, liveweight dry matter intakes and heat production of the cows offered the four diet levels

	M	2/3 M	1/3M	Fast	SEM	Sig
Condition score	2.7	2.7	2.7	2.7	0.183	NS
Liveweight (kg)	542	525	507	516	13.8	NS
ME intake (MJ/d)	74.5	50.7	26.0	0	4.27	***
Heat production (MJ/d)	67.5	58.3	52.8	46.9	1.61	***
Heat production (MJ/kg $^{0.75}$)	0.603	0.533	0.497	0.446	0.0105	***

A significant relationship ($P<0.01$) was observed between mean condition score of the cow during the experimental period and the fasting heat production. Condition score was responsible for proportionately 0.73 of the variation in fasting heat production between the cows. The relationship is defined as

FHP (MJ/kg $^{0.75}$) = 0.509 (s.e. 0.0155) – 0.025 (s.e. 0.0053) CS

Where FHP = fasting heat production and CS = condition score. The regression analysis between MEI and heat production gave an estimate of the efficiency of utilisation of ME for maintenance (k_m) of 0.68.

Conclusion Heat production declines with level of feeding and is influenced by the condition score of the animal.

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In vivo estimation of body composition of lactating dairy cattle from urea space measurements

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Introduction San Pietro and Rittenberg (1953) reported that urea appeared to meet all the requirements of a satisfactory tracer. Urea is non toxic, not foreign to the body and it shows an even and rapid distribution throughout the total body water without any physiological effect. For these reasons in addition to its easy and accurate measurement, urea is an ideal candidate tracer to estimate empty body water *in vivo*. Total body water volume (urea space) can be estimated by dividing the total amount of urea infused by the increase in plasma urea concentration from prior to infusion until 12 or 30 minutes after mean infusion time. Kock and Preston (1973) reported significant relationships between urea space measurements and percentage of empty body fat and water in cattle. However, Andrew *et al*. (1995) using 21 Holstein cows showed that prediction of empty body water using the urea space technique only explained 31 % of the variation. The objective of this experiment was to use the urea dilution technique to estimate the body composition of lactating dairy cows and produce relationships between urea space and body fat and protein content.

Materials and methods One hundred and eight lactating dairy cows were selected from the dairy herd at the Agricultural Research Institute of Northern Ireland. The cows were selected to represent the range of condition scores, genetic merit, stage of lactation and liveweight within the overall herd. Urea infusion studies were performed on all 108 cows. A blood sample was taken to determine urea blood concentration before infusion. Then a solution containing 20 % urea w/v dissolved in 0.9 % saline was administered during a 3 minute period through a catheter, inserted into the jugular vein. The volume injected provided 130 mg urea/kg liveweight. After infusion the catheter was flushed with saline and then removed. In order to know the exact volume of infused solution the syringes were weighed before and after infusion. Blood samples were collected from the coccygeal vein at 12 and 30 min after the mean infusion time. The blood was centrifuged and the plasma frozen for the subsequent determination of plasma urea concentration. All cows were slaughtered within two days of the infusion studies and all procedures were undertaken over a period of two weeks. At the slaughterhouse the following components were collected, carcass, head, pluck, hide, gut, udder and feet. The components were weighed, frozen and subsequently minced, subsampled and analysed for dry matter, total nitrogen, lipid and ash contents. Empty body water, protein and fat contents were determined for all cows. Simple linear regression relationships have been developed for urea space against empty body water, protein and fat.

Results The range in live animal and body composition data for the 108 cows is shown in Table 1. Table 2 gives the simple linear regression relationships derived between urea space and the empty body weights of the various chemical components.

Table 1 Live animal and body composition data for the dairy cows

	Mean	Minimum	Maximum	SD
Condition score	2.4	1	4	0.54
Liveweight (kg)	576	420	781	69.8
Stage of lactation (days)	149	21	472	70.2
Empty body water (kg)	250	194	318	28.3
Empty body protein (kg)	72.3	53.1	97.5	8.89
Empty body fat (kg)	42.3	16.4	129.8	20.6

Table 2 Relationships between urea space and components of body composition

	R^2	Sig
Empty body water (kg)	0.52	***
Empty body protein (kg)	0.48	***
Empty body fat (kg)	0.28	***

Conclusions The use of the urea dilution technique has the potential to estimate the body composition of lactating dairy cattle.

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An examination of metabolisable energy requirements of lactating dairy cows

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Introduction The present energy rationing system used in the U.K., the metabolisable energy (ME) system (AFRC, 1990), is an empirical model incorporating a factorial approach to estimate the requirements of animals. Axiomatically, when this approach is adopted each component of the system must be accurately determined. Recent studies at this Institute using regression techniques have indicated that ME requirements for maintenance (ME_m) are greater than that predicted using the equations of AFRC (1990), while the efficiency of use of ME for lactation (k_l) is in line with the latter predictions (Kirkland and Gordon, 1999). There is a paucity of recent data relating to the efficiency of use of ME for tissue gain during lactation (k_g), and the efficiency of use of body tissue for milk production (k_{rl}). The objective of the present study was to use regression techniques with data obtained from several recent calorimetric trials at this Institute to predict the ME_m and efficiency factors of lactating dairy cows offered different diet types.

Materials and methods The data were drawn from three studies with Holstein Friesian dairy cows and encompassed a total of 83 measures of total energy balance determined using indirect calorimetry. The diets in each trial were offered as a total mixed ration and included a straw-concentrate diet (0.18:0.82 DM basis), a lucerne hay-concentrate diet (0.30:0.70 DM basis) and a grass silage-concentrate diet (0.65:0.35 DM basis). The data represented a broad range in intake and production levels with ME intake (MEI) ranging from 75.5 to 292.8 MJ/d, milk yield from 1 to 42.4 kg/d (mean 22.3 kg/d), milk energy outputs (E_l) from 2.79 to 123.69 MJ/d, tissue energy (E_g) from -46.68 to 36.24 MJ/d and live weight from 478 to 733 kg. A range of regression models was used to analyse the data, including; the regression of milk energy output corrected to zero energy balance ($E_{l(0)}$) on MEI, or ME available for production (ME_p); the regression of heat production (HP) or $E_l + E_g$ on MEI; and multiple regression analysis relating MEI to metabolic live weight (MW), E_l and E_g, or to MW, E_l and positive and negative E_g ($-E_g$ and $+E_g$ respectively).

Results The regression equations developed with the current data set are presented in Table 1. All the equations were highly significant ($p<0.001$) and had high associated values of R^2 (range 0.92 to 1.00). Estimates of ME_m ranged from 0.63 to 0.66 MJ/kg$^{0.75}$/d with a mean value of 0.65 MJ/kg$^{0.75}$/d. The derived estimates of k_l were within a small range (0.63 to 0.64) and had a mean value of 0.64, while the predicted values of k_g (Equations (2) and (3)) had a mean value of 0.68. The estimate of k_{rl} derived from multiple regression analysis (Equation (3)) was 0.80. All estimates had relatively small standard errors (in parenthesis).

Table 1 The regression equations developed and the derived estimates of metabolisable energy requirements and utilisation from the present data set[#]

	Equations	**R^2**	**ME_m**	**k_l**	**k_g**	**k_{rl}**
(1)	$E_{l(0)} = 0.63_{(0.011)}\,MEI - 0.391_{(0.0176)}$	0.98	0.62	0.63		
(2)	$MEI = 0.66_{(0.020)}\,MW + 1.567_{(0.0317)}\,E_l + 1.390_{(0.0578)}\,E_g$	1.00	0.66	0.64	0.72	
(3)	$MEI = 0.65_{(0.020)}\,MW + 1.561_{(0.0312)}\,E_l + 1.588_{(0.1140)}\,(+E_g) + 1.246_{(0.0916)}\,(-E_g)$	1.00	0.65	0.64	0.63	0.80
(4)	$E_l + E_g = 0.64_{(0.012)}\,MEI - 0.417_{(0.0192)}$	0.97	0.65			
(5)	$HP = 0.36_{(0.012)}\,MEI + 0.417_{(0.0192)}$	0.92	0.65			
(6)	$E_{l(0)} = 0.64_{(0.004)}\,ME_p$	1.00		0.64		
	Mean	**0.98**	**0.65**	**0.64**	**0.68**	**0.80**

[#] $E_{l(0)}$: MJ/kg$^{0.75}$/d and MJ/d in Equations (1) and (6) respectively; MEI: MJ/kg$^{0.75}$/d in Equations (1), (4) and (5), and MJ/d in Equations (2) and (3); MW: kg$^{0.75}$; HP: MJ/kg$^{0.75}$/d; E_l and E_g: MJ/d in Equations (2) and (3), and MJ/kg$^{0.75}$/d in Equation (4)

Conclusions The results obtained from the present study are in line with those determined earlier at this Institute, and indicate that the ME_m derived using regression techniques with modern cow genotypes is much greater (proportionately 0.35) than that predicted using the equations of AFRC (1990). The present data suggest an ME_m of 80 MJ/d for a 600 kg cow compared to 58 MJ/d calculated according to the current energy rationing system. The estimate of k_g derived, 0.68, suggests that concomitant tissue deposition occurs with a similar efficiency to that of milk production, while the determined values of k_l (0.64) and k_{rl} (0.80) are in line with the recommendations of AFRC (1990).

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The effect of graded levels of 'Greenwich Gold' on the performance of growing-finishing pigs

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Introduction. Using food industry liquid co-products can significantly reduce the cost of pig production (Scholten, van der Peet-Schwering, Verstegen, den Hartog, Schrama and Vesseur 1999) as well as reducing environmental pollution (Brooks, Moran and Beal 1999). Greenwich Gold (GG)(Amylum UK Ltd.) is a liquid residue, known in the trade as bottom stills, resulting from alcohol fermentation of wheat starch. Although the product is widely used by UK pig producers there is no published information on which to base a recommendation for maximum inclusion rate in diets for growing-finishing pigs. The objective of the study reported here was to assess the effect on growth performance and carcass quality of feeding pigs diets containing increasing levels of GG.

Materials and Methods. The trial was conducted according to a randomised block design, with four treatments, two sexes and seven replicates. Pig were fed, twice daily to appetite, on one of four liquid diets (20% DM) in which 0, 15, 22.5 or 30% of the dietary dry matter was supplied as GG. The GG contained (g kg^{-1} fresh material) DM 192; crude protein 6.25; NDF 1.6; ash 1.28 and DE 3.3 MJ kg^{-1}. The diets were formulated to provide (at a nominal 87% DM) 13.4 MJ DE kg^{-1} and 12 and 9.5 g lysine kg^{-1} in the grower and finisher diets respectively. Water was also available *ad libitum* throughout the trial from nipple drinkers. Fifty-six pigs (from PIC Line 15 hybrid sows X PIC Meatlink' boars) with an initial weight of 21.0 ± 1.7 kg were used. Each replicate consisted of four female pigs and four entire male pigs. Within each replicate and sex group, animals were randomly allocated to the four dietary treatments. The pigs were housed in two environmentally controlled performance test houses. Carcass data was obtained at slaughter (90 kg). Data was subjected to analysis of covariance using initial weight as the covariate. Analyses were conducted using Minitab (v 12.1).

Results. The results of the trial are summarised in Table 1. There was no significant effect of GG percentage in the diet on daily gain at any stage, nor was there any significant effect on P2 back-fat thickness or killing-out percentage. Including GG in the diet reduced dry matter (DM) feed intake. However, growth rate was maintained because the reduction in DM intake was mirrored by an improvement in DM feed conversion ratio. The DM food conversion ratio of pigs fed diets containing 30% GG (1.91) was significantly (P<0.01) better than that of pigs on the control diet (2.08).

Table 1. Effect of inclusion level of Greenwich Gold in the diet on the performance of growing-finisher pigs.

		Greenwich Gold %				
		0	15	22.5	30	SE_D
Daily gain (g)	Grower (20-45 kg)	565	573	573	599	27.2
	Finisher (45-90 kg)	941	901	893	935	30.9
	Overall (2-90 kg)	779	759	746	793	15.9
Daily DM intake (g)	Grower (20-45 kg)	954	918	950	925	26.1
	Finisher (45-90 kg)	2093^{abc}	1947^{a}	1983^{b}	1918^{c}	36.4
	Overall (2-90 kg)	1608^{a}	1511	1523	1504^{a}	33.0
DM FCR	Grower (20-45 kg)	1.72	1.64	1.68	1.55	0.067
	Finisher (45-90 kg)	2.24^{a}	2.18	2.24^{b}	2.07^{ab}	0.060
	Overall (2-90 kg)	2.08^{a}	2.00	2.05^{b}	1.91^{ab}	0.041
Carcass weight (kg)		63.57	61.83	62.22	62.42	0.97
Backfat P2 (mm)		11.00	10.73	11.20	10.16	0.68
Killing-out %		70.38	70.85	71.70	69.70	0.93

^{a,b,c,} Means with the same superscript differ at P<0.05 or greater

Conclusions. These results indicate that when diets are formulated to provide an equivalent supply of energy and lysine, at a given dry matter concentration, GG can be included in the diet at inclusion levels up to 30% without any loss in growth performance or deterioration in carcass quality.

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Utilization of three biodegraded agro-industrial by-products (AIBs) by layers

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Introduction In Nigeria, the need to improve poultry production cannot be overemphasized because increase in population has necessitated an increase in the demand for animal protein. Increased egg production holds a unique position in bridging this animal protein supply (Longe, 1984). But the conventional energy sources like maize and sorghum are very expensive making feed to account for about 60% of cost of egg production. Alternatives are now being searched for in agro-industrial by-products because these by-products can be biodegraded to improve their energy value for poultry feeding.

Materials and methods Three agro-industrial by-products, rice husk (RH), brewer's dry grain (BDG) and palm kernel meal (PKM) were biodegraded for 10 days using a fungus, *Trichoderma viride*. The degraded products were included at 20, 30, and 40g/100g levels in the diets of layers such that it allowed for a graded reduction of maize in the diets. 12 diets were formulated. The control diet for each of the by-products contained no biodegraded by-product while the rest were the test diets containing the biodegraded by-products at the levels stated above. Three hundred and sixty (360) laying Nera Black birds were allocated to the diets in a complete randomized design with 30 birds per treatment and 10 birds per replicate. Feed intake estimation and egg collection were carried out during a 12 week laying period.

Results After degradation, the metabolizable energy of RH, BDG and PKM increased from 5880, 8316 and 9135 to 10,941, 12,087.2 and 12478.2 MJ kg^{-1} respectively while their crude fiber decreased from 30, 20, 12 to 20.25, 5.80 and 8.35 g/100g respectively. The crude protein increased from 4.00, 18.00 and 18.00 to 6.04, 36.32 and 29.03 g/100g respectively. Diets with 20g/100g biodegraded RH, BDG and PKM exhibited better percentage hen day production of 71%, 64% and 72% respectively. Birds on these diets also produced significantly ($P<0.05$) higher number of eggs and had significantly ($P<0.05$) better feed efficiency. Eggshell thickness, height and quality were not significantly affected ($P>0.05$) by inclusion of the biodegraded by-products. Belewu (1999) has reported increased feed intake of sorghum stover following biodegradation with fungi. The observed enhanced egg production on the biodegraded diets is attributable to the improved metabolizable energy and protein and reduced crude fiber of the by-products. Inclusion of the biodegraded product at 20g/100g gave the best result indicating that complete degradation was not achieved and therefore inclusion of the AIBs at levels over 20g/100g caused a depression in feed intake and egg production.

Table 1. Performance of layers on diets based on biodegraded agro-industrial by-products.

	RH Diets					BDG Diets					PKM Diets				
	1	2	3	4	$SE_{\bar{x}}$	1	2	3	4	$SE_{\bar{x}}$	1	2	3	4	$SE_{\bar{x}}$
Parameters															
Feed intake (g/bird)	124^{a}	138^{a}	124^{a}	104^{b}	±5.11	151^{a}	111^{b}	92^{b}	89^{b}	±8.21	147^{a}	121^{b}	125^{b}	108^{b}	±7.27
Feed Efficiency (%)	.27	.27	.27	.30	±0.01	.34	.24	.31	.23	±0.01	.27	.23	.31	.48	±0.02
Egg weight (g)	57.96	56.45	55.43	59.11	± 1.90	57	53	49	52	±1.88	58	53	52	49	±1.72
Shell thickness (cm)	.22	.20	.23	.18	±0.02	.22	.24	.21	.23	±0.02	.25	.24	.24	.25	±0.02
Hen day production (%)	69.48^{a}	70.64^{a}	63.89^{a}	51.72^{b}	±2.52	63^{ab}	64^{a}	48^{c}	55^{bc}	±2.32	74^{a}	72^{a}	63^{b}	38^{c}	±2.48
No of eggs	40^{a}	45^{b}	40^{a}	32^{c}	±1.20	39^{ab}	40^{a}	26^{c}	34^{b}	±1.10	45^{a}	44^{a}	34^{b}	24^{c}	±1.12

Conclusion Results show that inclusion of biodegraded RH, BDG and PKM at 20g/100g of diet allowed for a reduction in the level of maize from 50g/100g to between 30 and 40g/100g; a reduction that caused a lowering of the feed cost.

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Effect of barley variety, year and location of production on overall and ileal digestibility in growing pigs

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Introduction Variety and location of production can affect the nutritive value of barley grain (Valaja *et al* 1997). Riviera and Dandy are the most commonly produced varieties of barley in Northern Ireland and it was an aim of this study to compare their nutritive value and to examine the effect of location of production. The growing and harvesting conditions have been shown to affect chemical and physical properties of cereal grain (Rogel *et al* 1987). A further aim was therefore to compare the nutritive value of Riviera and Dandy produced in 1998 (McCann *et al* 2000) and in 1999.

Materials and methods Twelve pigs (Large White x Landrace) were fitted with PVTC cannulae (Van Leeuwen *et al* 1991) and randomised to 12 diets in a 2 x 6 factorial, 4 period crossover design. The factors were: barley variety (Riviera and Dandy); location of production (sites 1, 2, 3, 4, 5, 6). The diets contained g/kg: barley 650, soyabean meal 283, tallow 30, titanium dioxide 1.5, minerals / vitamins etc. 35.5. Each balance period comprised a 5 d pre-feed, 7 d faecal collection and a 2 d (2 x 12 h) ileal collection. Apparent overall (OD) and ileal (ID) digestibility coefficients were determined and the results were analysed by REML using Genstat 5.

Results OD of DM (0.843, SED 0.003) and NDF (0.665, SED 0.006) for Riviera were higher ($P < 0.001$) than for Dandy (0.836 and 0.646 respectively). ID of NDF (0.490) for Riviera was higher ($P < 0.001$, SED 0.012) than for Dandy (0.453). Location effects were observed (Table 1), mean values for OD ranged from 0.83 to 0.86 for DM, 0.82 to 0.87 for CP and 0.73 to 0.83 for oil and mean values for ID ranged from 0.63 to 0.67 for DM, 0.71 to 0.76 for CP and 0.77 to 0.87 for oil. ID of NDF for both Riviera and Dandy were improved ($P < 0.05$) in 1999 cf. 1998 (0.490 vs. 0.387 for Riviera and 0.453 vs. 0.407 for Dandy). OD of NDF for Dandy was also improved (0.646 vs. 0.608). No significant year effects were found for the other parameters.

Table 1 Overall and ileal digestibility of CP and oil for Riviera and Dandy produced at 6 sites (d.f. 11)

		Riviera						Dandy							
Site		1	2	3	4	5	6	1	2	3	4	5	6	SED	P
OD	DM	0.84	0.84	0.86	0.83	0.84	0.86	0.83	0.84	0.83	0.83	0.84	0.84	0.008	< 0.001
	CP	0.87	0.82	0.87	0.84	0.82	0.85	0.84	0.85	0.85	0.84	0.85	0.83	0.010	< 0.001
	oil	0.82	0.77	0.82	0.79	0.73	0.79	0.77	0.79	0.81	0.82	0.83	0.81	0.021	< 0.001
ID	DM	0.67	0.67	0.67	0.65	0.64	0.66	0.65	0.66	0.66	0.64	0.63	0.66	0.016	NS
	CP	0.76	0.71	0.77	0.73	0.72	0.74	0.72	0.74	0.74	0.73	0.73	0.73	0.016	NS
	oil	0.87	0.80	0.87	0.85	0.77	0.87	0.83	0.87	0.84	0.81	0.82	0.86	0.032	< 0.05

Conclusions Riviera was superior to Dandy in terms of OD of DM and NDF. ID of NDF was also higher for Riviera. This supports the findings by McCann *et al* (2000). The location effects cannot be fully explained by climatic conditions due to the lack of any significant difference between the year of production except for NDF. The results of this study are consistent with Waldron (1996) who reported that harvest year effects were smaller in comparison with variety differences.

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The effects of barley variety, the location of production and enzyme addition on overall and ileal digestibility in growing pigs

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Introduction Spring barley is the most widely produced cereal in Northern Ireland. Riviera and Dandy are the main varieties but there is a lack of information regarding their nutritive value. Variety and location of production affect the nutritive value of barley (Valaja *et al* 1997). An aim of this study was to determine the nutritive value of Riviera and Dandy and also to examine the effect of location of production. Cereal grains contain a high level of non starch polysaccharides (NSP). The major NSP present in barley are β-glucan and arabinoxylan and supplementation with exogenous enzymes has been shown to increase digestibility to different extents (Yin *et al* 2000). A further aim of this study was to examine the effects of a mixture of β-glucanase and xylanase on these two varieties.

Materials and methods Twelve pigs (Large White x Landrace) were fitted with PVTC cannulae (Van Leeuwen *et al* 1991) and randomised to 12 diets in a 2 x 3 x 2 factorial, 4 period crossover design. The factors were: barley variety (Riviera and Dandy); location of production (sites 1, 2, 3); enzyme (β-glucanase 0.75 g/kg and xylanase 0.5 g/kg, Finn Feeds Ltd) absent or present. The diets contained g/kg: barley 650, soyabean meal 283, tallow 30, titanium dioxide 1.5, minerals / vitamins etc. 35.5. Each balance period comprised a 5 d pre-feed, 7 d faecal collection and a 2d (2 x 12 h) ileal collection. Apparent overall (OD) and ileal (ID) digestibility coefficients were determined and the results were analysed by REML analysis using Genstat 5.

Results Riviera gave higher OD and ID coefficients than Dandy except for OD of NDF (Table 1). OD of oil (0.825) for Riviera (site 2) was higher ($P < 0.001$, SED 0.014) than for site 1 (0.798) or site 3 (0.793). ID of DM (0.719), protein (0.799), oil (0.833) and energy (0.742) were higher ($P < 0.001$, SED 0.014, 0.020, 0.019, 0.014 respectively) for Riviera (site 2) than for site 1 (0.662, 0.728, 0.781 and 0.684 respectively) or site 3 (0.644, 0.747, 0.788 and 0.693 respectively). OD of oil (0.788) for Dandy (site 1) was higher ($P < 0.001$, SED 0.014) than for site 2 (0.747). ID of NDF (0.474) for Dandy (site 1) was higher ($P < 0.001$, SED 0.015) than for site 3 (0.430). Enzyme addition reduced ($P < 0.01$, SED 0.024) OD of NDF for Riviera (site 1) (0.524 vs. 0.441). ID of NDF was also reduced ($P < 0.001$, SED 0.008) by enzyme addition (0.492 vs. 0.449).

Table 1 The effect of variety on overall and ileal digestibility (d.f.5)

	Overall Digestibility				Ileal Digestibility			
	Riviera	Dandy	SED	P =	Riviera	Dandy	SED	P =
Dry matter	0.846	0.837	0.004	< 0.05	0.685	0.644	0.008	< 0.001
Protein	0.857	0.844	0.005	< 0.001	0.758	0.720	0.011	< 0.001
NDF	0.635	0.644	0.010	NS	0.488	0.453	0.008	< 0.001
Oil	0.805	0.768	0.008	< 0.001	0.801	0.734	0.011	< 0.001
Ash	0.539	0.534	0.011	NS	0.235	0.158	0.026	< 0.001
Energy	0.850	0.840	0.004	< 0.05	0.706	0.668	0.008	< 0.001

Conclusions The results indicate small but important differences in digestibility of the two varieties, particularly at the ileal level and also differences due to location of production which cannot be readily attributed to climatic factors. The variety difference confirms previous observations (McCann *et al* 2000). The lack of effect of enzyme supplementation contrasts with the report of Yin *et al* (2000) but agrees with that of Thacker *et al* (1992).

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The effect of variety and location of production on the chemical composition of barley

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Introduction Variety and location of production affect the chemical composition of cereal grain (Valaja *et al* 1997). There are several varieties of Spring barley produced in Northern Ireland (NI) and in the Republic of Ireland (ROI). However, there is no information in the literature concerning the chemical composition of these varieties nor is there any information regarding location of production. The main aim of this study was to examine the effect of variety and location of production on the chemical composition of barley grown in these countries. Specific weight is traditionally used to predict nutritive value. However, a number of research reports including that of Stewart *et al* (1997) have not found a strong relationship between specific weight and chemical composition or nutritive value. For this reason the use of specific weight as a predictor of chemical composition was studied.

Materials and methods Barley samples (n=63) were obtained from locations throughout NI and ROI. Samples included the two most commercially produced varieties in NI (Riviera and Dandy, n=8) and ROI (Crusader and Lamba, n=7) grown at a range of locations and samples of different varieties (n=11) from the variety testing trials grown at Crossnacreevy (site 1) and Coleraine (site 2). Specific weight was measured, the samples milled (1.0 mm screen) and analysed for DM, ash, CP, NDF, B-Oil, gross energy, starch, β-glucan and *in vitro* viscosity. Significant differences were determined by paired t-tests and simple regression.

Results The proportion of the chemical components (n=63) ranged (g/kg DM) from 94-169 for CP; 178-398 for NDF; 15-24 for oil; 17-52 for ash; 434-631 for starch; 22 - 55 for β-glucan; 3.5-20.2 cps for *in vitro* viscosity; 45-64 g/hL for specific weight; and 18.3-18.9 MJ/kg for GE. The CP content of Riviera was significantly lower ($P < 0.05$) than that of Dandy (Table1). The starch content of Dandy was significantly lower ($P < 0.001$) than that of Riviera. There were no significant differences between the starch contents of the two ROI varieties but the oil content of Lamba was lower ($P < 0.01$) than that of Crusader. A location effect was observed between barley grown at site 1 and site 2 (n=11) in terms of contents of starch ($P < 0.01$) and *in vitro* viscosity ($P < 0.01$). There was a poor relationship between specific weight and any of the chemical components (Table 2).

Table 1 Mean chemical composition values and physical parameters for the barley samples (DM basis)

	CP (g/kg)	NDF (g/kg)	Oil (g/kg)	Starch (g/kg)	β-glucan (g/kg)	GE (MJ/kg)	Sp Wt (kg/hL)	Viscosity (cps)
Riviera	119	259	21	607	34	18.8	56	4.5
Dandy	132	267	21	523	32	18.8	57	5.5
SEM	3.9*	16.8	1.1	8.3***	4.7	0.11	1.2	0.54
Crusader	117	219	22	568	36	18.5	58	6.7
Lamba	127	251	19	556	37	18.5	59	8.7
SEM	5.1	23.6	0.5**	8.6	2.2	0.05	1.9	1.48
Site 1	118	228	20	542	34	18.6	56	4.4
Site 2	113	223	20	583	30	18.5	56	6.1
SEM	3.8	5.2	0.9	9.2**	2.0	0.05	0.58	0.42**

*P < 0.05 **P < 0.01 ***P < 0.001

Table 2 Relationship between specific weight and chemical components

	CP (g/kg)	NDF (g/kg)	B-Oil (g/kg)	Ash (g/kg)	Starch (g/kg)	β-glucan (g/kg)	GE (MJ/kg)	Viscosity (cps)
R^2	0.21	0.27	0.00	0.17	0.24	0.12	0.14	0.12

Conclusions The main component showing significant variation was starch which was significantly lower for Dandy than Riviera across a range of locations and lower for a range of barley varieties at site 1. These differences could be important in the formulation of pig diets. This study supports that of Stewart *et al* (1997) in indicating that specific weight is not well correlated with chemical composition.

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Bushel weight of wheat and enzyme supplementation did not affect weaner pig performance

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Introduction Wheat is often the largest single ingredient in piglet diets and therefore variation in quality may have a large effect on piglet performance. Currently there is no rapid method for the nutritive assessment of wheat. The livestock feed industry traditionally uses bushel weight as a predictor of nutritive value; however this assumption has recently been challenged by a literature review (Miller and Wilkinson, 1998) and by a weaner trial (Miller, Toplis, Arnold, Cook and Marshall, 2000). The aim of this experiment was to compare two more extreme bushel weights of Riband (64 v 78 kg/hl) than used in the previous experiment (Miller *et al*., 2000) when fed to weaned piglets with and without a xylanase enzyme. In order to amplify possible differences wheats were uncooked and included in the test diets at an atypically high level. We hypothesised that 78 kg/hl bushel weight would outperform 64kg/hl bushel weight (which is below the standard accepted by feed mills for feed wheat) and that enzyme supplementation would improve the performance of both wheats.

Materials and methods One hundred and twenty eight crossbred piglets (62.5% Large White, 25% Landrace, 12.5% Duroc) were weaned into fully slatted flat deck pens. The piglets were weaned at 21 ± 0.2 days of age (mean ± SEM) and 6.3 ± 0.19 kg liveweight. Piglets had not received creep feed prior to weaning. Eight piglets were allocated to each pen (1.37 m x 1.43 m) on the basis of litter, liveweight and sex. The experiment was a 2 x 2 factorial design with Riband fed at 2 bushel weights, 64 and 78 kg/hl, with or without enzyme. Proximal analysis of the two wheats was DM 883 and 885 g/kg, crude protein 118 and 109 g/kg, oil-B 26 and 24 g/kg, NDF 228 and 168 g/kg for 64 and 78 kg/hl respectively. Four pens were randomly allocated to each of the four treatments. All diets contained zinc oxide and were formulated to contain 500 g/kg wheat, 16.25 MJ DE/kg, 16.25 g total lysine/kg using the same formula as in the previous trial (Miller *et al*., 2000). The enzyme was added to provide 5,500 xylanase units/kg feed and 600 β-glucanase units/kg feed. Piglets were individually weighed at weaning and 7, 14 and 20 days after weaning. Food and water were provided *ad libitum* throughout the 20 day trial period. Data were analysed using the GLM procedure of Minitab 12.2.

Results Piglet performance during the experiment is given in Table 1. Neither growth rate, feed intake, nor feed conversion ratio were different between the treatments during any period of the trial. End weight after the 20 day trial period was not different between treatments.

Table 1 *Piglet start weights, overall average daily gains (DLWG), average daily feed intakes (ADFI), feed conversion ratio (FCR) and weights at the end of the trial for piglets receiving diets containing wheats of differing bushel weights with or without enzyme supplementation after weaning.*

	Riband 64 Minus	Riband 64 Plus	Riband 78 Minus	Riband 78 Plus	SEM	Enzyme	Bushel weight	Inter-action
Start wt (kg)	6.3	6.4	6.4	6.2	0.42	n.s.	n.s.	n.s.
End wt (kg)	12.3	11.7	11.9	11.8	0.24	n.s.	n.s.	n.s.
DLWG (g/day)	300	269	278	273	12.1	n.s.	n.s.	n.s.
ADFI (g/day)	300	291	291	282	10.1	n.s.	n.s.	n.s.
FCR	1.00	1.08	1.05	1.03	0.023	n.s.	n.s.	n.s.

*n.s. No significant difference between treatments.

Conclusions The low bushel weight wheat did not reduce piglet performance, neither did enzyme inclusion improve it. Despite the uncharacteristically high inclusion of uncooked wheat, piglet performance was typical for the experimental unit for this age of pig. The use of bushel weight as an indicator of nutritive value must be seriously questioned.

Acknowledgement This work was funded by a BSAS summer studentship, Primary Diets Ltd. and Frank Wright Ltd.

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Use of Sunflower Seed Meal (SSM) in broiler ration

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Introduction Soyabean meal (SBM) is used extensively in poultry rations. As the cultivation of soya bean is limited in Iran, annually a large amounts of SBM is imported. In recent years cultivation of some oil seed such as sunflower seed (Heliantus annus) is undertaken in some provinces of Iran. This plant will grow in cooler and dried climates than the soya bean or cotton plants. It contains 45 percent oil and a good source of protein and B-group vitamins. Due to high fibre content and low lysine content, use of sunflower meal (SSM) is limited in poultry feeds, but dehulled SSM is suitable for broiler feeds (Church, 1988 and Scott *et al*., 1982). The objective of the present study was to investigate the effect of SSM on performance of broiler chicken.

Materials and methods After determining the chemical composition of SSM, (Table 1) four diets were formulated with either 0.0, 50, 100 or 150 kg/t of SSM. These diets were isocaloric (12.12MJ/Kg) and isonitrogenous (215 g/kg CP). The main ingredients in control diet (1) were about corn (65%), SBM (27%) and fish meal (5%) . In the other diets (2,3,4), 5, 10 and 15 percent SSM were used respectively. In this experiment battery cages were used and in each cages 10 one - day old chicks were placed. During the experiment, feed intake, body weight gain, feed conversion ratio (FCR) and mortality were measured weekly. Data from this experiment were subjected to one-way analysis of variance and Duncan's range test by using Mstatc software (Mstat directory, 1990). There were 3 replicates of 10 chicks in each treatment.

Results Data are given in table 2. Use of SSM up to 100 g/kg in grower (3-6 weeks) and whole period (0-6 weeks) had statistically significant effects on feed intake, body weight gain ($p<0.05$). These data are in agreement with findings of Zatari *et al* (1990) and Vieria *et al* (1992).

Table 1 Chemical composition of SSM

nutrients	Kg/t
DM	915.3
CP	359.4
EE	12.1
CF	257.0
ASH	68.0
NFE	303.5
GE(MJ/Kg)	19.15
ME_n(MJ/Kg)	5.10
Ca	04.1
P	07.2
Na	00.2

Table 2 Effect of SSM on performance of broiler (0-6 weeks)

Treat	feed intake(g)	Body weight gain (g)	FCR
diet1 (0 kg/t SSM)	3560.3 a	1680.1 a	2.12 b
diet2(50 kg/t SSM)	3540.1 a	1623.0 a	2.18 ab
diet3(100 kg/t SSM)	3520.6 a	1641.0 a	2.15 ab
diet4(150 kg/t SSM)	3373.4 b	1516.0 b	2.22 a
S.E.M	32.68	25.78	0.02
F- value	6.77 *	7.41 *	1.97

Means with different superscripts in each column are significant different ($P<0.05$).

Conclusions The results of the present study demonstrate that about 30.5% SBM protein could be replaced by SSM protein without any adverse effect on growth and FCR. This is equal in use of 10% SSM in the diet which supplies about 3.6% of the dietary protein. Due to high crude fibre content, when SSM protein replaced about 46% of SBM, the growth was significantly impaired as compared to control birds. According to data obtained from this experiment use of SSM at the level of 10% is recommended for broiler ration.

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Effect of acute nutritional restriction on periovulatory oestradiol and IGF-I in beef heifers

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Introduction Severe negative energy balance (NEB) in the postpartum period of dairy cows may be associated with declining fertility but the mechanisms by which nutrition influences reproduction are complex, poorly understood and confounded by lactation. Hence, both chronic and acute nutritional restriction of beef heifers have been used as models to examine the effects of NEB on ovarian and endocrine responses in the absence of lactation. Plasma IGF-I concentrations gradually decreased until the onset of anoestrus (Stagg *et al*., 1999) but concentrations may be confounded with stage of the oestrous cycle, especially around ovulation (Mackey *et al*., 2000). Therefore, the aim of this study was to examine the effect of nutritional restriction on periovulatory oestradiol (E_2) and IGF-I concentrations.

Materials and Methods Eighteen individually fed beef heifers exhibiting regular oestrous cycles and with a mean (±SEM) liveweight and body condition score of 402±9kg and 2.8±0.1, respectively, were fed a grass silage and concentrate diet supplying the energy for 1.2 times maintenance (1.2 M) and synchronised using an intravaginal Controlled Internal Drug Releasing (CIDR) device with a 10 mg E_2 benzoate capsule for 8 days. One day before CIDR withdrawal, heifers were given an injection of synthetic prostaglandin to cause luteolysis of any existing corpora lutea present and allocated to feed allowances supplying the energy for either 0.4 M (n=9) or 1.2 M (n=9). Six days after ovulation of the dominant follicle (DF) present at CIDR removal heifers were given a second injection of prostaglandin to induce luteolysis and allow ovulation of the first DF of the subsequent oestrous cycle. All heifers were returned to a diet supplying the nutritional requirement for 1.2 M following emergence of the second follicle wave after CIDR withdrawal, after approximately two weeks. Ovarian scanning was conducted once daily throughout and twice daily during each periovulatory period. Blood sampling was conducted twice daily throughout and at four-hourly intervals during each periovulatory period. Plasma was assayed for E_2 and IGF-I by RIA. Hormone profiles were aligned to both day of di*et al*location and the day of highest E_2 concentration for each periovulatory period. Statistical analysis was conducted using PROC GLM with repeated measures, and regression analysis was used to establish the relationship between concentrations of E_2 and IGF-I at time of highest E_2 concentration (SAS, 1988).

Results In the first periovulatory period concentrations of E_2 increased in the period immediately prior to ovulation ($P<0.0001$) but there was no effect of diet ($P>0.10$). There was an interaction between diet and day on concentrations of IGF-I ($P<0.05$) where restriction to 0.4 M resulted in a more rapid decline following ovulation (Figure 1).

In the second periovulatory period (Figure 1) there was a significant diet x day interaction on the concentration of E_2 ($P<0.05$), but this was influenced largely by anovulation in 4/8 heifers restricted to 0.4 M where E_2 concentrations remained low ($P<0.01$). Where ovulation occurred there was no such interaction on concentrations of E_2. IGF-I concentrations in this period were affected by both diet ($P<0.05$) and day ($P<0.0001$), but there was no effect of diet where ovulation occurred ($P>0.10$).

At the time of highest E_2 concentration (day 0), there was a linear relationship between concentrations of E_2 and IGF-I ($P<0.01$; $R^2=0.27$) described by the equation IGF-I = 164.6 + 14.8 (E_2).

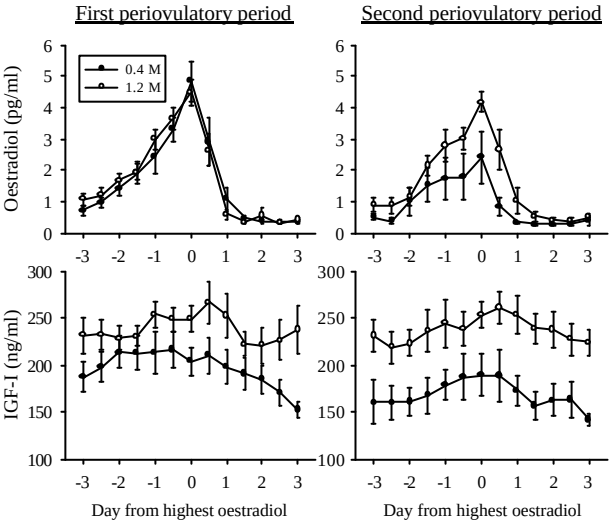

Figure 1 *Effect of diet on periovulatory oestradiol and IGF-I concentrations.*

Conclusions This study indicates that, like oestradiol, plasma IGF-I increases in the periovulatory period of cattle. However, both hormones are influenced by level of nutrition and are significantly lower preceding the onset of anovulation. Such effects indicate possible nutrition-reproduction interactions and may be important for studying negative energy balance in the postpartum period of high-yielding dairy cows.

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Glucose metabolism of *in vitro*-produced bovine embryos in cell-free and co-culture systems

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Introduction *In vitro*-produced (IVP) bovine embryos are used in a wide range of biotechnologies but develop less well than their *in vivo* counterparts and can give rise to foetal/neonatal anomalies after embryo transfer. The quality of bovine IVP embryos and the systems in which they are produced are traditionally assessed in terms of morphological and developmental criteria; namely, embryo grade and blastocyst formation rate. Lane and Gardner (1996) showed that mouse embryos selected for transfer on the basis of a low glycolytic activity (conversion of glucose to lactate), measured non-invasively, were 4 times more likely to implant than those selected randomly. Comparable data are not available for bovine embryos. The aim of this study was to assess linear glycolytic index of cattle blastocysts *in vitro* as a marker of viability. We have measured glucose consumption and lactate production by individual bovine IVP embryos grown in cell-free conditions and in a novel co-culture system (Orsi *et al*., 2000) involving confluent bovine oviduct epithelial cell monolayers on permeable supports. This preparation allows the epithelial cells to be fed by a nutritionally-rich medium via the physiological, basal, route, while the apical medium, containing the embryos, is more dilute, mimicking oviduct fluid.

Materials and Methods IVP bovine embryos were generated by fertilisation of *in vitro*-matured oocytes (n=493) from abattoir-derived ovaries (Thompson *et al*., 1996). Putative zygotes were cultured in groups in either synthetic oviduct fluid (SOFaaBSA), or amino acid and glutamine-supplemented KSOM (KSOMaaBSA). For epithelial cell/embryo co-culture, the basal medium was 1:1 DMEM:Ham's F12 supplemented with 5% serum; the apical medium was SOFaaBSA or KSOMaaBSA. Glucose consumption/lactate production by individual day 8 blastocysts (n=116) was determined microfluorometrically (Gardner and Leese, 2000) after short-term incubation (15 min) in 50nl KSOM drops containing 0.5mM glucose as the sole energy substrate.

Results Glucose uptake was significantly lower in co-culture than in cell-free culture, and independent of the medium used ($P<0.001$). Embryos grown in cell-free SOFaaBSA had a significantly higher glucose consumption than all other groups ($P<0.05$). Lactate production was significantly higher in KSOMaaBSA ($P<0.01$) than SOFaaBSA, regardless of culture system. Glycolytic index (proportion of glucose converted to lactate; %±SEM; Figure 1), for KSOMaaBSA and SOFaaBSA-derived embryos in cell-free culture was 89.3±8.0 and 50.5±3.6, respectively, and 96.1±9.1 and 92.0±14.7 for embryos grown in co-culture with KSOMaaBSA and SOFaaBSA, respectively. Glycolysis was significantly affected both by system and medium ($P<0.05$), with that for SOFaaBSA cell-free culture being significantly lower than for co-culture groups ($P<0.05$).

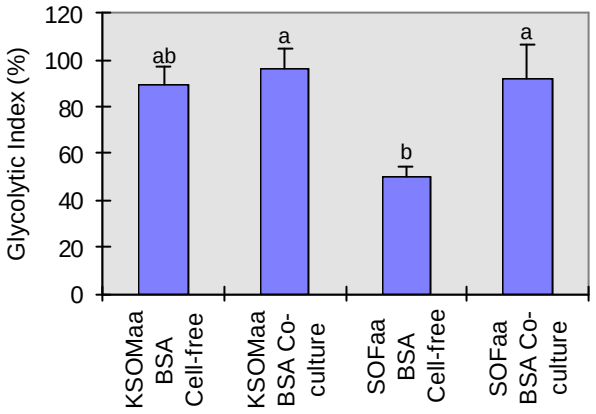

Fig. 1: Glycolytic index of bovine blastocysts generated in cell-free conditions and by co-culture in SOFaaBSA and KSOMaaBSA

Conclusions The metabolism of bovine preimplantation embryos is influenced both by the culture medium and the presence of somatic cells. Upregulation of glycolysis is a metabolic perturbation associated with cultured murine embryos under stress. On this basis, cell-free SOFaaBSA is the culture system which generates embryos with the least altered metabolism. Further refinement of this novel co-culture system on the basis of embryo biochemistry is required. Non-invasive assessment of embryo metabolism may allow selection of the most viable embryos for transfer as well as being a useful endpoint in the optimisation of *in vitro* production systems.

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A comparison of the fertility of Holstein Friesian and Norwegian Dairy Cattle under low and high nutrient input systems

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Introduction Until relatively recently, breeding programmes for Holstein Friesian (HF) dairy cattle have focused selection procedures on increasing milk output with little emphasis on secondary traits such as fertility. As a result the fertility of the Holstein Friesian dairy animal is currently declining in the UK. This decline in fertility causes particular problems in seasonal calving dairy herds, where compact calving is crucial to overall performance of this system. In the Norwegian dairy cattle (NC) breeding programme, emphasis has been placed on a multi trait selection procedure including fertility and disease resistance. There is evidence that this selection procedure has resulted in improved fertility within the NC population. The present study is part of an overall programme comparing the performance of HF and NC cattle with respect to food intake, animal performance, nutrient utilisation, behaviour, health, fertility and longevity. The objective of the present study was to investigate possible differences in fertility between the two breeds when offered either a high or a low nutrient input diet based on grass silage.

Materials and Methods Thirty-two HF heifers ($PIN_{(00)}$ £44) and thirty-two NC heifers (total merit index = 10.1) were purchased in Holland and Norway respectively and arrived at the Institute approximately one month prior to calving. The Dutch animals were representative of the top 1% of the UK HF population and the NR animals represented the top 5% of the NR cattle population in Norway. The heifers had a mean age at calving of 25.5 and 25.8 months for the HF and NC heifers respectively. The mean calving dates of the two groups of animals were 16 February and 10 February and the mean post-calving weights were 502 and 473 for the HF and NC heifers respectively. Post calving animals were blocked and allocated to either a low or high input system, based on grass silage (Keady *et al*. 2000). Milk samples were taken twice weekly for progesterone analysis from calving until the animals were confirmed in calf. Animals with prolonged anovulation post postpartum, determined by milk progesterone <3 µg/l for 45 days or more were given fertility treatments. The breeding season commenced on the 11th April and lasted for 12 weeks. To remove sire effects, animals across breeds and treatments were allocated to insemination with either HF or NC semen. No fertility treatments were given to animals for the first six weeks of the breeding season. After six weeks, fertility treatments were given to animals with no observed oestrus, to help maximise the number of animals in calf in the short breeding period.

Results Whilst small numbers of animals are involved in the present study, results indicate a trend towards fewer days to onset of luteal activity with the NC animals compared with HF animals (Table 1). There is also evidence that more of the HF animals had prolonged anovulation postpartum. No difference was observed in conception rate to first and second insemination, for either all the animals on the experiment, or those animals that had onset of luteal activity by day 45. Level of nutrient input did not appear to have any effect on the fertility parameters recorded.

Table 1 The effect of input system and genotype on reproductive performance

	Input Systems (IS)				Sem	Significance		
	High		Low					
Breed (B)	**HF**	**NC**	**HF**	**NC**		**B**	**IS**	**B*IS**
All animals								
Number of animals	16	16	16	16				
Days to onset of luteal activity	38.5	28.7	38.1	32.3	4.32	NS (P=0.078)	NS	NS
Days to first observed heat	49.6	49.5	50.2	37.9	4.73	NS	NS	NS
Days to first insemination	69.8	75.5	72.6	80.8	4.34	NS	NS	NS
Conception rate to first and second service (%)	87.5	87.5	75.0	68.8				
Number of animals with prolonged anovulation postpartum	6	3	7	4				
Animals not in calf at end of breeding period (number)	2	1	4	3				
Animals with onset of luteal phase by day 45								
Number of animals	10	13	9	12				
Conception rate to first and second service (%)	80.0	84.6	88.9	75.0				

Conclusions This study suggests that onset of cyclicity after calving occurred more quickly with NC than with HF cattle. Furthermore, there was a trend towards a lower incidence of prolonged anovulation with NC cattle. These effects may be related to reduced mobilization of body reserves of NC cattle in early lactation (Keady *et al*. 2000).

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The effect of breed and parity on the relationship between condition score and live weight in dairy cows

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Introduction Size, or its commonly used proxy live weight, is a necessary input when calculating the energy requirements of an animal. It is also a major factor in determining the intake capacity of an animal. The sole use of live weight as a determinant of size incorporates the implicit assumption that body fat and body protein mass are equivalent for the purposes of calculating energy requirements and intake capacity. Recent evidence indicates that this is not so in either case (Birnie et al., 2000; Friggens et al., 1998). The use of live weight may be acceptable where it can be reasonably assumed that there is a stable relationship between body fat and protein. However, when making breed or parity comparisons there is no reason to assume a stable relationship between body fat and protein. Meaningful comparisons can be made if live weights can be adjusted for differences in body fat content. In the applied context this means adjusting to a standard body condition score. This study provided the opportunity to examine the relationships between condition score and live weight in three breeds across three parities.

Materials and methods The data used for this analysis were collected within an on-going five year experiment carried out at the Danish Cattle Breeders Organisation farm, Ammitsbol Skovgaard. Animals enter the experiment as heifers 8 weeks before expected calving and remain in the experiment until mid way through second or third lactation. Three breeds are represented in the experiment; Holstein Friesian (HF), Danish Red (DR) and Jersey (J). The cows are housed throughout the year and fed total mixed rations based on whole crop wheat and a standard dairy concentrate. Milk yield, milk composition, and feed intake are recorded daily. The dataset used here comprised a total of 229 lactations with 110 HF, 69 DR, and 50 J, respectively. Live weights were recorded weekly throughout lactation and the dry period, condition score was measured 15 times per lactation cycle with fortnightly intervals around calving. Condition score was measured in half units on a scale of 1 to 5 (Kristensen, 1986 derived from Lowman et al., 1976). Using all condition score records and the associated live weight measurements, estimates of the kg live weight associated with a unit change in condition score were obtained from a mixed model for live weight in which condition score was fitted. Cow was fitted as a random effect and fixed effects of breed and parity were also included.

Results There was a highly significant relationship between live weight and condition score ($P<0.001$). There were significant effects of both breed ($P<0.001$) and parity ($P<0.001$) on the intercept of the relationship between live weight and condition score (Table 1). There was no significant effect of breed on the slope of the relationship. There was a significant difference in slope between first and subsequent parities ($P<0.05$; Table 1). There were no significant interactions between breed and parity.

Table 1 Predicted mean values of coefficients for the relationship between live weight and condition score as affected by breed and parity. Coefficients are expressed as the marginal change relative to a Jersey cow in third lactation. Standard errors are shown in parentheses.

	Breed			Parity		
	J	DR	HF	1	2	3
Intercept (kg)	366	+175	+200	-52	-27	+0
	(11.1)	(10.2)	(9.3)	(13.6)	(5.3)	
Slope (kg/unit CS)	31	+0	+0	-9.0	+0	+0
	(2.6)			(3.9)		

Conclusions The results of this study provide the necessary information to permit live weight to be adjusted to a standard degree of fatness, allowing for breed and parity effects. As expected, there was an increase in kg live weight per unit condition score corresponding to an increase in size with increasing parity. Contrary to expectations, there was no effect of breed on kg live weight per unit condition score despite clear size differences between the breeds. An explanation for this lack of breed effect could be that differences in internal fat distribution between the breeds cancelled out the expected size effects for the breeds used in this study.

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Genetic correlations between 305-day and monthly test day milk yield records in primiparous Iranian Holsteins

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Introduction Traditionally, in most dairy cattle breeding programmes genetic evaluation of dairy sires and cows has been primarily based on 305-day lactation yield. To provide 305-day lactation yields many partial lactations have to be extended by adjustment factors resulting in overestimation or underestimation of 305-day yields which in turn leads to biased prediction of breeding values. Over the past decade there has been a considerable interest in using monthly test day records instead of 305-day lactation yield to predict breeding values of dairy cattle as early as possible and also to increase genetic gain through reducing generation interval. The main objective of present research was to estimate the genetic correlations between 305-day and monthly test day milk yields in Iranian Holstein dairy heifers.

Material and Methods Data comprised 53673 monthly test-day milk records from 6101 Iranian Holstein dairy heifers calving between 1983 and 1995 distributed in 174 herds from different climatic regions of Iran. Milk records were adjusted for the heterogeneous variance using the procedure outlined by Ibanez *et al*., (1996). Genetic correlation between 305-day and monthly test day milk yields were obtained by using a multivariate restricted maximum likelihood procedure under an animal model. In the model for 305-day lactation yield, herd-year-season of calving (CG_{ik}, fixed), calving age ($Cov1_{ijkl}$, covariable) and lactation length ($Cov2_{ijkl}$, covariable) were fitted to account for environmental factors. For monthly test day yields, the effects of herd-year-season of production (CG_{ik}, fixed), age at test day ($Cov1_{ijkl}$, covariable) and days in milk ($Cov2_{ijkl}$, covariable) were fitted. Furthermore, for both traits the random effect of cows (a_{jk}) was also included to take account of direct additive genetic effect. The animal model was as follows:

$$y_{ijkl} = CG_{ik} + \sum_{R=1}^{2} b_R * (Cov1_{ijkl})^R + \sum_{R=1}^{2} d_R * (Cov2_{ijkl})^R + a_{jk} + e_{ijkl}$$

Results Genetic, phenotypic and environmental correlations between 305-day and monthly test day milk yields are presented in Table 1. In general, genetic correlation between 305-day and monthly test day milk yield increased from the first stages of lactation towards the middle of lactation then decreased as lactation stage advanced. The same pattern was also obtained for the phenotypic and environmental correlations which were generally smaller than corresponding genetic correlations at the same monthly test day. The results in the present study are in close agreement with those obtained by Pander *et al*., (1992).

Table 1 Genetic (r_g), phenotypic (r_p) and environmental (r_e) correlations* between 305-day and monthly test day milk yields (correlations within parameters with similar superscript are not significantly different from each other at P<0.05)

	TD1	*TD2*	*TD3*	*TD4*	*TD5*	*TD6*	*TD7*	*TD8*	*TD9*	*TD10*
r_g	0.90^{a}	0.96^{b}	0.96^{b}	0.99^{c}	0.94^{d}	0.97^{e}	0.97^{e}	0.96^{b}	0.96^{b}	0.94^{d}
r_p	0.57^{a}	0.72^{b}	0.80^{c}	0.83^{df}	0.84^{de}	0.85^{e}	0.84^{de}	0.82^{f}	0.80^{c}	0.75^{g}
r_e	0.54^{a}	0.68^{b}	0.76^{c}	0.78^{d}	0.82^{e}	0.80^{f}	0.80^{f}	0.78^{d}	0.75^{c}	0.68^{b}

* All correlation coefficients are significant at P<0.05

Conclusion With respect to the high positive genetic correlations between 305-day and monthly test day milk yields it can be concluded that these traits are controlled by the same genes. This is of great value in dairy cattle breeding programmes as genetic evaluation of dairy cattle can be changed to using monthly test day records rather than 305-day lactation yield. By using monthly test day records not only are the effect of environmental factors on milk yield more accurately taken into account but also there will be no need to use extension factors for projecting partial lactations. More interesting is that the costs of breeding programmes are reduced as the selection decision is based upon the predicted breeding values obtained from test day model which is of particular importance for young bulls which can be selected earlier in breeding programmes.

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The Effect of Feeding Calcium Soaps of Fatty Acids on the Reproductive Physiology of Lactating Dairy Cows

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Introduction Although it is not a substantial constituent of dairy cattle feed, there has been much work on the effects of feeding supplemental fat on reproductive performance. Supplemental fat increases the energy concentration of the diet and may lessen the effects of the negative energy balance post calving. Adding fat has been reported to positively influence reproduction in a number of ways, for instance increasing plasma progesterone concentration and increasing the life span of the corpus luteum (for a review Staples *et al*., 1998). A number of studies have demonstrated the importance of progesterone in early pregnancy and in particular the timing and strength of the post ovulatory progesterone rise. The aim of this study was to establish the effect of a dietary supplement of fat, in the form of calcium soaps of fatty acids, on reproductive function and in particular on post ovulatory progesterone levels in lactating dairy cows.

Materials and methods Thirty multiparous Holstein-Friesian cows from the University of Nottingham's commercial dairy herd calving October to December 1999, were assigned to one of three groups, which were balanced according to time post partum (46 ± 4 days µ±se). A control group (n=10) received a total mixed ration containing grass silage, maize silage, brewers grains, bread dough, barley mineral mix and cereal soya. In addition two further groups were fed supplemental fat (calcium soaps of fatty acids, Megalac®, Volac Ltd.) at either 750g/cow/day (high group, n=10) or 375g/cow/day (low group, n=10). Throughout the thirteen-week trial period twice-weekly milk samples were collected to monitor normality of reproductive cycles (p.m., Monday and Friday). Samples were also collected on days 4, 5 and 6 following 1st AI (day of AI=day 0). Milk was assayed for progesterone by ELISA (Ridgeway Scientific). Progesterone profiles were used to define cycle problems (delayed onset of cyclicity – progesterone < 5ng/ml until > 65 days post partum; cessation of cyclicity – progesterone < 5ng/ml for > 2 weeks following a period of > 5 ng/ml; luteal cysts – progesterone > 5ng/ml for > 3 weeks). Body weight and condition score were recorded weekly for the first four weeks and then fortnightly during the remaining trial period. A blood sample was taken on day 5 following 1st AI for progesterone radioimmunoassay. On three consecutive days mid-trial feed intake was recorded on a group basis. All data were analysed by two-way analysis of variance using Genstat 5 (Lawes Agricultural Trust, 1997).

Results Fat supplementation did not affect energy balance, with results for body condition score and weight change showing little difference between groups. The high group did, however suffer greater ($P<0.05$) weight loss for a short period (15d) at the beginning of the trial compared with the control animals. Feed consumption was not significantly different among the groups. In terms of reproductive responses a similar lack of variation between groups was observed. No effect was observed in conception rate to 1st service or with problem cycles. Post ovulatory plasma progesterone concentrations (d5) showed a tendency to increase with fat supplementation, however differences were not significant and did not continue further into the cycle.

Table 1 Comparison of parameters in the three treatment groups.

	Feed Intake (kg/cow/day) (µ±se)	Day 5 Plasma Progesterone (ng/ml) (µ±se)	Day 5 Milk Progesterone (ng/ml) (µ±se)	Milk Yield (l/day) (µ±se)	Cows with Normal Cycles	Cows Conceived to 1st AI
High	58.9 ± 2.5	1.9 ± 0.3 (n=10)	4.4 ± 0.7 (n=10)	41.0 ± 1.1 (n=10)	5 (n=10)	4 (n=10)
Low	54.3 ± 2.1	1.6 ± 0.4 (n=7)	4.3 ± 0.7 (n=7)	38.7 ± 2.0 (n=10)	6 (n=10)	2 (n=10)
Control	57.7 ± 2.0	1.2 ± 0.2 (n=7)	4.2 ± 0.4 (n=6)	37.2 ± 2.5 (n=10)	6 (n=10)	4 (n=10)
Significance	NS	NS	NS	NS	NS	NS

Conclusion In conclusion, the current results show that a dietary supplement of calcium soaps of fatty acids did not affect either milk or plasma progesterone. Furthermore no variation was seen in conception rate or cycle abnormalities. The energy balance of the animals measured through body condition score and weight change, also showed no major effects of fat supplementation.

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Comparison of changes in peripheral plasma inhibin in relation to estrous cycle between cows and buffaloes

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Introduction Lower reproductive efficiency of buffalo than that of cattle hampers efficient utilization of tropical buffaloes. Heat detection in buffaloes poses problem because overt signs in buffaloes are of less intense and of shorter duration than in cattle. Moreover, buffalo tends to exhibit overt signs during night or early morning and most farmers are ignorant of physical signs of estrus. Inhibin, a glycoprotein hormone produced by ovarian granulosa cells, suppresses production and / or secretion of FSH through negative feedback effect at pituitary level (Burger, 1992). There is no information available on comparison of peripheral plasma inhibin profiles in Sahiwal cows and Murrah buffaloes reared under identical feeding and management condition. The present study was, therefore, designed to compare inhibin profiles in Sahiwal cows and Murrah buffaloes reared under tropical condition.

Materials and methods Non-pregnant, non-lactating Sahiwal cows and Murrah buffaloes (5 each ; 5-6 year old having body wt. between 550-600 kg) were selected from National Dairy Research Institute animal herd and were fed on the same standard feeding schedule as practised in the general herd of the institute. Blood samples were collected once daily for thirty two days during winter months of January and February in heparinised tubes through jugular venipuncture. Plasma was harvested and stored frozen at - 20^0C. Estrus was detected by visual symptoms, parading a vascetomized bull twice daily and confirmed by plasma progesterone estimation (Kamboj and Prakash, 1993). Inhibin levels were measured by double antibody RIA (Palta et al., 1996). The sensitivity of inhibin assay was 16 pg/tube. The intra- and inter-assay coefficients of variation were 11.0 and 13.2, respectively. The differences in inhibin profiles between species and stages of cycle were studied by ANOVA.

Results Out of five cows, two animals exhibited anestrus based on progesterone profiles and hence excluded from analysis. The pattern of plasma inhibin were quite similar in cows and buffaloes, with peak being exhibited on Day 2 prior to estrus and minimum values on Day 9 of the cycle in buffaloes and on Day 11 of the cycle in cows. Peripheral inhibin levels in cycling cows increased from 0.47 ± 0.07 ng/ml on Day 4 prior to estrus to reach a maximum concentration of 0.59 ± 0.03 ng/ml on Day 2 prior to estrus. Thereafter the mean inhibin levels showed a decline to reach a low of 0.40 ± 0.01 ng/ml on day 11 of the estrous cycle. In buffaloes peripheral inhibin concentrations increased from 0.38 ± 0.04 ng/ml on Day 4 prior to estrus to reach a maximum concentration of 0.52 ± 0.05 ng/ml on Day 2 prior to estrus. Then mean inhibin levels showed a decline to reach a low of 0.29 ± 0.03 ng/ml on Day 9 of the cycle in buffaloes. In cows and buffaloes, peripheral inhibin concentrations which were lowest (0.43 ± 0.02 and 0.34 ± 0.02 ng/ml, respectively) during midluteal phase of estrous cycle increased ($P < 0.05$) to 0.52 ± 0.03 and 0.44 ± 0.04 ng/ml, respectively, during late luteal phase and then further to the highest value of 0.53 ± 0.02 and 0.49 ± 0.04 ng/ml, respectively, during periestrus phase, following which these declined to 0.50 ± 0.02 and 0.39 ± 0.03 ng/ml, respectively, during early luteal phase. The overall mean inhibin levels in cows were 0.47 ± 0.01 ng/ml compared to 0.38 ± 0.02 ng/ml in buffaloes.

Conclusion The results of the study demonstrate that inhibin levels were significantly higher ($P<0.01$) in cows compared to buffaloes throughout the cycle. It is observed that the number of follicles in buffaloes are less in comparison to cows (Taneja et al., 1988). It has also been reported that superovulatory treatment in buffaloes have led to fewer number of ovulations as compared to cows (Taneja et al., 1988; Madan et al., 1990). Since inhibin secretion is correlated with growth and development of antral follicles, lower inhibin levels in buffaloes could be due to fewer number of antral follicles in that species.

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Synchronisation of oestrus using a 14 day progestagen sponge treatment in the absence of a corpus luteum does not reduce fertility in ewes

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Introduction Progesterone and progestagens are widely used to synchronise oestrus in sheep, however 15 to 30 % of ewes fail to maintain a pregnancy following the first service after oestrous synchronisation and the reason(s) for this failure rate is unclear. In commercial flocks, the progestagen treatment commences at a random stage of the oestrous cycle and in some ewes luteolysis may occur soon after treatment commences, leaving the ewe solely dependent on the exogenous source of progestagen which promotes the development of persistent follicles (Flynn *et al*., 2000). These persistent follicles may be up to 14 days of age when they ovulate whereas the age of follicles at ovulation in natural cycles is 4 to 8 days. In cattle, it is clear that ovulation of these old follicles results in a decrease in fertility. The aim of this study was to examine the effect of ovulation of aged follicles on fertility in cyclic ewes by using treatments based on a 14-day, progestagen synchronisation protocol that is known to produce persistent follicles.

Materials and methods During the breeding season, commercial mule ewes were assigned to Group S (n = 98) or Group M (n = 100). All ewes were given an intravaginal sponge containing 60 mg medroxyprogesterone acetate (Veramix sponge, Upjohn) on Day 6 of a presynchronised cycle and were then given a luteolytic dose of prostaglandin (0.5 ml i.m., Prosolvin, Intervet). The sponge was left in place for 14 days in ewes in Group S (single sponge) but was replaced with a new sponge on Days 11 and 16 in ewes in Group M (multiple sponges). The sponge was removed from all ewes on Day 20. These treatments were designed to result in the ovulation of **aged follicles in Group S** and **follicles of normal age in Group M** (Flynn *et al*., 2000) and this was confirmed in a parallel study using ultrasonography. Raddled entire rams were introduced to the ewes 48 hours after sponge removal at a ratio of 1:10. In the 3 hours immediately after ram introduction each ram mount was observed. The number of ewes mated was determined at 3, 6, 24 and 72 hours and the rams were withdrawn after 72 hours. In order to determine which ewes failed to conceive, raddled entire rams were reintroduced to the ewes 14 days after the first service. Date of lambing and number of lambs born was recorded. Data were analysed using the general linear models or the categorical modelling procedure in SAS.

Results There were fewer ewes bred in Group M at 3 and 6 h after ram introduction than in Group S (Table 1) and the rams mounted ewes in Group S more frequently than ewes in Group M ($P<0.05$). By 180 minutes after introduction of the rams 73 ewes in Group S had been mounted 591 times and 38 ewes in Group M had been mounted 220 times. There was no difference between groups (Table 1) in the proportion of ewes which stayed pregnant to first service or lambed to first service. Similarly, there was no difference among groups in the number of lambs born per ewe, the proportion of ewes lambing to repeat services or the proportion of ewes that were barren or aborted. When the time of first mating was considered for the ewes that failed to remain pregnant to first service, a higher proportion of ewes failed to remain pregnant as the time to first service increased ($P<0.05$) but there was no difference between groups.

Table 1
Time of first mating and success of pregnancy. Values in rows with no common superscript are different ($P<0.05$).

	Group S	Group M		Group S	Group M
Ewes synchronised	98	100	Ewes that did not repeat (pregnant Days 14 to 40)	86^{x}	81^{x}
Ewes bred by 3 h	73^{x}	38^{y}	Ewes lambing to first service (lambed Day 142 to 152)	84^{x}	79^{x}
Ewes bred by 6 h	86^{x}	58^{y}	Repeat breeding ewes lambing to subsequent services	11^{x}	18^{x}
Ewes bred by 24 h	98^{x}	93^{x}	Ewes that were barren or ewes that aborted	3^{x}	3^{x}
Ewes bred by 72 h	98^{x}	99^{x}	Lambs per ewe (mean ± sem)	2.06 ± 0.08^{x}	1.99 ± 0.06^{x}

Conclusions The present experiment shows that (within the confines of a 14-day oestrus synchronisation protocol) the ovulation of prolonged lifespan follicles does not compromise fertilisation rates, litter size or the birth of live lambs. Ewes that ovulated older follicles came into oestrous earlier than ewes that ovulated younger follicles, presumably due to the older follicles producing more oestradiol. However even when considering only ewes bred during the first three hours after ram introduction, the proportion of ewes remaining pregnant to first service did not differ between groups which ovulated older or younger follicles. We conclude that when luteolysis occurs at the beginning of progestagen synchronisation, ewes will ovulate aged follicles but that this does not have an adverse effect on fertility.

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Improving lamb performance from Welsh Mountain Sheep breeding groups

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Introduction: Sheep group breeding schemes have gained in popularity in recent years. The objectives of group breeding schemes are to improve desirable traits such as carcass conformation, weight and maternal ability whilst maintaining local type. However, if group breeding schemes were to work together then a larger genetic pool of performance recorded animals would be available to facilitate improvements. The objectives of this project were therefore twofold, 1.) was to improve traits such as weight and carcass conformation and 2.) to demonstrate the benefits of cooperative groups working together. In this project, group breeding schemes from North and Mid Wales have been working together with ram performance testing groups from North, South and Mid Wales to improve results from Welsh Mountain breeding schemes.

Methods: The project was conducted in two phases. During Phase 1, sires and dams from established group breeding schemes in North, South and Mid Wales were cross-mated (using MOET). Two rams and 12 ewes were obtained from each of three established group breeding schemes (CAMDA, CAMP and Llysfasi) and one ram was obtained from each of three ram performance testing groups (Bangor, Ceredigion and Rhayader). A total of 9 sires and 36 embryo donors were used each year for two years. The ewes were super-ovulated, and allowed to mate with a selected sire. Eggs were subsequently flushed at 6 days after mating and implanted to recipient Welsh mountain ewes. The recipient flock was maintained at Pwllpeiran. Performance of the Phase 1 progeny was monitored and the results have been previously reported (Ap Dewi *et al*, 2000). In Phase 2, six ram lambs from phase 1 were selected as breeding rams. Three of the rams were pure-bred (i.e. within schemes) and three others were inter-bred (between schemes). The rams were jointly selected by members of the co-operative groups and were considered acceptable to be used by all groups. Each ram was then mated to 50 Welsh Mountain ewes. Performance of the Phase 2 progeny was monitored from birth through to weaning for all lambs. Finished weights and carcass weights were also recorded for all male progeny. The collected data was then analysed by a one-way ANOVA where only variance due to ram type (pure-bred or cross-bred) was identified.

Results: Liveweight records were obtained for 288 lambs from Phase 2 (year 1). The mean liveweights for lambs born to either a pure-bred ram or an inter-bred ram are given in Table 1. Lambs from inter-bred rams were significantly heavier ($P<0.01$) at birth (3.78 kg), shearing (17.83 kg) and weaning (27.48 kg) compared to lambs from pure-bred ram (3.47, 16.35 and 25.72 kg for birth, shearing and weaning respectively). Mean finished and carcass weight of males are also given in Table 1. Male lambs from inter-bred rams were significantly heavier ($P<0.001$) at finishing than those from pure-bred rams (30.60 *vs* 28.22 kg respectively). Carcass weights were also significantly heavier ($P<0.01$) for male lambs produced from inter-bred rams than those from pure-bred rams (12.94 *vs* 11.93 kg respectively).

Table 1: Mean birth, shearing and weaning weight (kg) for all lambs produced from either pure bred rams or inter-bred rams along with the mean finished weight and carcass weight of male lambs produced from either pure-bred rams or inter-bred rams

	Pure bred	Inter bred	sed	SIG
Birth weight	3.47	3.78	0.088	***
Shearing weight	16.35	17.83	0.496	**
Weaning weight	25.72	27.48	0.483	***
Finished weight	28.22	30.60	0.684	***
Carcass weight	11.94	12.93	0.335	**

Conclusion: Inter-bred rams produce heavier lambs at birth and this difference in weight was maintained at shearing and weaning. Consequently, lambs from crossbred rams were heavier at finishing and had heavier carcass weights. These results suggest that significant genetic improvement can be achieved within breeds over a short period of time if a large genetic pool of performance recorded animals are used in selective breeding programmes. This can be achieved by co-operative groups working together.

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Genetic analysis of birth weight and related traits in Dorset Down and Hampshire Down sheep

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Introduction In recent years genetic improvement programmes for sheep in the UK have been primarily directed towards the improvement of growth and carcase traits. The possible correlated responses in other production traits, that may not be of such obvious economic importance, have not been fully assessed. Birth weight is such a trait.
Although of no direct economic importance, birth weight is an important trait in sheep production. There is a known relationship between birth weight and lamb survival, with lambs of both low and very high birth weight being at greater risk. There have, however, been very few genetic studies of the trait. The aim of this study, which is part of a larger project covering a number of breeds, was to estimate the direct and maternal heritability of birth weight and its genetic correlations with 8 week weight in the Dorset Down and Hampshire Down breeds. In addition the genetic trend in birth weight over a fourteen year period is assessed.

Materials and Methods The data used in the study were collected as part of the MLC/Signet Sheepbreeder service over the period 1985 to 1998 and were from commercial pedigree flocks. 6100 and 1945 individual animal records, representing the progeny of 148 and 71 sires were available for the Dorset Down (DD) and Hampshire Down (HD), respectively. The records included birth weight and adjusted 8 week weight. Pedigree information was available for all animals. The data was initially analysed using a sire model to identify significant fixed effects, interactions and covariates. It was then analysed with ASREML (Gilmour, Cullis, Wellham and Thompson, 1999) using an animal model, including the maternal genetic effect and permanent and temporary (litter) environmental effects of the dam, where significant. A bivariate model was fitted to estimate the genetic correlation between birth weight (Bwt) and 8 week weight (8wt). The genetic trend in birth weight was estimated by regressing the mean estimated breeding value (EBV) against year of birth, for both direct and maternal effects.

Results The results of the analysis of Bwt and 8wt are summarised in Table 1. The maternal genetic effect was significant for both Bwt and 8wt in both breeds. The temporary environmental effect of the dam, or litter effect, was also quite large for both traits in both breeds. The permanent environmental effect of the dam was less important.

Table 1 Summary of variance ratios (±s.e.)

	Birth weight		8 week weight	
	DD	HD	DD	HD
h^2_a	0.09±0.026	0.22±0.074	0.08±0.026	0.11±0.057
h^2_m	0.19±0.033	0.15±0.037	0.04±0.021	0.17±0.035
c^2	0.08±0.024	-	0.07±0.020	-
t^2	0.17±0.019	0.23±0.037	0.23±0.025	-

h^2_a: direct genetic variance/ phenotypic variance
h^2_m: maternal genetic variance/ phenotypic variance
c^2: permanent environmental variance/ phenotypic variance
t^2: temporary environmental variance/ phenotypic variance

Table 2 Phenotypic and genetic correlations (±s.e.)

	DD	HD
r_p	+0.41±0.019	+0.35±0.036
r_{ad}	+0.23±0.137	+0.52±0.170
r_{am}	+0.51±0.091	+0.24±0.197

r_p phenotypic correlation,
r_{ad} genetic correlation for direct effect on birth weight
r_{am} genetic correlation for maternal effect on birth weight

Both phenotypic and genetic correlations were positive and moderate in both breeds. There was a small positive, but significant ($P<0.001$), genetic trend in the maternal effect on birth weight for the Hampshire Down breed of 7g per year (±1.2**)** , but no significant trend in the direct genetic effect. In the Dorset Down there was not a significant genetic trend in either direct or maternal effects.

Conclusions Birth weight is a trait with a moderate heritability for which maternal genetic effects are important. Both direct and maternal genetic effects on birth weight are positively correlated with later weights, however, there have been only small genetic changes in birth weight since 1985 in the Dorset Down and Hampshire Down breeds. The results of this study suggest that although birth weight is likely to increase as a consequence of selection for improved growth, the changes are likely to be small and beneficial, resulting in a decrease in the number of low birth weight lambs that are born.

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The effect of crossbred ewe type and ram genotype on lamb output and carcass quality

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Introduction In view of the stratified nature of the sheep industry, changes in breeding policies in the hill sector impinge on the performance of the lowland sector. Carson *et al* (2000) investigated the effect of choice of ram and ewe breed on lamb output and carcass quality in the hill sheep sector. First cross (F1) ewes were produced as a result of crossing Scottish Blackface and Cheviot ewes with a range of ram breeds. The primary objective of the present study was to provide information on the performance of these crossbred females in terms of lamb output and carcass quality and thus determine the impact of changing hill genetics on all strata of the sheep industry. Within the terminal sire breeds significant improvements in lean growth potential have been made through genetic improvement schemes. The secondary objective of this study was to provide information on the effects of using high lean growth index rams on lamb growth and carcass quality.

Material and methods The experiment was carried out over a period of three years on lowland farms (n=4 year 1, n= 5 year2) located across Northern Ireland. Four crossbred ewes types were obtained from the study by Carson *et al* (2000) were used. These were Blue Leicester X Scottish Blackface (BLXB), Texel X Blackface (TXB), Suffolk X Wicklow Cheviot (SXC) and Texel X Wicklow Cheviot (TXC). On each farm, in each year of the study groups of 20 of each crossbred ewe type were allocated to one of four mating groups balanced for liveweight and condition score. The mating groups comprised Suffolk and Texel rams. Within each of these ram breeds there were high lean growth index rams obtained from Sire Referencing Schemes and rams purchased from pedigree sales and selected on the basis of visual appearance (Control). Over the course of the study a total of 14 high lean index and 15 control rams were evaluated. Lambs were weighed at birth, at regular intervals until weaning and were then slaughtered at 36, 44 and 52 kg. Due to the unbalanced nature of the experimental design, the data was analysed using the Genstat REML (Residual Maximum Likelihood) procedure. This fitted fixed effects for farm and the various ram x ewe breed combinations.

Results Over the three years of the study BLXB (Mule) ewes were more prolific and produced a greater output of weaned lamb (P<0.001) than the other three crossbred ewe types. TXB, SXC and TXC ewes had similar levels of prolificacy and produced similar weights of weaned lamb. Lambs from Mule ewes had a poorer conformation classification (P<0.001) than lambs from the other three crossbred ewe types. Fat depth over the *L. dorsi* was lower in lambs from TXC ewes compared with the other crossbred ewe types (P<0.05). Lambs from high lean index rams reached a cold carcass weight of 19 kg 14 days earlier than the control rams (P<0.01) and were of a better conformation classification (P<0.05). Lambs from Texel rams had a greater dressing proportion and conformation classification score than lambs from the Suffolk rams (P<0.001).

Table 1 The effect of crossbred ewe type and ram genotype on lamb output and carcass quality

Crossbred ewe type‡	No lambs born per ewe lambed	Output of weaned lambs/ewe lambed (kg/ewe)	Days to slaughter†	Dressing proportion (g/kg) †	Conformation classification†	Fat depth over the *L. dorsi* (mm) †
BLXB	1.73[b]	46.5[b]	205	436	2.75[a]	2.5[b]
TXB	1.47[a]	39.6[a]	208	443	3.15[b]	2.4[ab]
SXC	1.46[a]	39.4[a]	208	440	3.04[b]	2.6[b]
TXC	1.41[a]	38.2[a]	207	442	3.12[b]	2.2[a]
Sem	0.032	1.33	4.4	2.3	0.046	0.10
Significance	***	***	NS	NS	***	*
Ram genotype						
High lean index	1.37	35.0	200	441	3.06	2.4
Control	1.43	34.9	214	440	2.97	2.4
Sem	0.029	1.09	3.2	1.7	0.034	0.07
Significance	NS	NS	**	NS	*	NS
Suffolk	1.40	34.9	206	433	2.89	2.5
Texel	1.40	35.0	208	448	3.14	2.4
Sem	0.027	1.16	3.1	1.6	0.032	0.07
Significance	NS	NS	NS	***	***	NS

† Results adjusted to a constant cold carcass weight of 19 kg; ‡ prolificacy and weaned lamb output mean results of ewe lambs, hoggets and mature ewes; carcass quality mean results of ewe lambs and hoggets; #mean results of ewe lambs and hoggets

Conclusions Mule ewes were the most prolific of the four crossbred ewes examined. However, the lambs were of poorer conformation classification. The use of high lean index rams improved conformation classification of the lamb carcass and reduced the length of time to reach slaughter weight by up to 2 weeks. The use of Texel rams improved dressing proportion and conformation classification compared with Suffolk rams.

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Effect of long and short-term protein nutrition on the metabolic status, body composition and reproductive performance of gilts

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Introduction Reproductive failure is a source of major economic loss to the UK pig industry, accounting for 0.5 of all first parity gilt cullings (MLC Pig Year Book, 1995). Previous research (Cameron *et al*, 1999) has shown that rearing gilts on a diet formulated to support maximal protein deposition has beneficial effects on ovulation rate at 3rd oestrus. The aim of the current experiment was to develop further the previous model to assess long and short-term effects of protein nutrition on reproductive performance, body composition and metabolic status.

Materials and Methods Two Diets based on Wheat, Barley, Soya, Fishmeal and Premix were formulated; Diet 1 to meet the nutrient requirements for maximal protein deposition rate (PDR) and Diet 2 for a PDR 0.8 of maximal. 8 x 50 kg live weight commercial white hybrid gilts were allocated to one of four dietary treatments, with dietary cross-overs of group 3 and 4 taking place on week 6 (See tables 1a and b). Diets were fed individually on a twice daily basis with feed allowances adjusted weekly as determined by dietary treatment (Diet 1 being fed to previously obtained feed intake data for the genotype when achieving maximal PDR and diet 2 being fed according to assumed live weight using the function $FI = 0.01W^{0.75}$). Pre and post – prandial blood samples were taken over 5 days from 4 gilts per treatment at 3rd oestrus for analysis of Blood Urea Nitrogen (BUN) and Glucose. A nitrogen balance trial was carried out on the remaining 4 gilts per treatment in order to determine differences in nitrogen retention between diet 1 and diet 2, with 3 x 5 day total collections on weeks 4, 8 and 12. All gilts were slaughtered at 12 days post 3rd oestrus. P2 backfat depth and *Longissimus dorsi* area were measured as an indication of body composition and *Corpora lutea* counted as a measure of ovulation rate . Data were statistically tested using one and two–factor ANOVA as appropriate**.**

Table 1a *Nutrient specification of diet 1 and diet 2*

Diet	1A	1B	1C	2A	2B	2C
Week of trial	1 to 4	5 to 8	9 to 12	1 to 4	5 to 8	9 to 12
CP (g/kg)	200	167	147	169	118	100
DE (g/kg)	14.6	14.4	14.2	13.3	13.3	13.1
LYS (g/kg)	12.7	10.4	9.5	10.5	7.6	6.4

Table 1b *Experimental design*

Group 1	Group 2	Group 3	Group 4
Diet 1	Diet 2	Diet 1/2	Diet 2/1

Results. Highly significant effects of diet on glucose ($P < 0.001$) and BUN ($P < 0.001$) profiles were found, whilst nitrogen retention data showed that gilts fed diet 1 retained significantly more nitrogen than those fed diet 2 ($P < 0.01$) (Table 2). No significant effect of diet on growth rate or body composition was found. Ovulation rate was shown to differ with dietary treatment (Table 3), although due to variation within group 1, the results were not significant.

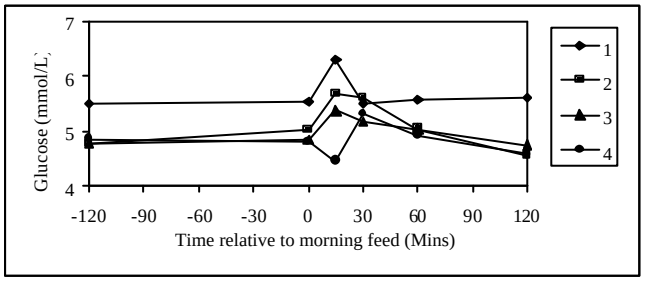

Figure 1 *Pre and Post-Prandial Blood Glucose Profiles*

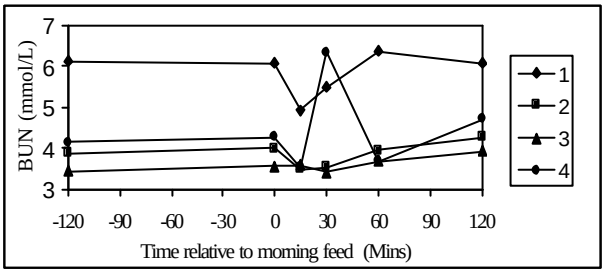

Figure 2 *Pre and Post-Prandial BUN Profiles*

Table 2 *Effect of dietary treatment on nitrogen retention*

N retention (g)	Diet 1	Diet 2	s.e.d	Fpr
Collection 1	187.4	152.1	4.1	<0.001
Collection 2	233.7	184.8	12.4	0.009
Collection 3	246.4	158.7	8.04	<0.001

Table 3 *P2 depth, L.dorsi area (LD) and Ovulation rate(OR)*

	Diet 1	Diet 2	Diet 3	Diet 4	s.e.d	Fpr
P2 (mm)	17.2	16.1	15.5	17.4	1.46	0.62
LD (cm^2)	43.0	46.3	44.2	41.4	3.06	0.45
OR	14.9	12.8	14.1	14.2	1.09	0.55

Conclusion Although not significant, ovulation rate data showed trends, with optimal results when gilts are fed to achieve a maximal protein deposition rate as compared to 0.8 of maximal, and with short term increases and decreases in dietary protein having only an intermediate effect. Blood urea nitrogen and glucose data indicate a more favorable metabolic state in gilts reared for maximal protein deposition rate, which in turn is responsible for improved reproductive performance. However, no effect of body composition on reproductive performance was found.

Acknowledgements The support of the MLC is gratefully acknowledged.

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In vitro differentiation of a cloned bovine mammary epithelial cell

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Introduction The accurate replication *in vitro* of bovine mammary gland function would be of enormous benefit to studies investigating the control of milk synthesis and secretion. However, progress towards this end has been slow, partially because of the biological variability in the cell preparations produced to date. In particular, the heterogeneous nature of primary cultures of mammary epithelial cells (MEC) makes them unstable for repeated experimentation over time, while for immortalised cell lines *in vitro* the level of milk protein production is either very low or non existent; if it does occur it has been independent of *in vivo* factors known to regulate mammary growth and milk secretion. The aim of this project was to establish a bovine MEC non-immortal clone, which is both responsive to mitogens and functionally responsive to lactogenic hormones and an appropriate extracellular matrix.

Materials and methods Mammary tissue from a 200-day pregnant Holstein cow was minced and digested using type II collagenase (600 units/ml) in Dulbecco's modified Eagle's medium (DMEM) containing 10% foetal calf serum (FCS). The resultant cell suspension was plated on plastic. Growth medium (GM) consisted of DMEM supplemented with 17.5% FCS, sodium acetate (5 mM) and transferrin (10μg/ml). Cultures were maintained at 37°C in 5% CO_2 in a humidified incubator. On the second passage, the primary culture cells were diluted to 5 cells/ml in GM, and 200μl/well was aliquoted into a 96-well plate. Six days following plating, wells with a single colony of rapidly growing epithelial-like cells were passaged when about 50% confluent. In this way a series of MEC clones resulting from a single parent cell were established. Growth of one of these clones on plastic was determined using GM, or GM supplemented with either or both insulin like growth factor 1 (IGF-1; 100 ng/ml) and epidermal growth factor (EGF; 10ng/ml). Cell density was determined in triplicate for 8 days, from an initial density of 1.2×10^4 cells/cm^2. The significance of the difference between control, IGF-1 and EGF treatments on each day of the incubation were determined by ANOVAR.

Differentiation media (DM) was similar to GM, but contained 5% FCS, 2 μg/ml prolactin, 3 μg/ml cortisol and 2 μg/ml insulin. Differentiation and milk protein production of the clone were determined by suspending the cells in DM, supplemented with progesterone (10 ng/ml), onto a thin film of a collagen/laminin matrix (Matrigel; Collaborative Biochemical Products, Bedford, USA). This media was then replaced with DM without progesterone 2 days later. Media were replenished every other day and the culture was maintained for 16 days. Cells plated onto a Matrigel film, but with GM rather than DM, were used as the control. After about 8 days of culture, alveoli-like structures had formed with a distinct lumen with both GM and DM. Incubating the cells with calcium free DMEM containing EGTA caused the tight junctions between the cells to break open, releasing the contents of the lumen. This process necessitated that the culture was then discarded. The lumen extract was analysed for α-casein by ELISA after 4, 8, 12 and 16 days of incubation. Each incubation duration and treatment was repeated 6 times. Differences between the GM and DM treatments for α-casein levels were determined by ANOVAR.

Results On plastic the cells had a monolayer, cobblestone, epithelial-like morphology. Growth of the cells on plastic is depicted in Fig. 1; by day 5, the IGF-1 and EGF significantly increased cell density ($P<0.05$), though there was no significant additional effect with both factors combined. When the cells were plated on Matrigel, alveoli-like lobule structures formed, regardless of the incubation media used. However, α-casein was found in the lumen of these structures only with DM (Fig. 2), with lower levels in the surrounding media (data not shown). No stimulation of casein production above the minimum detectable dose of the ELISA was detected for GM.

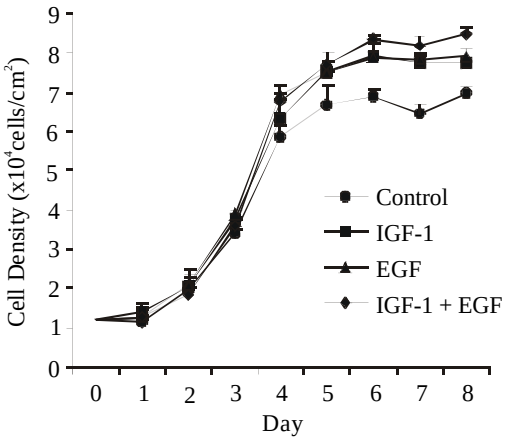

Fig. 1. MEC cell density on plastic with GM, supplemented with either IGF-1 or EGF or both.

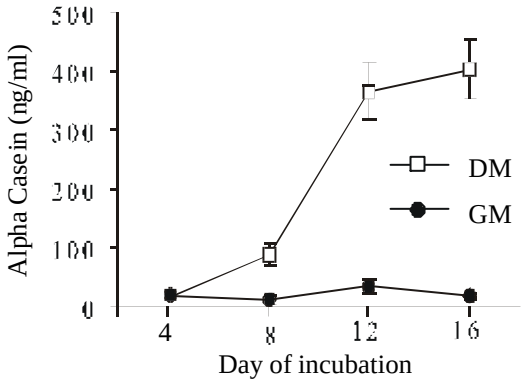

Fig. 2. Levels of α-casein in the lumen contents of MEC incubated on Matrigel with GM or DM

Conclusions This MEC clone is responsive to IGF-1 and EGF, which are thought to be mitogens for mammary growth *in vivo*. Additionally, given appropriate extracellular-matrix and lactogenic hormones, the clone can be stimulated to form alveoli like structures, which produce α-casein, a principal component of milk protein.

An introduction to endocrine disrupting compounds

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Introduction Endocrine disrupting compounds (EDC) include a diverse range of chemicals derived from industrial, agricultural and domestic processes (IEH, 1999). They include phthalates (used in the manufacture of plastics), alkyl phenols (present in detergents and surfactants), polychlorinated biphenyls (PCB; formerly used in electrical equipment), dioxins (released from incinerators), organochlorine pesticides and organohalogens (used as flame retardants).

The compounds have a wide range of chemical structures but all of them have the capacity to disrupt normal hormonal actions. In some cases they bind to steroid hormone receptors and so they can have weak oestrogenic or androgenic effects while others disrupt thyroid hormones or other physiological functions. Consequently, they may disrupt reproductive or immune function and can be carcinogenic.

Evidence of adverse effects on animal physiology is partly circumstantial and partly based on empirical studies, mostly involving laboratory rodents. Many reports in the literature indicate that reproductive disorders in wildlife species such as pelicans, birds of prey and polar bears are associated with abnormally high concentrations of EDC in their tissues. Furthermore, controlled studies involving the administration of large, arguably pharmacological, doses of EDC to laboratory rodents have demonstrated adverse effects on their reproductive or immune systems. Relatively little work has yet been done to determine the pattern of uptake and bioaccumulation of EDC in domestic species or the effects of EDC on the physiology of these species.

Properties of EDC The effects on physiological systems of EDC are dependent on their individual properties and these differ with chemical type (IEH, 1999). However, certain properties are common to many of the compounds. In general, the compounds are :

a) *persistent*. i.e. they are slow to degrade in the environment and are therefore present in the environment for long enough to enter the food chain. Examples of highly persistent compounds are PCB and the insecticide DDT, neither of which has been manufactured or used in the developed world for more than 25 years but which remain in the environment in biologically significant concentrations.
b) *hydrophobic and lipophilic*. Compounds with hydrophobic properties tend to come out of aqueous solution and become concentrated in organic matter, soil or silt; lipophilic compounds associate with fat depots and are readily accumulated in the fat depots of animals.
c) *biologically active at very low concentrations.* It should be noted that these compounds act on physiological systems at concentrations which are well below toxic concentrations and are generally less than 10^{-9}M.
d) *weakly associated with steroid binding proteins in the blood* of animals. Thus, although they may be present in animals in relatively low concentrations, most or all of the compound present may be biologically available, unlike endogenous steroids which are mostly attached to binding proteins and are not biologically available.
e) *able to act additively, contrarily or synergistically* and effects may be species-dependent. Furthermore, responses observed *in vitro* are not always apparent when studied *in vivo*. These properties make it difficult to predict the likely effect of EDC on animal physiology.

The effects of EDC on animal physiology depend in part on the age and physiological state of the animal. However, in general, foetal or neonatal animals are more susceptible to the adverse effects because the development of some components of their organs, particularly of the reproductive system, can be irreversibly affected by EDC. These effects on the developing organs can not only adversely affect reproductive function in the adult animal but can also make it difficult to determine the effects of EDC on physiological systems. There is also a possibility that adverse effects of EDC are expressed in subsequent generations (Safe *et al*., 2000)

Mechanisms of action EDC affect animal physiology through several mechanisms. Some compounds are known to bind to oestrogen receptors. PCB act via the aryl hydrocarbon receptor and some EDC act through mechanisms that are not receptor-mediated (Hansen, 1998). The potency of EDC on domestic animals will depend on the rate of uptake by the target species, the particular type of EDC and the extent to which it is metabolised, the stage of development and physiological state of the animal and interactions with other types of EDC. Clearly, their effects are likely to be difficult to predict accurately.

Conclusions EDC are highly ubiquitous and have the potential to affect the health and reproductive performance of both farm animals and humans. Relatively little is yet known of their bioavailability with repsect to these species or the precise nature of their effects.

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Epidemiological evidence of effects of EDC on ruminant reproduction

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Fifty percent of Dutch dairy farmers use surface water as the main source of drinking water for their cows during the grazing season. In many locations this surface water is in direct contact with a sewerage overflow. Belfroid and coworkers (1999) have demonstrated that this sewerage overflow surface water contains EDC, in particular xeno-oestrogens. The concentration of conjugated ethinyl-oestradiol, for example, in sewerage water was 0.02 µg/l. During the last decade the fertility of dairy cows has decreased dramatically. There is an increased incidence of cystic ovaries, an increase in the interval from calving to first insemination, an increase in the number of inseminations per pregnancy and more abortions have been recorded. These observations raise the possibility that EDC's adversely affect reproduction in dairy cows. Meijer et al. (1999) compared the production and fertility of cows on 397 farms that used normal surface water with the production and fertility of cows on 60 farms that used water in direct contact with sewerage overflow. The results showed clearly that cows drinking water that was in direct contact with sewerage overflow produced less milk and had a greater age at first calving. Moreover they exhibited a tendency towards a longer interval between calving and first succesful insemination and tended to have a higher rate of abortions. Boerjan et al. (2001) have recently addressed the issues of concentrations of xeno-estrogens present in soil, in plants, in water and in cow tissues, milk and blood, in order to assess the potential risk to ruminants of xeno-estrogen exposure through soil and water ingestion. Although data are sparse and concentrations of EDC in feed and water of ruminant animals are low, under certain circumstances such as sewerage overflow and sludge-fertilized pasture, they concluded that rates of ingestion of certain xeno-oestrogens may exceed levels at which there is no effect. Since most EDC's are lipophilic and become concentrated in adipose tissue, the mobilisation of fat during onset of lactation and pregnancy can expose animals to much higher concentrations of xeno-estrogens than are present normally in the environment. Important questions are: 1) how much of the EDC's stored in fat cells will enter the blood under certain circumstances, 2) how much of the EDC's in soil, water and plants will pass the rumen and enter also the blood and 3) how potent are the different EDC's i.e. what is the dose response relationship? In order to answer the last question an *in vivo* animal-model is urgently needed. We have developed an animal-model, based on the genital tract of an ovariectomized cow; the height and organisation of the endometrial lumen-epithelium, the rate of oedema in the stratum compactum and the electrical resistance of the cervical mucus are sensitive measures of oestrogenic activity.

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Empirical studies of the effects of endocrine disrupting compounds on male reproductive physiology

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Concerns have been raised about the potential adverse effects on reproductive health in farm animals, humans, and wildlife species from a range of environmental chemicals that disrupt normal hormonal actions. The alkylphenol polyethoxylates are non-ionic surfactants used in the manufacture of detergents, paints and herbicides. During sewage treatment, these compounds are broken down to short chain alkylphenol polyethoxylates, alkylphenol carboxylic acids and alkylphenols which bioaccumulate in the lipid of living organisms. The estrogenic nature of one of these compounds - octylphenol has been clearly demonstrated in cell culture, in a recombinant yeast screen with human estrogen receptor$_2$ and in animal studies. It is proposed that these endocrine disrupting compounds influence male adult reproductive potential by disrupting the development of the hypothalamic-pituitary-testicular axis during fetal life. We have recently identified that exposure to octylphenol for the second half of gestation decreases circulating concentrations of FSH during fetal life and the number of Sertoli cells of the testis and testis size at birth in comparison to control animals (Sweeney et al., 2000). However, the testes size, % interstitial space, semen volume, semen concentration and % live semen was similar in both treatment groups in the adult. In contrast animals exposed to octylphenol from birth to weaning (16 weeks of age) had a significantly greater number of primary and secondary abnormalities in comparison to controls and animals exposed to octylphenol for the second half of gestation. A number of the animals exposed to octylphenol from birth to weaning exhibited augmented sexual behaviour, while those exposed to octylphenol for the second half of pregnancy showed a suppression of sexual behaviour. The current data suggests the physiological effect of exposure to octylphenol is dependant on the time and duration of exposure. This has major implications for the determination of universal end-point measurements to assess exposure to endocrine disrupting compounds.

Empirical studies of effects of EDC on reproductive physiology (particularly in females)

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Over recent years increasing evidence suggests that xeno-oestrogens including alkylphenols, such as nonylphenol and octlyphenol (OP), may represent a threat to the health and reproductive function of humans, domestic animals and wildlife populations. To date most attention has focused on the effect of these endocrine disrupting compounds (EDCs) on male reproductive health. In contrast, hardly any attention has been paid to the potential adverse effects that these compounds may exert in females, where the end points are easier to measure than in males, and where oestrogen modulated diseases such as breast cancer, are of such importance to humans. This is also despite evidence from a number of species, including humans, that exposure *in utero* to the potent synthetic oestrogen diethylstilbestrol (DES) has profound and long-term effects on ovarian morphology, folliculogenesis, fertility, and pregnancy outcome of female offspring.

Investigations of the effect of *in utero* EDC exposure in female offspring are important as the full complement of eggs available in adult life is laid-down early in foetal development. Any perturbation of the programming of the foetal ovary may therefore alter the balance of follicle and oocyte development and so potentially compromise reproductive health later in adult life. This hypothesis is now supported by results from long-term animal exposure experiments and by data from laboratory based studies of somatic cell culture and gamete development and function in the presence or absence of a number of different EDCs.

We have used the foetal sheep as a model system to investigate the effects of exposure to pharmacological levels of DES and OP *in utero* on ovarian development and function. In this model environment, both direct and indirect exposure of the foetus to DES and OP, for varying periods, induced hypertrophy-hyperplasia of the ovarian interstitial tissue as evidenced by increased size, weight and vascularisation of the developing gonad. Furthermore, immunohistochemical staining of ovarian sections suggests that DES and OP exposure alter follicle population dynamics in the developing ovary. Both compounds stimulated the initiation of growth of an increased number of primordial follicles relative to control animals, but did not support increased preantral and antral follicle development. Not surprisingly these effects were less marked in animals exposed to OP which is less potent than DES. These observations suggest that follicle turnover in the foetus may be altered by exposure to EDCs. Furthermore, *in vitro* exposure studies indicate that it is the oocytes rather than the granulosa cells of maturing follicles which are susceptible to the oestrogenic effects of the EDCs.

Overall the results suggest that chronic foetal exposure to oestrogenic compounds may lead to a precocious depletion of the ovarian reserve, so compromising fertility and hastening the onset of reproductive failure in the adult. While this may not be a problem for short life span meat animals, these observations may have profound implications for the future reproductive health of long-lived farm species and women.

Biotechnology and Livestock Production

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For over thirty years livestock improvement programmes have exploited mainly statistical technology for the process of selective breeding. This has been safe, non-controversial, and highly cost-effective. The present revolution in knowledge of DNA now offers the possibility of much faster change in a wider range of traits, including meat quality and disease resistance which are difficult to measure in the live animal. However this new technology presents a threat as well as an opportunity. This paper examines the possible role of the new technology in genetic improvement, and considers the scientific strategy that the industry should adopt.

Present situation Today the goal for the pig industry is to produce high quality lean meat at minimum cost, in a manner acceptable to the public and which safeguards animal welfare. There is an inherently unstable world market for pig meat, a trend towards more complex multifactorial pig disease, and rising public concern over animal production.

BLUP now permits increased emphasis on less heritable traits such as reproduction. By allowing comparisons of genetic merit across herds it has changed the structure of nucleus populations which can now be geographically diverse. Through the Internet, database and BLUP methodology can easily be made available to closed nucleus herds around the world, encouraging a diversity of specialised selection objectives. Alternative breeds such as Meishan and Pietrain continue to offer options for genetic change. Estimated rates of genetic change in Cotswold populations are in the range 1-3%, with little sign of any biological limit. Before adopting technology to speed up genetic change, the industry should recognize that some 20-30% of the genetic potential that exists today is probably not expressed on commercial farms. The reasons are health, nutrition and management.

The new technologies The new technologies fall into the broad categories of manipulation of reproduction, marker-assisted selection, and gene transfer (GM). Some of the opportunities are described below.

Marker assisted selection In pigs a very large number of associations has been reported between DNA variants and performance. The majority of effects appear small and inconsistent across populations. The most consistent are for litter size and fatness, and arise from the "candidate gene" approach of understanding biological pathways rather than serendipitous hunting for DNA variants (so-called microsatellites). Disadvantages of markers are the small benefits in performance compared to the DNA typing cost, and the highly unpredictable association with performance.

DNA microarrays As many as 30 000 or more spots of DNA can be fitted on a microscope slide. When treated with any tissue, the RNA hybridises with DNA to give a fluorescent display indicating which genes are expressed. Thus *microarrays* will revolutionize detection of genes expressed in diseased versus healthy tissue and tough versus tender meat. This together with much cheaper DNA testing may allow a move towards marker assisted selection. The challenge will then be how to rationalise information on thousands of genes.

Reproduction Unlike cattle, the freezing of semen and embryos, as well as non-surgical embryo transfer, still have to be perfected in pigs. Semen sexing by flow cytometry using a stain and laser is used for IVF in cattle, but is too slow for pig matings. Some success has been reported in sperm separation by raising antibodies against X or Y (Blecher *et al*, 1999). *In vitro* meiosis to produce sperm and eggs has yet to be achieved.

Gene transfer In pigs gene transfer technology is being developed primarily to produce hearts and other organs for use in humans (xenotransplantation). This involves addition of human histo-compatibility genes and deletion of pig sugar genes. Deletion of the *myostatin* gene (GDF-8) in mice resulted in a doubling of lean growth rate, and a 3-4 fold increase in "ham" weight. (McPherron, A.C. *et al*, 1997). This mutation occurs naturally in double-muscled cattle, but not in pigs.

Gene therapy This involves insertion of DNA which is not incorporated into the germ line, and which is not passed on to offspring. For example modified growth hormone releasing hormone (GHRH) was recently introduced into young pigs, with a 37% increase in growth rate (Draghia-Akli *et al*, 1999). The promoter was altered to increase growth hormone production, and a further change made to reduce the rate of degradation of the GHRH.

Genomic imprinting This is a natural phenomenon which can modify gene function depending on whether the gene was inherited from the dam or sire. For example the IGF2 gene in humans, pigs and mice is maternally imprinted (Nezer, C. *et al* 1999). This means it is switched off in progeny when received from the dam, and is therefore inherited only via the sire. This mechanism might be used for example where genes for fatness are required to convey longevity to the dam, but her market offspring are required to be lean.

Cloning Following Dolly, the first cloned pigs were announced recently by PPL Therapeutics (Polejaeva *et al*, 2000). Dolly-style cloning potentially offers cheaper and easier gene transfer. Cloning to produce a totally uniform slaughter generation may be a long way off, and presents the challenge of continuing genetic improvement in the absence of genetic variation in the slaughter generation.

Putting the technologies together Together these technologies have the potential for dramatic change. For example, suppose a *myostatin* "knockout" could be introduced into Meishan pigs. The result could be nine extra piglets per sow per year with normal or even improved lean growth. Suppose then that genes for androstenone and skatole could be "knocked out" removing boar taint and the need for castration, and that a 100% male slaughter generation could be produced by semen sexing. The result could be a 20-30% overnight improvement in the efficiency of pig production.

A further option might be to produce the slaughter generation from surrogate mothers using frozen and cloned identical embryos from a "slaughter" line, as opposed to a sire line. In vitro meiosis would allow a complete cycle of genetic selection with sexual reproduction in the absence of a live animal. Embryos would be tested by microarray markers, and the best cloned to produce sperm and eggs for IVF. Ultimately of course it must surely become possible to "culture" meat without a live animal.

Risks of GM in animals What then are the risks of GM? Modification of the animal could lead to a transformation of a pathogen causing an epidemic. Perhaps toxins or allergies might be produced, but these could be detected by testing. Poorer welfare and production stress could result from faster lean growth. Most worrying would be the lack of knowledge to deal with unforeseen changes. For example it is difficult to argue in public that GM is safe, when billions of dollars have so far failed to find a solution to cancer (a genetic modification?), and a great deal of the basic biology of BSE remains unknown.

The key issues So what the key issues regarding biotechnology and livestock?

- Is gene transfer really going to be needed in farm animals? The new science of functional genomics seeks to understand how genes are controlled, and offers the promise of altering the expression of genes already there.

- Under what circumstances should the public be asked to accept gene transfer? Today perhaps for disease resistance to improve health, welfare and food safety, but not just to increase output.

- Are we exploring options for genetic synergies between animals and plants, such that any genetic manipulation can be preferentially targeted to plants? For example, can plants deliver oral vaccines as gene constructs in the plant genome?

- If Europe and North America decline to allow GM in animals, what would be our reaction to imported meat from say the Far East containing the *myostatin* knockout at dramatically lower cost?

- What will happen to farm animals and the countryside when the option exists to produce meat in vitro?

- Who will be responsible for genetic improvement of farm animals? Will this now pass to the biotech or pharmaceutical companies?

A scientific strategy for the livestock industry The main challenge today is to judge the correct balance of investment between present and future technologies. For a pig breeding company, this strategy may involve the elements:

(1) Maintain maximum genetic improvement using existing BLUP and statistical technology.

(2) Close the gap between today's genetic potential and observed performance on the farm through research on nutrition, health and management.

(3) Secure access to, and experience of, the new technologies in case they may be needed to safeguard the competitive position of the industry, or to deal with *unforeseen* events such as BSE.

To access the new technologies, Cotswold has created its own science base through a programme of research fellowships, shareholdings, strategic partnerships and alliances with both universities and biotech companies.

Much of the speculation and debate on biotechnology today is caused by the fact that our state of knowledge is in its infancy. With more complete knowledge it will become clear what is safe and what is sensible. At this stage therefore, the overriding requirement for the livestock industry is to advance the fundamental science of molecular biology. The knowledge gained will be our protection and opportunity for the future. The industry must also acknowledge its responsibility to

participate in the debate on the correct balance between technology, competitive advantage, society and the countryside. The industry has nothing to hide. Now is the time to begin.

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Food safety Issues : safe meat and a prosperous industry?

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The United Kingdom, and much of the rest of Western Europe, has seen a series of food "crises" over the last 10-15 years, which have shaken consumer confidence in the safety of the food they purchase. Such concerns have also led to the establishment of Food Safety/Standards Agencies in a number of European countries. The most serious food safety issue has been BSE, which has claimed the lives of many thousands of cattle and almost 100 people in the UK. The rise of BSE coincided with the appearance of highly pathogenic bacteria, such as *Salmonella* Enteritidis phage type (PT) 4 and *Escherichia coli* O157:H7. *Campylobacter* spp. also became highly important zoonotic pathogens during this period.

There is a public belief that the above bacterial pathogens, and BSE, gained a foothold in animal production as a consequence of the intensification of the industry. Although this is an over-simplification, there is no doubt that the presence of a large number of potential host animals in the same place at the same time facilitates both infection and horizontal transmission. Food safety cannot be separated from the economics of production, however, and with ever-falling profit margins farmers must find the most economical ways of producing animals. The unit costs of intensive production will often be lower than with extensive systems and thus it is highly likely that intensive animal production will continue. Does intensification necessarily mean unsafe? Is it possible to produce food at an affordable price that is free from microorganisms that are potentially pathogenic for man? These and other issues will be discussed with particular emphasis on *Salmonella*, *Campylobacter* and *E.coli* O157:H7.

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***Salmonella*, *Campylobacter* and *E.coli* O157 as foodborne pathogens**

There are over 2,400 different serovars of *Salmonella* and most can infect either food animals and/or man. The most frequently identified vehicles of human infection are contaminated poultry meat or eggs. Each year, there will also be outbreaks of salmonellosis where red meat is the vehicle of infection. The peak of human infection with *Salmonella* spp. in the UK was reached in 1997, when there were over 35,000 reported cases. It is believed that this is at least a threefold underestimation. Most of the rise in *Salmonella* cases in the UK over the last 10-12 years has been due to the appearance of a highly invasive strain of *Salmonella*, *S*. Enteritidis PT 4. This organism is able to infect the reproductive tract of chickens and from there contaminate egg contents, which is a direct threat to public health, or hatching eggs which leads to vertical transmission from breeding flocks. The bacterium is also highly invasive in broiler chickens and this can lead to contamination of chicken muscle tissues. *Salmonella* Enteritidis PT 4 was almost exclusively a poultry-associated pathogen and was only isolated rarely from red meat animals. There are *Salmonella* serovars, however, which show equally invasive behaviours in red meat animals. The most recent example of this was *S*. Typhimurium definitive type (DT) 104 which rose to prominence in the mid-1990's and was initially associated with cattle, although it later spread to pigs and sheep. This bacterium could also be isolated from chickens. Isolates of DT 104 were almost always resistant to at least five different antibiotics used for human treatment. DT 104 also caused a particularly nasty infection and a case control study by the Public Health Laboratory Service (PHLS) identified that 36% of infected people ended up in hospital and that there was a mortality rate of 3%. This is greatly in excess of what is seen with other *Salmonella* spp.

In recent years, both PT 4 and DT 104 have shown a marked decline in incidence in both animals and man. With the former, this was due to intervention by the poultry industry (see below) but with the latter it is believed that this just reflected the cyclic nature of *Salmonella* infections in animals and man.

Escherichia coli O157:H7 is almost exclusively found in ruminant animals where it is a transient coloniser of the intestinal tract. Carriage length will vary from animal to animal and there may well be cycling among animals within a herd. The acquisition of O157 does the animals no harm and the factors which control intestinal colonisation are not yet fully understood, although there are suggestions that particular types of diet may either encourage or limit carriage. *Escherichia coli* O157:H7 is a highly important human pathogen and although there are only approximately 1,000 cases in the UK each year, infection carries a high risk of mortality and O157 is the major cause of infective renal failure in children under five years of age. Infection also has serious consequences for the elderly and, in outbreaks involving this age group, mortality rates of up to 40% have been observed.

Campylobacter spp. are the most frequently isolated bacterial enteric pathogens in the UK and in most of the developed world. A variety of vehicles are associated with infection but the most important are contaminated chicken meat, unpasteurised milk and recreational and drinking waters. *Campylobacter* spp. can be isolated from all food animals. In poultry, the principal species present is *Campylobacter jejuni*, although *C.coli* and *C.lari* may also be present. *Campylobacter jejuni* can also be isolated with high frequency from bovines, but in pigs *C.coli* is the more common isolate. Red meat is only rarely identified as a vehicle for human infection with *Campylobacter* spp. This may be

associated with the fact that, in these animals, *Campylobacter* spp. are confined to the surface of carcases and the greater chilling time given to red meat will cause a marked reduction in the numbers of viable *Campylobacter* cells present. This is not the case with chicken and the rapid throughput of poultry processing plants, coupled with packaging soon after slaughter, facilitates the survival of *Campylobacter* spp. and it is not unusual to find that poultry carcases will carry over one million cells of these bacteria.

Simple control measures?

Despite increasing competition from overseas, it is probable that for the foreseeable future British farmers will continue to supply the bulk of the UK market with fresh meat. It is important for the public health and the long-term security of the animal production industry that farmers and food processors take every practical step to deliver pathogen-free meat to the consumers. Food safety and quality are becoming increasingly important issues and are ones which provide the farming industry with interesting challenges.

There are essentially three ways in which farm animals and products derived from them can be rendered pathogen-free. The first, and probably the most successful option, is vaccination and this has been used with great success recently by the UK poultry meat and egg industries. It is the opinion of PHLS that the fall in *S.* Enteritidis cases seen in England and Wales since 1997 was due to the vaccination of laying hens by the major UK egg producers. In this instance a heat-killed, iron-starved culture of *S.* Enteritidis PT 4 was used. The broiler industry also vaccinated breeder flocks and this, coupled with culling of infected flocks, led to a marked reduction in the *S.* Enteritidis contamination of poultry carcase. Such interventions can be at high cost, however, and it is estimated that the UK egg industry has spent over ten million pounds vaccinating flocks. It is not always possible to recover the costs of such intervention measures from industry customers, however, and this inbalance needs to be addressed.

Another commonly used intervention measure is to protect animals from contact with potential pathogens by improved biosecurity. This is clearly most easily achieved where animals are housed throughout their lifetime. Studies in broiler production have demonstrated that the most frequent source of *Campylobacter* in growing flocks is the external environment. Flock colonisation results when people carry in *Campylobacter* on either their boots or clothing. Intervention measures in Scandinavia, where boots are dipped in disinfectant and/or changed along with clothing, allows flocks to remain *Campylobacter*-free until slaughter. This is a low-cost system which should be adopted by the poultry industry worldwide, where possible.

A number of UK producers have demonstrated that it is possible to produce *Campylobacter*-free chicken, provided there is strict attention to detail. Biosecurity is undermined, in part, by the practice of thinning where a proportion of the flock is removed at 4-5 weeks of age. During this process, transport crates will be taken into the broiler house. These are frequently contaminated with *Campylobacter* spp. and this may introduce these bacteria into a previously negative flock. This practice requires review. Strict biosecurity has also been demonstrated in Scandinavia to protect pig herds from *Salmonella* infection.

Biosecurity is more difficult to maintain where animals are on pasture. A particular focus of recent concern has been the colonisation of sheep and cattle with *E.coli* O157. Measures are not yet available to the animal industry to remove easily and effectively these bacteria from the intestinal tract once colonisation has been initiated. With this particular bacterium, the most effective control measure is the delivery of clean animals to the slaughterhouse. This is a highly important measure and one which should not be ignored by the farming community. It is no longer acceptable for heavily contaminated animals to be delivered to the abattoir. Although good abattoir hygiene can help to remove, and may possibly eliminate, O157 from sheep and cattle carcases, this process is greatly facilitated by clean animals. This and other matters will be discussed.

Understanding the Consumer

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We can produce the tenderest, tastiest, highest welfare beef, pork and lamb in the world but if the consumer wants to buy chicken and fish, so what!

The changes that have taken place in what consumers can purchase and what and where they eat are nothing short of phenomenal and on-going.

In the 1950s families ate at least one cooked meal together every day, as they had for most of the century. The focus of this meal, lunch, dinner, tea or evening meal, depending on where you lived, was meat and two veg. All of this was cooked from scratch by the housewife, who had probably bought all the ingredients freshly that day.

What a contrast to today, only 50 years later:

- How often do families eat together?
- What has developed in addition to meat and two veg?
- What cooking skills are available today?
- Who shops daily?
- What extra choice is there in the shops?
- How important is the 'evening meal' compared to other competitive activities?

At the heart of this is the consumer. It is fair to say that it is the consumer who is responsible for all our destinies. It is only by listening to consumers, understanding their feelings, interpreting this information and responding in an appropriate manner that we can survive and develop. This is true not only for the meat and livestock industries but for any market, and it is why the ubiquitous 'marketing department' is at the heart of so many successful brands and successful companies.

It's not by accident that companies like Unilever, P&G, Mars and Tesco continue to develop. At the core of their philosophy is a commitment to marketing and to the consumer. It is by identifying what the consumer wants and then developing and rigorously checking and re-checking strategies and activity plans that they continue to outperform their competitors in an ever more difficult market and trading environment.

So what lessons are there here that the meat and livestock industries can learn from to improve what they do?

All sectors of the industry must talk to and understand the consumer. On the surface a geneticist, to use an example, has little to do directly with the consumer. So how do they know which element of the animal's performance to look to improve? They could be looking in the wrong direction and miss a major opportunity. Talking to and understanding the consumer can reduce this hit and miss approach. History is littered with great ideas that were just not wanted!

At the MLC we are continually talking to the consumer in all areas, including Research and Development, in an effort to understand more and more about their motivations and reasons why. Obviously this manifests itself in the form of consumer advertising but the breadth of work is much greater than this.

In the last year we have carried out consumer research on attitudes to GMs, organics, meat eating quality, animal welfare, and nutritional understanding. Each of these are important issues and key to our future

But where does this lead us? What learnings must we take forward in understanding the consumer and moving our industry forwards?

Firstly that there are rarely black and white answers. Why, if animal welfare is so important, is lamb consumption declining and poultry increasing? OK so the answer here is relatively simple, lamb is fatty, full of bone and expensive, whereas chicken is low in fat, frequently boneless and cheap. But the lesson is in the interpretation. Animal welfare is important and the 'manipulation' of animals just to increase productivity is rarely acceptable to consumers.

Secondly for the consumer a key word is trust. Post BSE consumers have had to confront their fears, and fortunately meat, and beef in particular, remains on the menu. The recent FSA survey illustrates the lack of overall trust in the food chain which only a decade ago was probably taken for granted. Every element of the industry must work to rebuild this fragile trust and certainly do nothing to damage it further.

Thirdly the move to convenience and the increased competition are irreversible. Do not try and turn the clock back. Yes, we have to produce the best but increasingly we must look at ways of improving the variety of ways in which it can be presented if we are to maintain our development.

Fourthly we are working in a global market. In the medium and longer term we have to be able to compete, we are unable to be fortress Britain. But knowledge is an international commodity and any technical advantage will be short-lived before it is caught up. We must therefore never stand still and think the job is done.

So where does all this lead us? From my point of view I find it reassuring that there is such a massive 'industry' supporting the future of our industry and looking to make significant developments and drive it forward. However, I also feel rather intimidated. The team and the resources which we possess at the MLC are small in comparison yet a lot of the responsibility for developing the consumer lies with us. An advertising spend of £6 - 8 m may sound enormous. However, against the overall size of our market, £14 bn, and the vast range of products we supply both in and out of home, it is small. By comparison, Kellogg's spend over £60 m per annum on their range of cereals.

However, as stated, it is the consumer who is responsible for all our destinies. It is the consumer who we will continually work with to understand better and to project a positive image for red meat through a variety of channels and provide the best possible future environment for red meat.

Meat Tenderisation – The Role of Calpains

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Introduction In recent years there has been a shift in emphasis in livestock production away from increased muscle growth towards improved meat quality. The final eating quality of meat depends on a number of organoleptic properties including appearance, colour, fat content, taste, texture and tenderness. Whilst colour and fat content are important in influencing meat purchase, consumer studies indicate that it is the degree to which muscle tenderises after slaughter that is the most important factor contributing to overall meat quality (Warkup *et al*, 1995). Despite efforts to standardise breeding, husbandry, nutrition, transport, lairage and slaughter regimes, ensuring a consistently tender product still remains difficult to control or predict. The problem is international, with beefsteak toughness a major concern in the USA and pork toughness difficult to eradicate in the UK. The tenderisation process involves complex changes in muscle metabolism in the immediate post slaughter period and is dependent on genetic makeup, protein complement, metabolic status and environmental factors such as physiological stress. In the early postmortem period, glycogen depletion, lactic acid accumulation, pH decline and rate of entry and exit into rigor can all influence the ultimate tenderness of the meat some 8 - 20 days later following a period of conditioning (Goll *et al*, 1995). However, the main determinant of ultimate tenderness appears to be the extent of proteolysis of key target proteins within muscle fibres (Taylor *et al*, 1995). Research in all major livestock species has pointed to the calpain proteolytic enzyme family being a major factor responsible for key peptide bond cleavage (Koohmaraie, 1996). Whilst opinion is divided as to which isoform of calpain is the most important under specified conditions, most workers agree that the major factor is the level at slaughter of the specific calpain inhibitor calpastatin. The evidence for this is reviewed here, highlighting potential means of regulating the system in order to assure a consistently high quality tender product.

The calpain system The calpain system comprises two ubiquitous μ- and m- isoforms, active *in vitro* at low and high calcium ion concentration respectively, a growing number of tissue-specific variants, all the products of different genes, and a specific endogenous inhibitor, calpastatin (Suzuki *et al*, 1995; Sorimachi *et al*, 1997). The system is highly sensitive to fluctuating levels of calcium ion, pH and temperature, all of which change rapidly in the immediate postmortem period. Micro- and m-calpain consist of a large 80 kDa subunit and a small 30 kDa subunit, both of which can be readily truncated at their N termini, thereby modifying their membrane-binding properties and calcium-requirement. The extent to which this autolytic modification is physiologically significant has been difficult to assess and reports suggest that separation of the subunits is all that is necessary to render both μ-and m-large subunits fully active at low physiological (<1 μM) concentrations of calcium (Suzuki *et al*, 1995). Of the 'novel' calpains, nCL1 or p94 is of particular interest, given that it is expressed almost exclusively in skeletal muscle and that the lack of expression of a functional p94 gene in human populations is responsible for limb girdle muscular dystrophy type IIA (Richard *et al*, 1995). The p94 polypeptide closely resembles the large subunits of μ- and m- isoforms, albeit with three additional inserted sequences, one of which confers instability by exposing the polypeptide to an intramolecular autolytic cleavage event. This instability has so far prevented the isolation of p94 by conventional chromatography so that its enzymological properties remain unestablished. However, it is known that p94 is tightly bound to the giant elastic protein titin in a region that corresponds to the sarcomeric N2 line (Labeit and Kolmerer, 1995). This region is known to be susceptible to postmortem cleavage. Consequently, p94, either alone or in conjunction with μ- and m-calpain, may have an important function in meat undergoing conditioning.

Calpastatin is a five-domain inhibitory protein of predicted molecular weight 77 kDa (Takano *et al*, 1988; Lee *et al*, 1992). It is expressed in all tissues expressing calpains and in skeletal muscle at a higher level of activity than calpains themselves. Of the five domains, the N terminal leader (L) domain does not appear to have any inhibitory activity, but may be involved in targetting or intracellular localisation, whilst the other domains (I – IV) are highly homologous and are each capable of inhibiting calpain. Although there are at least eight calpain genes (Braun *et al*, 1999), it is believed that there is only a single calpastatin gene. It is predicted that the gene exceeds 100 kb and that considerable tissue-specific microheterogeneity is thought to occur as a result of alternative splicing (Takano *et al*, 1999; Takano *et al*, 2000). The microheterogeneity of calpastatin in different cells and tissues (Arnold *et al*, 1995) may determine its intracellular localisation and its physiological role.

Calpains and postmortem proteolysis The apparent requirement of the calpains for supra-physiological concentrations of calcium and the natural excess of calpastatin in most muscle types has, in the past, led to the dismissal of a role for calpains in postmortem tenderisation. Other proposed candidate enzymes have included the lysosomal cathepsins, whose acid pH optima were presumed to suit the low pH conditions that occur postmortem due to glycolysis and lactic acid accumulation, and the cytosolic multicatalytic ATP dependent protease or proteasome. However, neither of these enzyme groups have been shown to cleave the spectrum of protein substrates known to be degraded *in situ* during the conditioning process, based on SDS PAGE. This contrasts with the calpains, whose substrates are believed to include a number of myofibrillar, Z line and costamere proteins, but significantly exclude the major myofibrillar proteins actin and myosin and the major Z line protein α actinin, all of which remain intact during the normal tenderisation process (Goll *et al*, 1991, 1992; Koohmaraie, 1994; Taylor *et al*, 1995). The calpain system is therefore likely to be functional postmortem and have a role in determining the proteolytic events associated with meat tenderisation. However, the time frame within which the most significant proteolysis occurs has proved difficult to ascertain. Both μ-calpain and calpastatin decline relatively rapidly postmortem while tenderisation continues for up to 4 weeks, depending on the species. Nevertheless, there is now considerable evidence linking the calpains to tenderisation in beef, lamb and pork. Correlations have shown that the different tenderisation rates between species (beef < lamb <

pork) relate inversely to the ratio of calpastatin:calpain (beef >lamb > pork) (Koohmaraie *et al*, 1991). Infusion of $CaCl_2$ in beef carcasses increases the rate at which the meat tenderises, whilst infusion of the calpain inhibitor, $ZnCl_2$, reduces the rate of tenderisation in both beef and lamb (Geesink *et al*, 1994; Koohmaraie, 1990).

Although arising from growth rather than meat quality studies, the observation that livestock treated with β-adrenergic adrenergic agonists developed pronounced hypertrophy of the predominantly fast fibre muscle types has added to the evidence for calpain involvement in muscle protein turnover. These effects were accompanied by significant changes in the extractable activity of calpain and calpastatin. Decreased μ-calpain and elevated calpastatin in treated animals suggested that myofibrillar degradation could be reduced by a β receptor-mediated mechanism (Higgins *et al*, 1988; Wang and Beermann, 1988; Bardsley *et al*, 1992). The β-agonist effect was also evident at the level of mRNA expression, suggesting that the activity measurements were in fact reflecting changes in gene expression and not merely some other altered property of the hypertrophying muscle (Parr *et al*, 1992; Killefer and Koohmaraie, 1994). Whilst β-agonists were clearly shown to induce muscle hypertrophy, they did so at the expense of producing a tougher meat product than in untreated animals (Kretchmar *et al*, 1990; Wheeler and Koohmaraie, 1992). Bardsley *et al*. (1992) suggested that the β agonist effect on calpains could be a reflection of the natural stress hormone response involving adrenaline oversecretion, which could happen in livestock during production, or in the transport and lairage events leading up to slaughter. Data from porcine LD has shown that elevated plasma adrenaline increases calpastatin activity and expression, implying that the link between stress and meat toughness may indeed be mediated via the calpain system (Sensky *et al*, 1996; Parr *et al*, 2000).

The sequence of proteolytic events leading to conditioning have led to a general consensus that calpain activity is the most likely causative agent for specific peptide bond cleavage postmortem. Since both μ- and m-calpain appear to be capable of cleaving the same target proteins it is difficult to determine unequivocally which is likely to be most important during the postmortem conditioning period. In general, the m- isoform persists longer than the less stable μ- in ageing muscle from all species studied, including pig (Sensky *et al.*, 1996). Despite the potential for p94 to play an important role in postmortem tenderisation, unequivocal evidence for this is lacking at present. In porcine LD, p94 expression does not differ between tough and tender animals and shows no correlation with 8 d shear force values (Parr *et al*, 1999b), whereas recent reports in ovine and bovine muscle shows a positive correlation between p94 and tenderness (Ilian *et al*., 2001). In general, the most important component of the calpain system with respect to meat tenderisation is currently considered to be calpastatin.

Relationship between calpastatin and meat toughness Studies on a random selection of commercially slaughtered pigs have shown that a high level of calpastatin in the first few hours after slaughter is associated with an increased incidence of toughness (Sensky *et al*, 1998; Parr *et al*, 1999a). This is consistent with relationship between calpastatin in the muscle of ruminant species 24 h after slaughter and the degree of tenderization achieved after conditioning, with differences in calpastatin accounting for 40% of the variation in tenderness (Shackelford *et al*, 1991; Koohmaraie *et al*, 1995). By monitoring calpastatin at these times it should therefore be possible to predict whether or not any given carcass will tenderise to an acceptable degree. This provides an opportunity to grade carcasses at a much earlier time postmortem than is currently possible. Measurement of calpastatin using specific antibodies has shown that a number of bands appear on SDS PAGE, suggesting that proteolytic processing may occur, a fact that may be of considerable importance when considering how calpastatin may be regulated. In porcine LD, the principle bands run at molecular weights of 172 and 135 kDa (whole muscle) and 135 and 80 kDa (muscle extracts). Of these bands, the intensity of the extracted 135 kDa isoform correlates most closely with toughness, identifying over 70% of ultimately tough carcasses (Figure 1; Parr *et al*, 1999a). It has also been shown that short term β-agonist treatment in pigs leads to a differential increase in the 135 kDa immunoreactive band, suggesting that expression of 135 kDa can be modified and may be stress sensitive (Parr *et al*, 1999b). Furthermore, isoelectric focussing of the 135 kDa protein reveals multiple bands, which may be minor splicing or phosphorylated variants, possibly truncated by proteolytic editing. The identification of these isoforms and the mRNA species from which they originate may lead to an even closer predictor of toughness/tenderness.

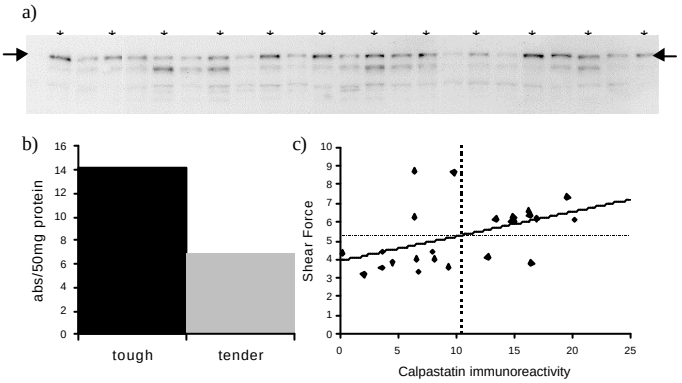

Figure 1. a) Immunoblot of tough (*) and tender pork samples taken 2 h postmortem after probing for calpastatin. The 135 kDa band is indicated (→). b) Quantification of 135 kDa calpastatin band intensity in tough and tender samples. c) Relationship between 135 kDa calpastatin immunoreactivity and 8 d shear force values. Dashed/dotted lines represent mean values.

Calpastatin is also known to vary between different breeds within a species in a manner consistent with the prevalence of meat toughness associated with each breed. For example, the callipyge sheep, characterised by increased hypertrophy of specific muscles, notably in the hind quarters, produces extremely tough meat and has a high level of calpastatin in the muscle at slaughter (Koohmaraie *et al*, 1995). β-agonist treatment does not induce further hypertrophy nor does it raise calpastatin any higher in these animals, suggesting that the muscle growth rates in the callipyge sheep are maximal leaving no room for further increases in response to β-agonist treatment (Koohmaraie *et al*, 1996). The mechanism by which the callipyge gene causes muscle hypertrophy is therefore likely to be similar to that by which β-agonists act. Duroc and Large White pigs provide another instance where calpastatin has been shown to differ between breeds within a species. Immunoblotting techniques reveal that the 80 kDa calpastatin band is more abundant in Duroc genotypes than

it is in Large White breeds (Sensky *et al*, 1999), a fact that may be of significance given the reduced incidence of toughness seen in Duroc pigs.

Extensive intraspecies studies in cattle of diverse genotype have demonstrated the heritability of the link between calpastatin level and beef toughness (Shackelford *et al*, 1994). Given the potential this has for marker-assisted breeding programmes, a number of groups have now identified calpastatin gene polymorphisms and have attempted to link these to growth and meat quality (Lonergan *et al*, 1995). A major problem in this approach is the fact that the size of the calpastatin gene inevitably means that most polymorphisms will tend to be intronic. Furthermore, the measurement of calpastatin inhibitory activity in muscle extracts is problematic because the protein tends to be unstable postmortem, and quantitative measurements will differ with different methods of extraction and assay. Additionally, the purified protein exhibits anomalous molecular weights on SDS PAGE, compounded by the fact that calpastatin can exist in a number of alternatively-spliced isoforms or proteolytically-processed fragments (Takano *et al*, 1999). The capacity of any one of these potential variants to inhibit one or both calpains under conditions likely to prevail in postmortem muscle has not yet been established. Whilst polymorphisms in the calpastatin gene have been detected in cattle and sheep, the significance of this with respect to toughness remains to be clarified (Killefer and Koohmaraie, 1994; Palmer *et al*, 2000). A QTL for beef tenderness has been identified (Keele *et al*, 1999). However, the QTL is located on chromosome 15, not on chromosome 7, where the calpastatin locus is part of a linkage group. This illustrates that care should be exercised when ascribing the control of meat tenderness solely to calpastatin.

Thus there is evidence that variations in calpastatin that relate to meat toughness/tenderness are subject to environmental and genetic regulation. Understanding the mechanism by which such regulation can control calpastatin function is therefore of paramount importance to further improvement of meat quality.

Regulation of calpastatin On the basis of the data linking β-adrenergic stimulation to increased calpastatin and increased toughness, a physiological mechanism for increased calpastatin expression is likely to relate, at least in part, to the β-receptor/cAMP/protein kinase A axis. Given that this pathway is activated as part of the natural stress response, this mechanism would go a long way towards explaining the molecular and biochemical link between stress and poor meat quality. Support for this has arisen recently with the publication of a partial calpastatin gene structure in bovine (Cong *et al*, 1998). A potential promoter was identified and shown to contain a cAMP responsive element (CRE) indicating sensitivity to β-receptor linked signalling pathways (Figure 2). Transcription from this promoter generated a novel mRNA transcript that included sequence from three additional N terminal exons. These transcripts indicated that the Leader (L) domain of the corresponding protein was longer than previously believed, containing an extended Leader sequence (XL) incorporating the peptide encoded by the additional exons. The XL-sequence was shown to contain four potential phosphorylation sites based on the amino acid sequence deduced from bovine calpastatin cDNA, rendering it susceptible to posttranslational modification. This means of regulating calpastatin is also likely to be of significance in attempts to influence postmortem tenderisation, given that phosphorylation by cAMP-dependent kinase *in vitro* can alter the specificity of the inhibitor for the calpain isoforms (Salamino *et al*, 1994).

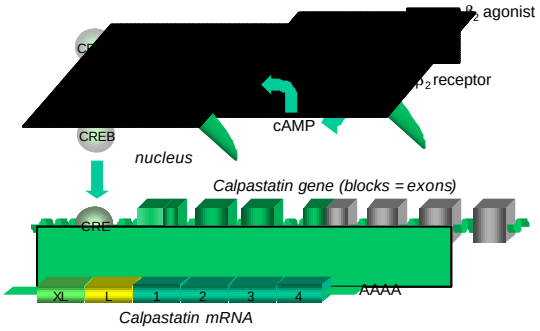

Figure 2. Hypothetical mechanism of β_2-agonist induced changes in skeletal muscle calpastatin expression via phosphorylation of the CRE binding protein (CREB).

It has recently become evident that the region in the calpastatin that encodes the XL sequence of the calpastatin protein is subject to alternative splicing mechanisms (Takano *et al*, 2000). Furthermore it is likely that there are a number of alternative promoters in the calpastatin gene and that transcription from these could vary between different tissue or muscle types (Takano *et al*, 2000; Bardsley *et al*., 2001). Another potential promoter has been identified in the porcine calpastatin gene that contains several potential cAMP responsive elements (CRE), which would reinforce the idea that calpastatin expression is sensitive to adrenergic stimuli (Parr *et al*, unpublished). The potential physiological consequences of calpastatin alternative exon usage include effects on mRNA stability and rate of translation, post-translational modification and the targetting of nascent proteins to subcellular loci where contact between inhibitor and calpain is restricted. In the postmortem period, the alternatively-spliced isoforms could be localised in different regions of muscle fibres or be degraded at different rates.

As well as regulation by β-adrenergic pathways there is evidence that calpastatin is responsive to changes in the IGF/GH axis. Recent studies have shown that somatotropin-induced growth in pigs actually resulted in a selective downregulation of calpastatin mRNA transcripts (Ji *et al*, 1998). This implies that elevated somatotropin would tend to produce meat of low shear force. The mechanism by which this impacts on calpastatin is likely to involve the calcineurin signalling pathway and is the subject of continued research.

It is known that under certain conditions, calpain itself (Doumit and Koohmaraie, 1999; Sensky *et al*., 2001) can degrade calpastatin to functional peptides which still retain inhibitory activity. Recent *in vitro* experiments in ovine skeletal muscle extracts have shown that calpain can induce the disappearance of 135 kDa calpastatin and that this correlates with loss of inhibitory potency (Doumit and Koohmaraie, 1999). In pigs, analysis of the 135 kDa and 80 kDa calpastatin bands in muscle extracts has shown that the intensity of the 80 kDa band is proportional to increased calpain activity (Sensky *et al*., 2001). Whilst this doesn't alter overall calpastatin activity, the fact that the 80 kDa band intensity also correlates negatively to 8 d shear force suggests that cleavage of calpastatin permits calpain mediated tenderisation of skeletal muscle to proceed more effectively (Sensky *et al*., 2001).

Conclusions Until recently the meat industry did not know if cathepsins, proteasome or calpains were involved in the proteolytic phase of the tenderisation process. It had long been suspected that stress played a role in determining meat toughness, but the underlying mechanism by which this could be achieved biochemically was unknown. The close relationship between elevated calpastatin expression and increased toughness in livestock species has led to extensive research on the role this protein has to play in the tenderisation process. The current knowledge of the structure of the calpastatin gene and its potential regulation by β-agonist/cAMP pathway mechanisms has shown that the presumed link between stress and toughness can be explained at a molecular level. Therefore, any efforts that can be undertaken to minimise stress arising from production and slaughter procedures can only be of benefit to the livestock industry. In addition, by monitoring calpastatin in the early posmortem period it is theoretically possible to predict whether or not a carcass will fail to tenderise adequately and this has the potential to be incorporated into meat quality assurance schemes. It is clear that calpastatin may be regulated in a number of ways and attention is therefore currently focussed on determining how calpastatin can be regulated in a more controllable fashion by investigating the pathways that impinge on calpastatin expression. Such research could lead to improved nutritional or husbandry regimes, more accurate testing methods and to the possibility of identifying one or several DNA markers associated with toughness that could be incorporated into breeding programmes aimed at reducing the incidence of toughness in British meat.

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Muscle lipids and meat quality

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Introduction Lipids are present in muscles as structural components of the muscle membranes, as storage droplets of triacylglycerol between muscle fibres and as adipose tissue (marbling fat). These lipids, or more precisely their fatty acids, contribute to a wide range of quality attributes. For fresh meat these are colour stability, drip loss and the development of oxidative rancidity. Meat colour and level of marbling are the two major factors relevant to the selection of meat by the consumer. Eating quality of meat is judged by texture and flavour. The former depends, in part, upon marbling fat which also contributes to juiciness, and flavour depends upon products from the thermal oxidation of lipids. These breakdown products react with other meat constituents to produce flavour and odour compounds. Finally, nutritional quality depends upon the fat content of the meat and it's fatty acid composition.

Tenderness The presence of adipose tissue as marbling fat between muscle fibre bundles can weaken the structure so that it is broken down more easily during chewing. The relationship between muscle fat content and tenderness is not clear. However, studies in Denmark showed that 20g/kg of total lipid gave maximum tenderness (Bejerholm and Barton-Gade, 1986). After correcting for structural lipid this is equivalent to approximately 12.5g/kg of triacylglycerol, most of which will be in the marbling fat. A survey of pork chops from UK supermarkets in 1995 gave values for total fatty acids in all of the muscles combined of 22g/kg equivalent to 17g/kg in marbling fat (Enser, *et al*., 1996). However, other studies of particularly lean pigs have produced values of 5g/kg of ether extractable lipid, most of which consists of marbling fat and which would indicate a potential problem with texture (Wood *et al*., 1996). In beef cattle in the USA a fat level in excess of 30g/kg is considered the minimum for good eating quality (Smith *et al*., 1984). This is close to the amount found in the UK supermarket survey of beef *m.longissimus* and in a recent study of carcasses weighing 334kg from Charolais cross steers (Scollan *et al*., 2001). Other muscles would mostly have slightly higher levels of marbling fat but clearly beef animals produced on a high forage diet are close to the minimum level recommended in the USA. Young bulls finished on concentrates at 12-14 months of age may have less than 20g/kg intramuscular fat (Enser *et al*., 1998) but other factors controlling texture, that are related to age and rapid growth, are likely to compensate for lower marbling. Marbling fat may also contribute to the perception of juiciness and enhance the perception of flavour since many flavour volatiles are lipid soluble.

Lipid oxidation and colour The attractiveness of meat to the purchaser is mainly related to colour, after perceived economic value. As meat ages, it turns brown as the myoglobin is converted to oxidized metmyoglobin and is rejected by the consumer. Lipid peroxidation increases the rate of metmyoglobin formation and conversely metmyoglobin acts as a catalyst of lipid peroxidation so that in beef muscle displayed under oxygen permeable film, lipid oxidation and metmyoglobin levels were closely correlated (Anton *et al*., 1996). Lipid peroxidation depends upon the degree of unsaturation of the fatty acids and the levels of the antioxidant vitamin E (α-tocopherol) and prooxidants such as free iron. Increasing the degree of unsaturation of the fatty acids results in a decrease in colour and oxidative shelf-life. In a study to increase the content of n-3 PUFA in beef, Richardson *et al*., (1997) observed that minced muscle from steers fed 3% fish oil had more rapid colour deterioration (saturation) and higher levels of lipid oxidation (thiobarbituric acid reacting substances) than muscle from steers fed Megalac, bruised, whole linseed or a mixture of linseed and fish oil (Table 1). This difference in shelf-life resulted from relatively small changes in muscle fatty acids. Total PUFA in the phospholipids were similar for meat from the fish oil or Megalac (control) fed steers but a doubling of the amounts of the main fish oil PUFA, 20:5 n-3 (EPA) and 22:6 n-3 (DHA) decreased shelf-life even when accompanied by a halving of the content of 20:4 n-6 (arachidonic acid).

Table 1 *Effect of dietary lipid on colour (saturation) and lipid oxidation (TBARS) of beef muscle mince after 10 days simulated display in modified atmosphere packs.*

Treatment	TBARS	Saturation
Megalac	0.623 ± 0.074^{a}	19.58 ± 0.37^{a}
Linseed	1.114 ± 0.268^{a}	18.28 ± 0.48^{a}
Fish oil	3.623 ± 0.436^{b}	15.89 ± 0.56^{b}
Fish oil/Linseed	1.281 ± 0.321^{a}	18.58 ± 0.61^{a}

[ab] Means within columns with different superscripts differ significantly ($P<0.05$).

Flavour During cooking, chemical reactions occur between fatty acids, amino acids and carbohydrates and their degradation products such as aldehydes and ketones, ammonia and hydrogen sulphide to give a large number of compounds that can contribute to meat flavour (Mottram, 1998). Heat-induced lipid peroxidation produces many reactive products such as aliphatic aldehydes that are substrates for thermal synthesis reactions. As with oxidative rancidity, the more double bonds in the fatty acid the greater susceptibility to oxidative breakdown during cooking and the greater the quantity of volatile products formed. For example, when steers were fed dietary supplements of Megalac, linseed, fish oil or a mixture of fish oil and linseed, total volatiles from cooked meat (ng/40g meat) were 2516, 6008, 14902 and 9828 respectively (Enser *et al*., 1997). The rapid oxidation of highly unsaturated fatty acids results in free radical attack on less susceptible fatty acids such as oleic acid and there is an increased content of thiazoles and thiozolines synthesized from oleate-derived saturated aldehydes (Elmore *et al*., 1997, 1999). Many of the compounds

produced in cooking have no flavour or odour so that as well as acting as precursors for flavour compounds, the fatty acids can also modulate flavour by diverting reactants to other components. Differences in the phospholipid fatty acids between grass and grain finished steers results in different types of flavour volatiles since the grass finished animals contain higher levels of α-linolenic acid and the n-3 PUFA synthesized from it and the grain finished animals contain higher levels of linoleic acid and other n-6 PUFA (Larick and Turner, 1990). In lamb, differences between the contents of these two fatty acid groups contribute to the differences in flavour between milk and concentrate finished lambs and those finished on grass (Sanudo *et al.,* 2000) (Table 2). This trial compared the eating quality of three types of lamb carcasses purchased in Spain and one in the UK. The lamb in Spain consisted of Spanish Merino which were milk and concentrate fed, with the ewes housed with the lambs during suckling, Rasa Aragonesa fed milk and concentrates but with the ewes allowed to graze during the day and Welsh Mountain lambs exported to Spain and all having light (10kg) carcasses. The "early lambs" were UK carcasses from the South West of England weighing 17kg. The contents of linoleic acid and arachidonic acid (20:4 n-6) were higher in the Spanish lamb and most n-3 PUFA were higher in the British grass finished lamb. The slightly higher n-3 PUFA in the Rasa Aragonesa compared with the Merino resulted from the ewes access to fresh forage. Taste panels in Britain and Spain found that the grass-fed lamb had more intense 'lamb' flavour.

Table 2. Effect of diets and breed of lambs on the polyunsaturated fatty acid composition of *m.longissimus.*

Breed	Spanish Merino	Rasa Aragonesa	Welsh Mountain	Early Lamb
Feed	Concentrates	Concentrates	Grass	Grass
Fatty acids		mg/100g muscle (mean SE)		
18:2 n-3	202 ± 11	186 ± 9	89 ± 4	108 ± 7
18:3 n-3	13.4 ± 0.6	20.7 ± 3.2	67.1 ± 4.5	50.2 ± 8.3
20:4 n-6	67.6 ± 4.4	57.1 ± 3.6	35.4 ± 3.3	29.6 ± 1.7
20:5 n-3	8.4 ± 1.0	14.6 ± 2.5	26.9 ± 1.0	21.4 ± 2.4
22:5 n-3	15.4 ± 0.9	19.2 ± 1.5	26.8 ± 0.8	22.8 ± 2.2
22:6 n-3	6.5 ± 0.9	9.8 ± 1.6	9.0 ± 0.7	9.7 ± 1.4

Human nutrition The recommendations for the amount and type of fat in the human diet in relation to coronary heart disease (CHD) and stroke were set out in the COMA report (Department of Health, 1994). Similar recommendations were made in other countries (Table 3). In addition to cardiovascular disease, these recommendations are also relevant in terms of obesity and diabetes, both of which are risk factors for CHD, and also for cancer. However, in considering the possible role of meat lipids in disease it must be remembered that most diseases are of complex aetiology and that fat is only one risk factor. The proportions of fatty acids of different types supplied by meat to the average UK adult are: saturated fatty acids 0.23, trans unsaturated fatty acids 0.18, monounsaturated fatty acids 0.31, n-6 PUFA 0.20 and n-3 PUFA 0.19 (Gregory *et al*., 1990).

Table 3. COMA recommendations for fat consumption

1.	Total fat to contribute less than 35% energy
2.	Saturated fat consumption to be less than 10% energy
3.	Trans unsaturated fatty acids to be less than 2% energy
4.	Polyunsaturated fatty acids to increase to a maximum of 10% energy
5.	Consumption of n-3 long-chain PUFA to be doubled
6.	Cholesterol intake to be kept below the present 245mg/day

The first recommendation of COMA was that fat consumption should be decreased from 40% of dietary energy to 35%. In fact muscle, if consumed without any adhering adipose tissue, can easily contribute to this aim since, as discussed above for texture, most muscles have less than 50g/kg lipid and hence can be classed as a low fat product However, even a lamb or pork chop or sirloin steak can be included in meals that meet dietary guidelines (Enser *et al*., 1996). It is the consumption of "hidden" fat in burgers, pates, sausages and the like that contribute to the high fat intake from meat since a high proportion of people discard adipose tissue on the plate. The saturated fatty acids, lauric (12:0), myristic (14:0) and palmitic (16:0) acid contribute to heart disease by raising plasma low density lipoprotein cholesterol whereas linoleic acid and α-linolenic acid lower it thereby decreasing he risk of heart disease. Stearic acid (18:0) does not affect plasma cholesterol concentrations. However, it may contribute to thrombosis, the final event in CHD that produces the heart attack. It is therefore included in the ratio of PUFA to saturated fatty acids (P:S) used to assess fat quality in terms of human nutrition and which has an acceptable value of 0.4 or above for the diet as a whole. The P:S ratio for pig muscle is generally above this value but for muscle from cattle and sheep is around 0.1 or less.

Another consideration in addition to increasing the intake of total PUFA is the relative levels of those derived from linoleic acid, the n-6 or ω6 series, and those derived from α-linolenic acid, the n-3 or ω3 series. The longer-chain PUFA act as precursors for oxidized derivatives called eicosanoids and these function a regulators of many physiological processes. In heamostasis, thromboxane A_2 produced from arachidonic acid (20:4 n-6) is a powerful

clotting agent whereas thromboxane A_3 from eicosapentaenoic acid (20:5 n-3) is much less active. The levels of the two eicosanoids depend on the quantities of their precursor fatty acids in the phospholipids of blood platelets. These amounts, in turn, depend upon the relative amounts of linoleic acid and α-linolenic acid in the diet since these are the precursors of the longer-chain PUFA. It is believed that primitive man evolved with a ratio of 18:2/18:3 of 1 in his diet but because we now consume large quantities of linoleic acid in vegetable oils the ratio ranges from 7-20. This contributes to heart disease by raising 20:4 n-6 and increasing the thrombotic tendency and contributes to autoimmune disease like arthritis because the leukotrienes produced from arachidonic acid stimulate the immune responses more than those from 20:5 n-3.

Because of these competitive metabolic effects between n-6 PUFA and n-3 PUFA the recommended level for the ratio of linoleic to α-linolenic acid is 4 or less. Muscle from forage finished cattle and sheep usually has an n-6:n-3 ratio below 2 whereas for pigs it is nearer 20. However, the presence of C20 and C22 n-3 PUFA in the muscle albeit at a low level is important because of the few dietary items that contain them and the recommendation that their consumption should be increased to overcome the high n-6:n-3 ratio in the overall UK diet.

Fatty acids of considerable interest at present are the conjugated linoleic acids (CLA). One isomer, 9-cis, 11-trans is present in ruminant meats and milk. It is formed either in the rumen as the first step in biohydrogenation of linoleic acid or by Δ^9 desaturation in body tissues of transvaccenic acid, itself produced in the rumen. CLA isomers inhibit carcinogenesis, decrease atherosclerosis, modify the immune response and partition energy toward the growth of muscle rather than adipose tissue (Pariza, 1997). Hence it has potential value in human nutrition and animal production. Amounts in ruminant meat and milk can be altered by dietary means (Dhiman *et al*., 1999; Enser *et al*., 1999). However, the value of CLA in human nutrition and the amounts required for therapeutic effects remain to be established.

Conclusion A certain level of fat in muscle is necessary for optimum tenderness and juiciness and meat fatty acids are responsible for specific flavours resulting from changed production systems. Meat fat is important in human nutrition with n-3 PUFA and CLAs playing a beneficial role.

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Meat Structure and Quality

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This abstract is built from the work of many members of our research group in Copenhagen. The names in brackets indicate the main people involved in each element of the reported results

Introduction

For most common meats, the most important aspects of eating quality that determine overall acceptability are taste (flavour), texture (especially toughness/tenderness) and juiciness (water-holding). Unexplained variations in tenderness and water-holding are two of the consistent problems in the industry and sources of consumer dissatisfaction. A basic understanding of the causes of variability in toughness and water-holding is therefore important to the meat industry, because an understanding of the mechanisms controlling these aspects of eating and processing quality is desirable if we are to reduce undesirable variations and ensure high acceptability. Despite considerable research in the last century, we have met with only partial success in explaining the causes of such variations. This warns us that the complete picture is multivariate and complex. Toughness and water-holding are in fact both properties determined at several levels of structure within muscle tissue, from the molecular, through to macroscopic. Our current programme of research contains several related areas of work which aim to further our understanding of the structural mechanisms at several levels of organisation which cause variations in tenderness and water-holding of whole meat.

Structures involved in meat texture

Current models of meat texture concentrate on the proteolytic degradation of myofibrils as the greatest cause of the development of tenderness during post-mortem storage, with degradation of the extracellular (collagenous) component playing little role. Whilst there is no broad agreement of which proteins within the myofibrils are the principle substrates of the major proteolytic enzymes, recent attention has been directed towards the cytoskeletal proteins (Taylor et al, 1995; Wheeler et al 2000). These studies show that cytoskeletal proteins are degraded during conditioning of meat.

Identifying the location of cleavage sites in the desmin molecule caused by specific enzymes (Caroline Baron)

We have recently investigated the way in which the principle cytoskeletal protein in the intermediate filaments of skeletal muscle (desmin) is cleaved by the two major enzyme systems in muscle, calpains and cathepsins. Both μ- and m-calpain quickly degrade the isolated protein. Sequencing of the fragments shows that the primary mode of attack by calpains is cleavage of the non-helical regions of the molecules, which are responsible for the interactions building the individual molecules into the polymeric structure of intermediate filaments. Calpains therefore seem to rapidly depolymerise the desmin molecules, but have only limited ability to cleave within the alpa-helical rod domain of the molecule. In contrast, cathepsin B appears to have the ability to sequentially break the desmin molecule down into very small fragments and amino acids.

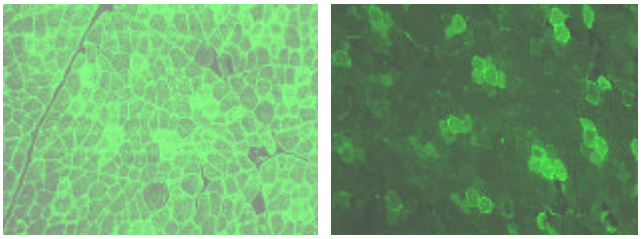
We are currently using the yeast two-hybrid molecular biology technique to identify as many substrates for calpains as possible by scanning for interactions between the binding domain of μ- and m-calpain against a complete muscle cDNA library *(Anna Larsen)*

Degradation of cytoskeletal proteins varies between muscles (Heater Morrison, Moira Lawson)

We have studied degradation of cytoskeletal proteins during post-mortem conditioning by inmmunohistochemical labelling of transverse cryosections from five porcine muscles; *longissimus, iliocostalis, semitendinosus, semimembranosus* and *psoas major.* In agreement with previous findings (Morrison et al, 1998), the intensity of immunolabelling for desmin using monoclonal antibody D33 decreased overall during 7 days conditioning, but in *longissimus* muscle the spatial distribution of this decrease was not uniform; labelling intensity was more quickly lost from type IIB fibres than types I+IIA muscle fibres.

In contrast to the *longissimus*, the gradual decrease in labelling intensity for desmin was more uniform across all fibre types in the other four muscles. Only in *semimembranosus* was there a suggestion of fibre type-specific reduction in labelling, although this trend was minimal compared to that seen in *longissimus*. The results suggest that cytoskeletal protein degradation in *longissimus* is atypical of the process in porcine muscles generally. As well as a fibre type-specific variation in desmin degradation seen in *longissimus*, there also appears to be a muscle-specific effect not solely due to muscle fibre type distribution.

Immunolabelling for desmin (D33 antibody) in porcine longissimus 1day (left) and 7 days (right) post mortem. The brightly labelled fibres at 7 days correspond to types I+IIa (Morrison et al, 1998)



Variations in cytoskeletal degradation due to fibre type composition (Mette Christensen)

Western blots of muscle homogenates reveal that the proteolytic degradation of desmin and troponin-T are faster in porcine *longissimus* and *semimembranosus* than in *semitendinosus, vastus intermedius* and *soleus* muscles. *Longissimus, semimembranosus* and *semitendinosus* muscles were predominantly composed of type IIb fibres, whereas *soleus* had a mixed composition and *vastus intermedius* composed mainly type I fibres. These inter-muscle differences in the rate of proteolytic degradation therefore cannot be explained solely by differences in fibre type distribution alone, but may also be influenced by other muscle-specific traits. To determine whether the local environment within the muscles affects the proteolytic behaviour of fibre types, type II fibres from each of the five muscles were isolated and the rate of proteolytic degradation of desmin and troponin-T determined. The results showed that the rate of proteolytic degradation of desmin and troponin-T in type II fibres varied between muscles, indicating that the local environment does influence the degradation of the individual fibres. The rate of postmortem proteolysis seems to depend more on muscle to muscle variations than on fibre type composition. (Christensen et al., 2000)

This may be explained by the fact that intracellular proteolytic enzymes are free to migrate out of the cell early (e.g. within 6 hours) post-mortem, and so can affect the proteolysis in neighbouring cells – which may be of a different fibre type. (Purslow et al, 2000) However, it is likely that the overall level of proteolysis in the whole muscle is related to the metabolism and pH development post-mortem, which in turn will be related to the proportions of different fibre types present in the muscle.

Structural basis of water-holding

In terms of water-holding, accepted models (Offer and Knight, 1988) emphasise shrinkage of the myofilament lattice within the myofibrils due to changes in pH and temperature post-mortem as the principle mechanism of drip development during storage of meat. This mechanism is undoubtedly the driving force of drip formation. However, as Offer & Knight realised, the shrinking myofibrils must be connected to the cell membrane in order for water to be transported extracellularly and to eventually appear as drip. The desmin-rich intermediate filaments and costameres provide such a connection. Kristensen & Purslow (2001) have shown that the proteolytic degradation of these cytoskeletal proteins reduces the ability for water lost between myofibrils to be transported to the extracellular space, so improving water-holding in later stages of post-mortem storage. (*Lars Kristensen*)

Structural basis of variability in drip loss (Annette Schäfer)

Unexplained variability in water losses from pork and poultry has considerable economic importance. In the past the pale, soft and exudative (PSE) condition in pig meat was identified as an extreme cause of drip loss. In Denmark, even after virtual elimination of PSE, problems of variability in drip loss have not been eliminated.

Studies within the last decade in the USA have revealed a large problem with excessive drip. A 1992 survey of 14 commercial plants (approximately 40% of their national capacity in 1992) revealed more than half the total production could qualitatively be classified as having excessively high drip. Only a small proportion of this was due to PSE; the rest had normal colour but was soft and exudative ("RSE"). Large variations between plants and between days in the same plants indicated that much of the variability could be due to environmental or animal handling procedures. Although variability in water-holding in Danish pork is not perceived to be so extreme, there is a need to identify causes and sources of variability in order to be able to control this quality attribute most effectively.

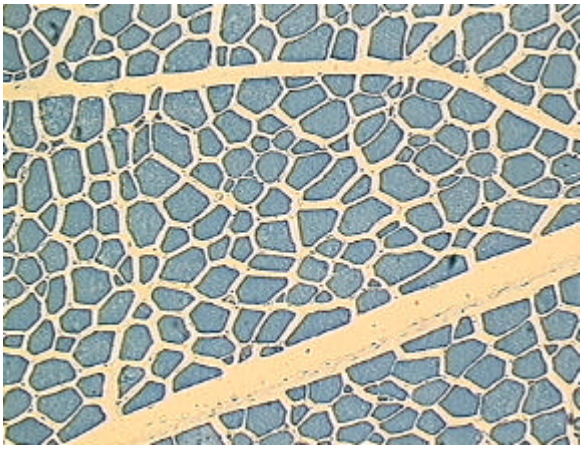
A survey by the Danish Meat Research Institute shows that variability in drip loss from pork produced in commercial Danish slaughterhouses is small compared to the US survey, but considerable variations still exist. Measures of paleness (L-value) and drip loss show that there is a continuous spectrum of variation; the division of meat into different classes (e.g. "PSE", "RSE", etc) is rather arbitrary, depending on the limits set. A hypothesis that handling and behavioural stress can lead to high drip loss was supported by studies of conventional versus "low stress" handling of pigs at a commercial Danish slaughterhouse. Significantly lower drip losses are found in animals subjected to less stress immediately prior to slaughter (Støier et al, 2001).

In an experimental model run by our collaborators at DIAS Foulum, the physiological stress on animals just prior to slaughter is being manipulated by running pigs on a treadmill (the exercise model). Pigs of various breeds show an increased drip loss and elevated L-value with exercise compared to unstressed controls. Increased stresses by electrical stunning also tend to increase drip loss compared to carbon dioxide stunning. There may be breed-related variations in the susceptibility of animals to stress; animals that are heterozygous for the halothane gene show increased tendency to excessive drip and pale meat colour after exercise.

Increased drip loss in this model population is generally associated with a faster pH decline post-mortem, although this may not be fast enough to produce the myofibrillar protein denaturation associated with PSE.

Structural studies by electron microscopy confirm that high drip loss in commercial meat is associated with a greater shrinkage of the lattice of thick and thin filaments within the muscle cells. Analysis by X-ray diffraction of filament spacing in a variety of muscles shows that the rate and amount of lattice spacing shrinkage also varies with sarcomere length; shortened muscle shows a higher shrinkage than stretched muscle (Schäfer et al., 2000).

Meat with high drip loss (whether pale or normal in colour) tends to have a more open structure, with greatly increased extracellular space development during the first 24 hours post-mortem.



Transverse section of a high drip loss (>10%) pork longissimus muscle 6 hours after death. Abnormally high amounts of spaces exist between muscle cells. Drip can more easily run out of the large channels between muscle fibre bundles. *(Photo: Annette Schäfer, KVL)*

Variations in electrical impedance of meat with time post-mortem are known to reflect changes in the permeability of the muscle cell wall. Our measurements of impedance indicate that fast pH decline and high drip loss may also be associated with rapid permeabilisation of the muscle cell wall. Increased sarcolemmal permeability obviously increases the ease of transfer of intracellular water to extracellular spaces and drip channels.

Variations in the degree of proteolysis of cytoskeletal proteins during the first 24 hours post-mortem are indicated by a previous study to be linked with high drip loss. Slow proteolysis of intermediate filaments and costameres may increase the co-operative lateral shrinkage of linked myofibrils and muscle cells. In meat with fast proteolysis the reduction in integrity of the cytoskeletal – extracellular linkages seems to reduce the opening up of large drip channels. Current work aims to quantify the relationship between NMR relaxation times and the structural location of water inside and outside the muscle cells.

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Computerised tomography for carcass analysis

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Introduction Computer tomography (CT) scanning is a method for non-invasive imaging of subjects developed for use in human medicine. It allows cross-sectional images, containing a wealth of information, to be obtained for a living animal (Davies *et al.*, 1987). These can be used to provide very accurate assessment of body composition in live animals in a welfare-friendly manner. Not only is accuracy improved but also a wide range of novel traits lend themselves to assessment and objective measurements can be collected rapidly, using mathematical algorithms for image analysis (Glasbey & Robinson, 1999; Glasbey *et al*., 1999).

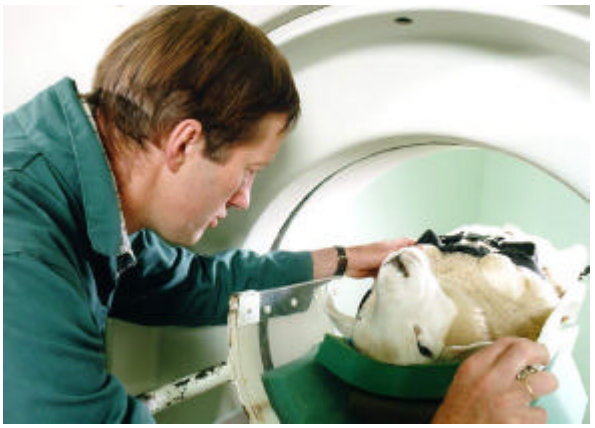

Plate 1. *Sheep being carefully positioned prior to scanning. Sheep are lightly sedated to minimise stress, and carefully restrained in a specially designed cradle. These procedures also reduce animal movement, and so maximise scan quality while minimising scanning duration.*

What does CT scanning animals involve? For CT scanning animals are gently restrained to minimise movement and ensure animal safety. For sheep, a mild tranquilliser is administered and animals lie on their back restrained by broad webbing straps and soft foam pads (see Plate 1). Pigs require higher levels of sedation, as they are powerful and less tractable in handling.

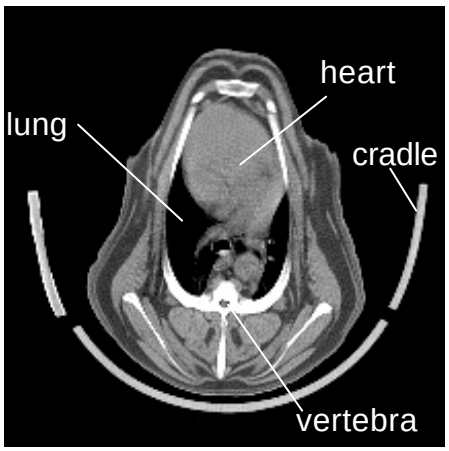

Plate 2. *Tomogram scan through the chest of a live sheep. The lungs are black because they are mostly air.*
The animal is lying on its back in a purpose- built cradle. Bone is white, muscle is light grey, fat is dark grey and air is black.

What do CT scans look like? Scans are produced of two types. The first, a ***topogram***, looks like a conventional X-ray image (not shown here). This is used to position the second type of scan, a ***tomogram***, on the basis of skeletal landmarks (Young *et al*., 1996). This tomogram is a two-dimensional reconstruction of a cross-section through the body. It makes use of information gained from absorption of a low dosage X-ray beam that passes through the subject from all angles around the body. The picture produced is a greyscale of density with dense tissues appearing lighter and less dense tissues appearing darker (see Plate 2).

Compared to other in vivo imaging methods, CT scans produce images with uniform and high resolution throughout the image. Coupled with excellent discrimination between fat, muscle and bone, on the basis of density, it is an ideal imaging method for detailed study of the carcass in live animals.

Visual inspection of images demonstrates the wealth of detail contained in just a few scans. In terms of carcass evaluation it is more "WYSIWYG (what you see is what you get)" than alternative methods. Tomography, be it X-ray CT or magnetic resonance imaging (MRI), produces data that allows objective assessment of the relative proportions between tissues (composition), distribution/ partitioning and shape of tissue units.

Carcass value Meat consumed by humans is predominantly made up of muscle together with some adipose tissue. It has been established that consumers in most western societies want their meat to have low or modest levels of visible fat (Wood & Fisher, 1990). In terms of eating quality, consumers rate tenderness as the single most important feature followed by juiciness and flavour (Wood, 1995; Thompson, 1998). Processors and retailers also want a product with reasonable shelf life to help in marketing and supply. At present carcass value in most markets is directly influenced by fatness but the other characteristics described influence value indirectly, or not at all (Jones, 1995; Price, 1995).

Trimmed fat and bone represent waste to the consumer and processor. A desirable carcass should have a high proportion of muscle. In addition, the location of fat affects value. Some cuts of meat contain seams of intermuscular fat, which are only seen when the customer cuts up cooked meat. In contrast, it is desirable in many markets to have highs of fat within muscle, the intramuscular or marbling fat, which is believed to indicate eating quality (Wood, 1995).

As with most products, size is an important determinant of carcass value. However, producers need to improve the image of sheep meat in terms of quality so other traits, some new, will receive increasing attention in the future (Dewar-Durie, 2000).

Why scan live animals? There are several ways to improve carcass quality in meat animals. Manipulating the environment, primarily through quantity and quality of nutrition, offers one avenue (Emmans *et al*., 2000). However, there are strong reasons why emphasis should be put on altering the genetic potential of animals for growth and development of their carcass tissues. Traditional selection, whereby the best animals are kept as parents for the next generation, offers permanent, cumulative gains that fit well within a sustainable livestock production system (Simm, 1998, Hill *et al*., 2000). Coupled with modern statistical approaches to breeding scheme design and genetic analysis substantial gains are possible (Simm, 1998). Modern breeding methods also provide the most economic way to select for improvement in a suite of traits.

Early research highlighted the potential of CT scanning for the study and evaluation of animals (Vangen & Skjervold, 1981; Young *et al*., 1987) and theoretical predictions presented a clear and convincing case for supplementation of ultrasound scanning with CT scanning of elite animals in breeding programmes. Such predictions forecast increases in genetic progress that may be as high as 50% (Simm & Dingwall, 1989) or closer to 100% (Jopson *et al*., 1995) through use of CT in combination with ultrasound scanning.

Carcass form – the comprehensive model Defining the carcass is not a trivial task. It is a complex shape and there are a variety of subtle differences between animals that simple descriptions will not take account of. It is worth reiterating that size is a dominant effect when it comes to describing differences between animals. It is also the easiest to measure objectively. We will devote most of our attention to other differences in form and the objective description of these. To do this we will work with a model of body form that we believe accounts for all major variations in carcasses.

The COMPREHENSIVE model of body form

The major determinants of body form can be separated into five main categories (with examples of possible measures):

1. Size – overall (e.g. bodyweight, frame size)
2. Proportions of major tissues (e.g. fat%, muscle to bone ratio (M:B))
3. Distribution and partitioning of tissues (e.g. %muscle in high priced cuts, %muscle in loin, %fat in subcutaneous depot)
4. Shape of tissue units e.g. muscularity (e.g. volume relative to length, width relative to depth)
5. Density of tissues (weight relative to volume, chemical composition).

Animal weight (category 1) is an all-encompassing integration of the volume and density of the three major tissues, their distribution and shape. However, since animals can be selected for slaughter at different stages of growth, our real interest is on the form of animals of a similar size or on how these other traits change with overall size during growth.

It is reasonable to expect that changes or differences in one factor are associated with changes in one or more of the others e.g. during growth the proportion of muscle changes relatively little whereas decreases in bone% are associated with increases in fat% (Butterfield, 1988). This will affect body form both directly (category 2) and through changes in shape of tissue units (category 4) concomitant with changes in the relative size of muscles compared to the bones they are associated with (Young & Sykes, 1987).

It is noteworthy that carcass composition, in terms of dissectable tissue weights (category 2), is part of this description of overall body form. Variation in the relative proportions of these three tissues affects geometric form since the tissues are not uniformly distributed throughout the carcass, with the muscle being intimately (and functionally) related to the underlying framework of the skeleton while fat tends to be more superficial.

Value of carcasses is affected by categories 1 and 2 of this model through carcass size and fatness, and commercial carcass classification systems focus on these two characteristics (Kempster, *et al*., 1982; Price, 1995). To what extent other components are valuable is less clear. Many classification systems assess "conformation" which is related to shape of the carcass overall (Price, 1995). This is a less than useful definition as it has many different interpretations and it is almost always associated with fatness within a genotype or feeding system (Kempster *et al*., 1982; Kirton *et al*., 1983; Butterfield, 1988; Jones, 1995). More useful is "muscularity" which refers to the shape of muscles (Butterfield, 1988). "Blocky" (thick relative to length) is the desired shape.

As animals grow, fatness increases and muscles become blockier (Young & Sykes, 1987; Butterfield, 1988; Price, 1995; Abdullah *et al*., 1998). Since carcass bone proportion decreases, muscle to bone ratio (M:B) increases as well (Young, 1989). Traditionally this "fleshing out" of the carcass as animals grew was highly desirable. However, with the modern trend against fat, traditional values for the lean carcass (blocky muscles and high M:B) are compromised since the most obvious way to reduce fat is select animals that are less mature. That this has occurred is borne out by the results of many breeding programmes that have reduced fatness by selecting animals that are less mature at a given age or size (Lewis *et al.*, 1996; Abdullah *et al*., 1998; Emmans, *et al*., 2000). Thus animals are larger but less mature, fitting neatly the theory of genetic size-scaling so elegantly described by Taylor (1985).

Since fatness is one dimension of classification, further classification on the basis of conformation implicitly measures some other aspect of body geometric form. This is assumed to be muscularity but it may also be due to M:B, muscle distribution or fat partitioning (e.g. more intermuscular fat underlying muscles).

Given that carcasses are cut up before the consumer sees the meat, some authors argue that shape is not important unless it is useful as a predictor of something else. However claims that this is the case for traits such as yield (%) of lean meat from a carcass have not been supported by research (e.g. Kirton & Pickering, 1967; Jackson & Mansour, 1974; Kirton *et al*., 1983; Price, 1995). Nevertheless, shape of cuts can affect the yield of consumer-ready cuts of a certain size, particularly for smaller animals such as sheep. The value of shape *per se* warrants further investigation.

Study of geometric form A strong argument exists for more rigorous examination of geometric form in animals and to put this into the context of our whole body form model. While shape has received less attention in research to date, it is the basis on which much subjective assessment of animals occurs. Lack of attention or lack of progress, in elucidating the nature of geometric form, in part reflects the complex shape of the body and its components and in part the large volume of data that would have to be collected to describe it objectively.

Single cross-sectional scans cannot be used to describe 3-dimensional shapes directly. Use of several CT scans across the carcass or within a region does allow this. This may be further refined by considering the distance between scans since it is known that animals vary in the number of skeletal elements making up the spine (Pálsson, 1939; Zhang & Siqin, 1998) which are used for locating cross-sectional scans.

Shape information is what remains once location, orientation and size features of an object have been dealt with. One commonly used shape statistic is a measure of compactness, which is defined to be the ratio of the area of an object to the area of a circle with the same perimeter (Glasbey & Horgan, 1995). Another statistic often used to describe shape is a measure of elongation. This can be defined in many ways, one of which is as the ratio of the second-order moments of the object along its major and minor axes. However, the description of shape is an open-ended task, because there are potentially so many aspects to an object even after location, orientation and size effects have been removed. We are investigating a number of different approaches too the description of shape including the use of landmarks (Dryden & Mardia, 1998) and warpings such as thin-plate splines and other morphometric methods (Bookstein, 1991), which consider image plane distortions needed to move landmarks to designated locations.

Such research requires detailed data from live animals, which CT can provide. There will be two significant outcomes from this work. Firstly we will have validated our model of body form and objectively described the relative impact of different components on body and carcass form. Secondly, these findings will be used to enhance current CT scanning services for commercial animal breeders such that carcass quality can be a focus in the breeding objective.

Most significantly, it is our intention to examine and describe links between carcass form and meat quality which require objective methods for comprehensive description of carcass form. This is essential information if the industry is to ensure that the focus is on meat as a consumer product at all levels of the sheep production, processing and retailing industry (Dewar-Durie, 2000).

Current scanning methods for the sheep industry Three years of research (1997-1999) conducted at SAC has demonstrated the value of CT. Table 1 summarises accuracies of prediction from a number of trials. Using ultrasound scan data we would expect prediction accuracies (R^2) in the order of 65% and 50% for fat weight and muscle weight respectively. A consequence of the increased accuracies shown for CT results is high heritabilities (0.40-0.50) for fat weight and lean weight, one of the reasons CT scan data helps breeders.

Our work has shown that a standard scanning approach using scans at just three specific anatomical locations (see Plate 3) is valid across a range of sheep genotypes and ages. From just these scans, objective measurements can be made of traits in all five categories of our model of body form. Presently the focus is on size of the major carcass tissues (categories 1 & 2) but we can also estimate muscle distribution. However, our research has found little variation in this as reported in the literature (e.g. Butterfield,

Table 1. Prediction accuracy of CT scans (R^2%, residual standard deviation and mean). Predictors are carcass tissue areas from two or three scans plus liveweight.

Carcass tissue		fat	muscle	bone
Prediction of		Weight (g)	Weight (g)	Weight (g)
Meat sheep	R^2%	99	97	89
	rsd	434	611	313
Predicted variable mean		8620	13880	4130
Hill ewes	R^2%	98	91	54
	rsd	400	562	225
Predicted variable mean		5102	13068	3815
Hill lambs	R^2%	92	86	73
	rsd	191	388	184
Predicted variable mean		2820	7930	2550

1988). We are presently looking to develop measures of tissue shape, muscularity specifically, based on these three scans and will investigate more optimal scanning protocols where the focus is on geometric form (categories 3 and 4). Preliminary findings show we can make useful predictions of intramuscular fat content from muscle density (R^2 c.50%). We are looking at ways to improve the accuracy of this prediction.

In 2000 a service was implemented to CT scan sheep for the breeding industry. Most gains are to be had from large populations and MLC agreed to subsidise scanning costs for the major meat sheep sire referencing schemes. Elite animals, identified on farm on the basis of ultrasound scanning, are CT scanned at three anatomical positions (Plate 3). These scans are processed using a variety of semi-automated image analysis routines to produce measures of fat, muscle and bone area in each scan. Together with liveweight, these data are used to predict total carcass tissue weight (see Table 1). These data are incorporated into genetic analyses run for the whole sire referencing scheme. As a result, more accurate estimates of genetic merit are obtained for the animals scanned and, most importantly, ALL their known relatives.

Experience gained in the development of image analysis software and through inspection of scans from close to 6000 sheep in the last four years has brought the issue of body form to our attention. There are clear differences between animals in terms of shape and these seem to relate to terms experienced breeders use to describe good "conformation". Plate 3 illustrates this for two animals from the same flock (other similar examples exist). In this case the better animal (A) has extreme development of muscle in the loin (middle) scan and a more compact shaped gigot (upper scan). It is also apparent that the better development of *M. longissimus dorsi* (the "eye muscle") apparent in the loin carries through to the forequarter (lower scan).

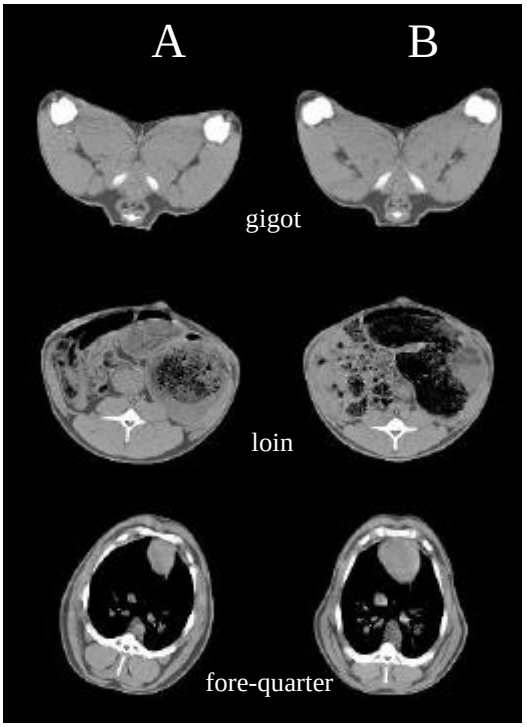

Plate 3. *Two sheep of contrasting conformation or muscularity (A vs. B). These were chosen as they had similar liveweight (63.0 vs. 62.5kg) and similar predicted carcass fatness (27%). They differ for M:B (4.21 vs. 3.77), muscle weight (18.9 vs. 17.4) and fat weight (8.6 vs. 8.1).*

Conclusions CT scanning will be an essential tool for the characterisation of carcass form in live animals. Using the model of body form presented, sophisticated descriptors of body form can be derived which will enable appraisals to be made of the relative importance of the different determinants of body form. These are fundamental to work that will establish the link between carcass merit and eating quality. Knowledge of these associations is critical for development of appropriate breeding objectives for sheep breeders.

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Milk Fat Composition and Nutritional Value

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Introduction

The perception of milk has changed over the past 25 years from one of being the ideal food to one of being detrimental nutritionally, mainly due to the fatty acid composition of its fat component. Now however, it has been discovered that milk contains a number of compounds, which may have positive nutritional benefits. It also appears that the association between saturated fatty acids in milk and effects on cholesterol may have been an oversimplification. It is accepted that the hypercholesterolaemic saturated fatty acids in milk fat are confined to lauric (C12:0), myristic (C14:0) and palmitic acid (C16:0) with the shorter chain saturated fatty acids and stearic acid having no cholesterol raising effect. Indeed bovine milk fat contains two fatty acids which may have important beneficial effects on human health, namely conjugated linoleic acid (*cis*-9, *trans*-11 linoleic acids – C18:2, CLA) and butyric acid (C4:0). Also monounsaturated fatty acids have been shown to be beneficial in altering the proportions of LDL and HDL cholesterol and it is possible to increase the concentration in milk of the principal monounsaturated fatty acid, oleic acid (C18:1), by optimising the diet of the cow. This paper will discuss nutritional strategies to optimise milk fat composition with particular reference to work from my own Research Centre in relation to oleic acid and CLA.

Increased Unsaturated Fatty Acids in Milk Fat

Initially work on increasing the unsaturated fatty acid content of milk fat concentrated on the polyunsaturated linoleic and linolenic acids. Feeding formaldehyde-treated casein and safflower oil, formaldehyde treated oilseeds such as mixtures of ground sunflower and soyabean seeds or full fat soyabean flour resulted in the production of milk fat containing up to 350 g of linoleic acid and 220 g of linolenic acid per kg of fatty acids. However, this milk fat was highly susceptible to lipid oxidation and its melting profile was unsatisfactory in that the fat became liquid at about 20^{o}C. The upper limit for the concentrations of these polyunsaturated fatty acids in milk fat is likely to be determined by their effects on the functionality and keeping quality of the fat.

It has been shown that dietary monounsaturated fatty acids have beneficial effects on plasma lipoproteins in humans (Mattson and Grundy, 1985). It is now accepted that oleic acid lowers LDL cholesterol without affecting HDL cholesterol, which is beneficial in man because there is an inverse relationship between HDL and atherosclerosis (Tall, 1992). Therefore, there has been interest in modifying milk fat so as to increase the concentrations of oleic acid, and reduce the hypercholesterolaemic fatty acids. Such an alteration would also result in fat with a more desirable melting profile because an increased ratio of oleic to palmitic acid results in softer milk fat.

We investigated the effects of feeding crushed full fat unprotected rapeseeds (FFR) and soyabeans (FFS) to cows on a basal diet of grass silage indoors (Table 1). The short and medium chain fatty acids (with 4 to 14 carbon atoms) were reduced, on average, by proportionally 0.18, palmitic acid by 0.24 and stearic and oleic acids were increased by 0.54 and 0.47, respectively. The resulting solid fat content at 10^{o}C was approximately 400 g/kg compared to approximately 520 g/kg in the milk fat from the control cows. This level of solid fat indicated that butter manufactured from the modified milk fat, while softer, would not be spreadable directly from the fridge. Feeding protected Canola, which is rich in oleic acid resulted in similar changes in the fatty acid composition of milk fat. Oleic acid was increased from 238 to 292 g/kg of total fatty acids, linoleic acid from 22 to 49 g/kg of total fatty acids and palmitic acid was decreased from 312 to 232 g/kg of total fatty acids (Ashes *et al.* 1992).

Milk fat produced at pasture, compared to indoors, is generally softer and has a higher ratio of oleic to palmitic acid. Butter made from this milk fat, though softer than that from milk fat produced indoors on grass-silage was still not spreadable at refrigeration temperatures. The effect on fatty acid composition and fat softness of offering FFR and FFS to cows on pasture was studied at Moorepark. The short and medium chain fatty acids were reduced, on average, by proportionally 0.34, palmitic acid by 0.21 and oleic acid was increased by 0.35. These fatty acid changes resulted in solid fat at 10^{o}C of 341 g/kg from which butter spreadable at refrigeration temperatures could be manufactured. Comparing the data from the control diets in the indoor and pasture studies shows that milk from cows at pasture had a substantially higher ratio of oleic to palmitic acid (1.21) than milk from cows indoors on grass-silage (0.58). The respective solid fat contents at 10^{o}C were 520 and 401 g/kg respectively. These data highlight the enhanced fatty acid composition of milk fat produced on pasture and the opportunity to optimise composition by relatively simple supplementation with whole oilseeds containing high proportions of 18-carbon fatty acids.

Table 1. The fatty acid profile (g/kg of total fatty acids) of milk produced on diets based on grass-silage and pasture either unsupplemented or supplemented with full fat rapeseed (FFR) and full fat soyabeans (FFS)

Basal Forage	Grass-silage			Pasture		
Oilseed Supplement	**None**	**FFR**	**FFS**	**None**	**FFR**	**FFS**
C4:0-C14:0	267	221	219	224	135	162
C12:0-C16:0	493	373	378	375	267	291
C16:0	324	243	246	238	181	197
C18:0	92	140	143	115	121	142
C18:1	187	281	268	288	427	351
C18:2	23	20	29	18	23	48
C18:3	5	4	5	7	5	9

Increasing the level of Conjugated Linoleic Acid (CLA) in milk fat

CLA refers to a mixture of positional and geometric isomers of linoleic acid involving conjugated double bonds in geometric variations of *cis-cis, cis-trans, trans-cis* or *trans-trans* at a number of positions in the carbon chain. The *cis*-9, *trans*-11 isomer is believed to be the biologically active form and the term CLA is used here to denote this isomer. CLA is produced naturally in the rumen as an intermediate in the biohydrogenation of dietary linoleic acid to stearic acid and in tissues by the action of the delta-9 desaturase enzyme on trans vacennic acid. It has been associated with important biological activities including anticarcinogenic activity, antiatherogenic activity, the ability to reduce the catabolic effects of immune stimulation, the ability to enhance growth promotion and the ability to reduce body fat. Because of these positive biological activities CLA is a target fatty acid for enhancement in milk. A recent study by our group demonstrated that milk fat triglyceride bound CLA in CLA enriched milk fat, obtained by supplementing cows at pasture with full fat rapeseed, was cytotoxic towards the human breast cancer MCF-7 cell line.

The CLA concentration in Irish manufacturing milk varies throughout the year, mainly due to dietary factors and is between 5.5 and 16 mg/g of fat. It is higher on pasture than on indoor diets and is related to the quantity of grass available in the diet. Some recent results obtained at our Research Centre indicate that milk fat CLA concentration may be affected by the grass cultivar grazed by cows but further evaluation of this is required. Supplementing the diet with oils containing linoleic acid resulted in increased CLA in milk fat (Dhiman *et al*. 1997, 1999). Oilseeds rich in linoleic acid were superior to those rich in oleic or linolenic acids in enhancing milk fat CLA (Kelly et al. 1998). At our Research Centre pasture fed cows supplemented with FFR and FFS had significantly higher CLA concentrations in their milk fat compared to cows supplemented with unmolassed sugar beet pulp. Recently it has been shown that supplementing cows with fish oil increased milk fat CLA (Offer et al. 1999; Donovan et al. 2000). The challenge nutritionally is to maintain a similarly high concentration of CLA in milk from indoor fed cows as is possible with pasture fed cows. In our studies substituting a portion of the grass silage fed indoors with pasture or supplementing it with a by-product feed consisting of a mixture of brewers grains and beet pulp increased milk fat CLA content. While the influence of cow breed may be small the cow to cow variation in milk fat CLA concentration can be quite large and, at least within a lactation, there is a significant correlation between concentrations of CLA in individual cow's milk fat at different sampling times. Milk fat CLA concentrations measured on different diets and with different breeds of cows, at Moorepark, are given in Table 2. Thus, milk fat containing elevated concentrations of CLA can be achieved by diet optimisation and cow selection and this could provide the Dairy Industry with an opportunity to carve out a new market niche for specialised dairy fat-containing products.

Other milk fat components with nutritional benefits

Butyric acid is a unique feature of milk fat from ruminant animals. About 0.33 of all milk triacylglycerols contain one molecule of butyric acid which occupies the sn-3 position. It inhibits cell growth and induces differentiation in a wide spectrum of cancer cell lines including those of the breast and colon (Parodi, 1999). In Irish manufacturing milk butyric acid concentrations were at their highest in March and April (45-47 g/kg of fatty acid methyl esters) and lowest in September (39 g/kg of fatty acid methyl esters). The seasonal variation is not very large and since butyric acid arises from de-novo synthesis, the opportunity to modify its concentration in milk fat by nutritional means is likely to be poor. Sphingomyelin accounts for about 0.33 of the total milk phospholipids in bovine milk. Ceramide and sphingosine both metabolites of sphingomyelin participate in major antiproliferative pathways of cell regulation that supress oncogenesis. Strategies for modifying the concentration of sphingomyelin in milk have not been considered.

Certain metabolites of eicosapentanoic and docosahexanoic acids posses antiatherogenic properties. The major source of these fatty acids in dairy cow diets is fish oil. Reports cited by Palmquist *et al*. (1993) indicated that fatty acids with greater than 20 carbon atoms reached levels of 60 to 70 g/kg of total fatty acids when diets were supplemented with fish oils. However, others estimated the apparent efficiency of transfer of these fatty acids into milk fat to be very low. The effectiveness of incorporating these fatty acids into bovine milk fat needs further study.

Table 2. The effect of different diets and breeds of cows on milk fat CLA concentrations measured in recent experiments at Moorepark.

Grass Cultivar (g/100g FAME[1])				
	Diploid early heading	Diploid late heading	Tetraploid early heading	Tetraploid late heading
	1.72[a]	2.16[b]	1.45[a]	1.72[a]
Supplementation of Pasture (mg/g of fat)				
	SBP[2]	FFR[3]	FFS[4]	
Day 11	10.7[a]	14.0[b]	19.7[c]	
Day 18	13.4[a]	17.2[b]	21.9[c]	
Day 32	16.6[a]	19.6[a]	24.0[b]	
Supplementation of Grass-Silage (g/100g FAME[1])				
	Grass-silage	Grass-silage + pulp and brewers grains		
	0.50[a]	0.78[b]		
Cow Breed (mg/g of fat)				
	Irish Holstein-Friesian	Dutch Holstein-Friesian	Montbeliardes	Normandes
Sample 1	17.0[ab]	14.7[a]	18.3[b]	15.5[ab]
Sample 2	17.9	16.7	18.6	17.2

[1]Fatty acid methyl esters [2] sugar beet pulp; [3] full fat rapeseed; [4] full fat soyabeans; within rows means not sharing a common superscript differ significantly (P<0.05)

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Gene Regulation of Muscle, Meat and Milk

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Meat and milk production from farm animals is dependent in part on fetal development and post-natal growth of specific tissues. This paper will review some of the systems controlling both muscle and mammary growth which are related to meat and milk production and some of the genes known to regulate these production traits.

Genes regulating muscle

Mature muscle size is dependent on a number of critical stages during development and growth. The stages are the determination of muscle progenitor cells, the proliferation of these progenitor cells, and finally the formation of multinucleate fibres and their subsequent growth. The first two phases occur during fetal development and the growth of fibres is initiated during late fetal development and continues throughout post-natal growth.

The formation of muscle progenitor cells is dependent on factors derived from gene products derived from surrounding tissues, in particular the neural tube, notochard, dorsal ectoderm and lateral plate mesoderm. Some of these factors have been identified: WINT, Sonic Hedgehog and Bone Morphogenic Protein 4 (1,2).

The induction of muscle cells involves the expression of muscle specific transcription factors, myogenic regulating factors (MRF), which are essential for the early development of muscle (3). There are four MRFs which have different functions. MyoD and Myf5 are involved in the regulation of myoblast and satellite cell proliferation of muscle fibres, whereas myogenin, and possibly MRF4, induce differentiation and formation of myotubes (3).

The second stage of muscle development is proliferation of myoblasts and then differentiation into fusion-competent myoblasts which eventually fuse to form myotubes. The number of myoblasts appears to determine the number of fibres per muscle and so muscle size. Myoblast proliferation can be manipulated by a number of growth factors such as fibroblast growth factor, insulin, platelet derived growth factor, transforming growth factor, epidermal growth factor and leukaemia inhibiting factor (4). A positive example is the insulin-like growth factor, which stimulates proliferation of myoblasts during fetal development and causes differentiation of myoblasts so they can fuse to form myofibres. Selection for high IGF-I in 12-week old mice results in their offspring having increased muscle mass and decreased fat, compared with the line selected on 12-week body weight which has a fatter carcass with a reduced muscle mass, when compared with the unselected controls (5).

A factor which inhibits myoblast proliferation is myostatin, a member of the TGFβ superfamily. The myostatin gene, which maps to the muscular hypertrophy locus, has been shown to be mutated in a number of cattle breeds exhibiting double muscling (6). This clearly indicates that manipulation of genes controlling determination and/or proliferation of myoblasts can have a dramatic effect on muscle mass and meat production in mature animals.

Myoblasts eventually exit the cell cycle and become terminally differentiated and fuse to form myotubes. The MRFs are the most characterised factors which have been shown to regulate the expression of muscle specific genes. There are now indications that, in pigs, polymorphisms in the MRFs are related to post-natal growth and weight of lean meat (7).

Terminal muscle cell differentiation also results in the sequential expression of muscle-specific genes, which includes the structural and contractile proteins found in muscle. Although polymorphisms have been found in some of these genes, such as myosin light chain -/- fat gene, no association has been found between these polymorphisms and muscle/meat characteristics in cattle.

The bulk of post-natal muscle growth results from satellite cell division and incorporation of their daughter cells into the multinucleated fibres, which then grow. Post-natal muscle growth can be significantly affected by nutrition and exercise and there appears to be a number of genes which are associated with muscle growth under these different conditions.

Local IGF-I gene expression increases with work induced muscle hypertrophy whereas in adult sheep IGF-II decreases with muscle growth associated with nutrition. Expression of the inhibiting gene myostatin increases with muscle atrophy but is not affected by growth controlled by the level of nutrition.

There have been reports of a number of animal models which show an abnormal increase in muscle growth after birth. The Callipyge sheep (8) and compact mouse (9) have extreme hindquarter muscling, which develops post-natally. The Callipyge gene has been localised to chromosome 18 and the compact mouse appears to be associated with a mutation in the pro-peptide region of myostatin.

There are undoubtedly many other undiscovered genes controlling various aspects of muscle growth which, when identified, may be of use for the future selecting and engineering meat producing animals of the future.

Genes regulating milk yield

From a physiological perspective, milk yield is determined by the number of secretory cells and the productivity of those cells. The latter parameter is determined not only by nutrient supply but also the proportion of cells which are actively lactating as opposed to being engorged with milk and quiescent (12).

The key question with regard to the establishment of the number of secretory cells at the start of lactation is - what determines the extent of udder growth (and by inference the number of secretory cells) and what are the genes involved? There is a possibility that rates of mitosis in the udder during pregnancy is important but there is little evidence that an increased rate of udder growth in pregnancy can enhance ultimate udder size except in Jersey cows of high genetic merit or in twinning sheep where, in both cases, enhanced udder size is greater in association with increased rate of growth in late pregnancy (10).

Ultimately the extent of secretory cell proliferation in ruminants may be determined simply by the size of the mammary fat pad – which is laid down early in foetal development. The boundaries of the fat pad may be the constraint which limits the number of secretory cells which can develop (22).

While much is now known about the genes underpinning the endocrine control of udder growth in terms of systemic hormones and local growth factors and cellular interactions in the udder, this information is not necessarily going to be useful in understanding how the size of the secretory cell population is determined. Currently, there are large gaps in our knowledge as to how the existence of a pregnancy is signalled to the udder to initiate full development and also as to how much of a constraint is the size of the mammary fat pad. Somewhere among the many QTL's (21) linked to milk yield will be the genes which give the answers. Such genes may be expressed in the mammary gland but may also be hormones expressed in other tissues such as placenta or liver.

In the lactating udder the regulation of milk output from the secretory epithelial cells is achieved through systemic and local pathways. The most famous of the systemic pathways is the somatotropin axis where bovine somatotropin is a key endocrine regulator. However, while the augmenting effects of bST on milk yield is well described, how this is achieved at the udder is not known, although recent research has demonstrated expression of the gene for a bST receptor in the secretory epithelium (17). The thyroid hormones also regulate milk synthesis (17) but again the exact mechanism of action at the udder remains to be established.

Local pathways regulating milk yield have been demonstrated. In particular, these pathways regulate milk yield in relation to milking frequency and it is likely that elements of the same pathway are linked to the persistency of milk production through effects on cell loss from the udder. Specific factors in milk which inhibit milk secretion have been reported. A feedback inhibitor of lactation has been partially characterized as a novel low molecular weight peptide (16) while a proteolytic cleavage product from β-casein has also been suggested to be part of the local pathway (18).

Loss of secretory activity will ultimately result in programmed cell death and increased cell loss from the udder. From a dairy production point of view the pathway of cell loss is less important than the triggers which ultimately lead to cell death. Whether FIL or the β-casein product are effectors in vivo remains to be established.

Genes regulating milk composition

Within the udder, synthesis of milk fat, protein and lactose is highly coordinated implying some commonality in the regulation of these very different metabolic pathways.

The level of water secretion into milk largely determines the fat and protein content of milk. The rate of water secretion is mostly determined by the rate of lactose synthesis, lactose being responsible for about 70% of the osmolality of milk. Differences between Jersey and Friesian milk in terms of fat and protein content can largely be ascribed to differences in relative rates of lactose synthesis, although exactly how this is achieved is unknown. One key gene in the pathway of lactose synthesis is the whey protein α- lactalbumin and manipulation of this protein in transgenic animals has resulted in variation in water secretion (11).

About half of milk fat is synthesized in the udder, the other half supplying mostly longer chain fatty acids being taken up from blood triglyceride. In future, as the human population gets more and more conscious of limiting energy intake it is likely that demand for milk fat will decline which may increase the demand for dairy animals which produce low fat milk. Certainly this trait is heritable (15). Candidate genes which might be involved in regulating milk fat output include acetyl CoA carboxylase and lipoprotein lipase.

One key gene involved in fat metabolism in the udder is the desaturase gene which not only enhances oleic and linoleic acid content of milk fat but is also involved in the formation of conjugated linoleic acid (CLA) a compound which may have anti-cancer properties (13).

The udder is one of the most active tissues in terms of protein synthesis and within the milk proteins there is a high degree of coordination of synthesis particularly within caseins and whey proteins. Much has been determined about transcription factors which regulate milk protein gene expression (20), but the regulation of translation remains more of a mystery although the nature of regulatory elements has been described in other tissues.

Among the bioactive components in milk, antibacterial, immunomodulatory, mitogenic and many other activities have been described, most of which are attributable to genes expressed in the mammary gland. There are also a wide range of genes involved in mineral transport and metabolism in the mammary gland. A key role for casein is to act as a carrier for calcium and phosphorus to provide for the enhanced requirements of the rapidly growing young animal. Calcium, in particular, is an important cell regulator and a series of transporters and binding proteins have been described in the mammary gland (14).

There are many, many other genes regulating milk synthesis and being regulated in the mammary gland. The nature of these genes will be exposed in the next 5 years or so. Finding out what these genes actually do will take a lot longer.

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An overview of Yak Production

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14-15 million yak (*Poephagus grunniens*, or *Bos grunniens*) provide the mainstay of life of several million people in the vast plateau (approx. 3 million sq. km) on "the roof of the world" - the provinces of Tibet, Qinghai, Sichuan and Gansu in western China, with the largest populations. Other provinces of China and countries, including Nepal, India, Bhutan, Mongolia (0.5 million yak) and some of the Russian Federation have smaller, but locally important populations. A small herd has existed at Whipsnade Wild Animal Park in England for about half a century and a population of 2-3 thousand has now grown in North America.

Yak provide meat, milk, transport, wool and hide, fuel (from their dung) and many by-products. They live mostly at altitudes from 3.5 thousand m to 5 thousand m) but lower in the more northerly latitudes. Typically, the vegetation grows from mid May to late August followed by a period when the wilted herbage remaining on the pastures provides a decreasing maintenance supply of feed. From January until May, starvation or near starvation is not uncommon and a 25-30% loss of body weight is usual. Supplementary feeding is not usually available. Yak have a number of anatomical and physiological features seen as adaptations for survival to the harsh climatic conditions and lack of feed over large parts of the year. Yak females calve for the first time at 4 or 5 years old and then every two years. (In the USA and Canada where yak are managed like conventional beef cattle on range, productivity is higher). Typically, adult yak females weigh 200-300 kg and produce between 200 and 400 litres of milk in the year of calving and about two thirds of that amount in the second year without calving again. Fat and SNF content of the milk is high.

There are two principal types of yak - the "grassland" or "Plateau" type (the majority) and the Alpine type in mountainous areas with deep valleys (where supplementary feed is sometimes available). For each type exist a number of breeds, but no clear evidence of the extent to which these breeds differ from each other genetically (except in terms of colour in some cases). Breeds (breed names) are generally local to different areas of the country and hence breed and environment are confounded.

Hopes of improving the productivity of the yak, and therefore the standard of living of the herders and their families, have to overcome difficulties compounded by custom, culture and religion, not only by lack of money and infrastructure. Increasing the availability of feed over the winter should be a first priority. Improved health care and disease control is limited by lack of veterinary services and finance. Changes in management practices to reduce overgrazing, especially in winter, is easier to advocate than to address, because animal numbers are still widely equated with wealth and status. Genetic improvement is inhibited by an absence of recording - but measures to avoid inbreeding may help in the short term, Strategies for crossbreeding (within yak species) might be developed if breed differences can be identified. Selection should be considered if nucleus groups with pedigree and performance recording could be set up along with an infrastructure that would permit the dissemination of improved genotypes. Hybridisation of yak with other species of cattle has been traditionally practised at intermediate altitudes to combine some of the extra productivity of the cattle and the adaptive characteristics of the yak and an unknown contribution of heterosis. The use of exotic breeds like the Holstein is advocated by officialdom and has a minor role to play in limited and favoured circumstances, but is not an overall solution for increasing productivity. Hybrid males are sterile.

A small population of wild yak still exists in parts of Tibet and Qinghai, but it is endangered. Although wild yak are protected by law they are still being illegally hunted and numbers are still declining. Potentially they are a useful genetic resource as they are substantially larger than domestic yak and crosses of wild and domestic yak appear to generate hybrid vigour.

An interesting question for the future is whether the yak has unique genetic factors that could provide a basis, through genetic engineering, for extending animal production to harsh, cold, inhospitable environments in other parts of the world.

Further reading: Cai, Li and Wiener, Gerald (1995). The Yak. Regional Office for Asia and the Pacific of the Food and Agriculture Organisation of the United Nations, Bangkok, Thailand. 237 pp. (ISBN 974-89351-0-8)

Animal Rights and Wrongs

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Animals are an object of concern, and, as we extend our control over the earth's resources, ever more so.

The old way of distinguishing humans from other animals - in terms of the soul, God's purpose, and the after-life - is no longer universally accepted, and the Darwinian theory of species has unsettled people's moral perceptions. Is there any way of making the distinction between humans and other animals, therefore, that could recommend itself to modern, secular, sceptical people who believe nevertheless in the difference between right and wrong?

Animals have minds, but the mind is organised on various levels of complexity: sensation, perception, desire, belief, and finally self-consciousness and the moral life that stems from it. Animals can attain to the fourth of those levels, but only if they attain to the last are they strictly on a par with us, from the point of view of moral judgement.

You cannot have it both ways in morality. You cannot say that animals have the benefits of the moral life, without also having the burdens. If they have rights, they have duties, and if they have self-consciousness, they must do what is right by others. In which case cats are serial murderers, mice are burglars, and foxes psychopathic vandals.

The only defensible position is that animals (at least, those which raise all the problems for us) have neither rights nor duties, and are strictly speaking under our protection. It is therefore permissible to keep them as pets, to raise them for food and to kill them when we have good reason.

This does not mean that we can treat them as we will. We have duties towards animals, even if they have no rights. But these duties depend on context and on the relation between us and them.

Morality springs from four sources: the moral law, which deals in rights and duties and which abstracts from the individual case; sympathy, which deals in fellow-feeling and which is biased towards the individual case; virtue, which controls our social emotions and makes us useful to others; and piety, which reminds us of our smallness, our dependence, and of the fact that we are judged. The first does not apply directly to animals; but the other three all have a bearing on our relation with them.

There are three kinds of relation which are important: to pets, to animals raised for their products, such as meat, milk, eggs and furs, and to animals in the wild.

Pets are honorary members of the human community, towards whom we have assumed a duty of care. Having made them dependent on us we have a duty to provide for them.

Domestic animals owe their lives to our use for them; but we must ensure that they have a life which is as fulfilling as is reasonable, given that use. Hence we ought not to keep pigs in battery pens; we ought not to slaughter animals inhumanely; we ought not to deprive them of a natural diet and a routine compatible with their nature.

Towards wild animals our duty is first to the species, and not to the individual. We must safeguard habitats, maintain the equilibrium of species, and be guided in our dealings by sympathy, piety and virtue.

If we take those arguments seriously, we shall find ourselves hostile to agribusiness, in favour of the old-fashioned and 'unhygienic' farmyard, and in favour of field sports (especially hunting with hounds and angling). We shall be suspicious of much that passes for the fishing of the seas, we shall prefer working companions to pets, and we shall be in favour of eating meat and raising animals for their skins. We shall offer only a hesitant endorsement of animal experiments and a hostile judgement of zoos. In the war between cat and bird we might reasonably favour the birds; but in that between man and rat we ought to stand by our species, provided we avoid the use of poison which, of all the crimes against the animals, is by far the most heinous.

The legacy of positivism and the role of ethics in animal science

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A slow but steady shift in perspective on the need to address ethical issues within the professional activity of animal scientists has been underway for almost twenty years. Increasingly the issue is less *whether* animal scientists should be discussing ethical issues in their classes, at their professional meetings and in their interactions with client groups, but *how* they should do so. This paper will provide some arguments in support of this general trend, and will make some suggestions about how animal scientists can increase their capacity to address ethical issues as part of their professional responsibility.

Looking back on positivism from the vantage point of the 21st century, we can see that it was, in fact, at the vanguard of the philosophical movement we now call 'postmodernism'. The sciences of the 18th and 19th centuries were committed to foundational methods of inquiry. Some, following Descartes, stressed logic and mathematics as the foundational basis of natural laws, and argued that science must proceed through a rigorous process of deduction from basic definitions and self-evident principles of reason. Others, following Locke and the empiricists, took the foundational metaphor more literally, and argued that science could be built, brick by brick, from observations of the world. 'Positivism' is a word that came to be used to describe a number of alternatives to the foundational view. Some stressed the role of prediction, while others stressed verification. What they shared was the view that the quality of a scientific claim should not be assessed by looking backwards at its origins or foundations, but by looking ahead, to replication by others, to confirming or corroborating experimental results, or to real-world technological applications. In short, the focus shifted from foundations to justifications.

Several commentators have suggested that animal scientists reflect a positivist attitude toward their role, and that positivism has provided a rationale for excluding ethics from the professional life of the animal sciences. Positivism was a pervasive and very influential philosophy of science in the early 20th century, and its influence on the practice of science in both teaching and research settings was complex. On the one hand, the main doctrines of positivist philosophy dealt with methods of inquiry and the testing or justification of theory. This made it seem as if positivism itself had little to say about ethics or ethical issues. On the other hand, positivism *was* an ethic for the practice of science, and the rationale for being a positivist drew upon many principles that should continue to guide animal scientists in their approach to ethical issues.

During the early stages of the change from to foundations to justification, it was crucial to develop criteria that were independent from moral, ethical, religious or political norms. Some of the most notorious failures of early 20th century science—especially in genetics—involved putative justifications in light of contested moral norms. Nevertheless, there are three senses in which science—including animal science—cannot be made wholly independent from ethical norms. First, science is a process of rational inquiry and as such is ethically committed to a procedure of argumentation and consensus formation that presumes rules, common goals and a conception of fair play. Second, there are certain phenomena that can only be defined in light of ethical or normative reasons why they are of interest. Arguably, both health and welfare are such phenomena. Third, the rationale for undertaking many scientific investigations is often contingent upon the pursuit of personal or social goals, and these goals can themselves be subjected to ethical evaluation.

In each case, it is possible and important to distinguish the role of ethical justification from justification in terms of scientific criteria that appeal to logical consistency, predictive power, replication and the like. Much of the motivation for configuring the disciplinary norms of the animal sciences is based on the judgment that preserving and strengthening the distinction between ethical and scientific justification is an important thing to do. But this judgment is itself an *ethical* judgment, rather than a scientific one. Those who argued for positivism at its origins were not shy about basing their arguments on ethical grounds, but positivism was, in effect, undone by its own success. By investing so much intellectual energy into the protection of scientific criteria for the justification of theories and their results, animal scientists deprived themselves of the intellectual resources that they need to justify this very investment. And that is not to mention the ability to justify the use of their theories and results in the pursuit of both individual and social goals.

People who lack the ability to justify their activity in ethical terms often fall back on power plays of one sort or another. When scientists do this, they lose credibility and trust, for the value of science to society depends in large measure on the shared belief that appeal to coercive, political or economic power has no place in scientific justification. What is even worse is when the decline of critical, ethical argumentation actually allows scientists to align themselves with ethically indefensible practices and goals. Arguably, the decline of capacity and willingness to undertake ethical justification has reached crisis proportions in the agricultural sciences during the last four decades. As I indicated at the outset, I believe that the corner has been turned, and that we have now entered a phase of rebuilding that capacity.

As I wrote in my 1999 paper in the *Journal of Animal Science* (Thompson, 1999), the way to work out of this situation is, above all, simply to do it. Bioethics is emerging as an interdisciplinary field in which people with disciplinary training in the natural and social sciences collaborate with philosophers to examine critically production and consumption practices, along with research, and to frame arguments about the ethical justifiability of these practices.

These arguments will come to naught if they are not challenged and critiqued aggressively (but respectfully) by others. But if the argumentation and debate takes place *within* the professional world of animal science, it will build capacity and provide a basis for both wiser and more articulate defense and justification of practices among producers, consumers and citizens.

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Ethical issues in animal biotechnology

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During the 20th century scientists have made genuine progress in explaining and modifying usefully the processes of life. The main leap forward was, of course, the rediscovery of Mendelian genetics at the beginning of the century. Since the 1930s this theory has been put to use in an ever more efficient and systematic way, particularly in breeding domestic animals with desired traits. Thus the animal breeder can now plan how he wants future generations of domestic animals to be, knowing that the plans will work.

A number of reproductive technologies have been developed to make selective breeding more efficient. Artificial insemination, freezing of semen, embryo transfer and trans-vaginal oocyte recovery followed by embryo production *in vitro*, are used increasingly to ensure that animals with good genetic potential produce more offspring than they would otherwise have had. These techniques reduce the generation interval, which means that the breeder's aims can be realised more rapidly. Looking ahead, sex selection is another reproductive technology which could prove useful in improving breeding efficiency.

But it is molecular genetics that holds the promise for a major leap forward in man's ability to control the processes of life. By means of so-called marker assisted selection it is possible to select highly specific traits at the genetic level. Moreover, through transgenesis, genes, and their phenotypic expression, may be moved across species barriers.

Until now molecular genetics has had relatively little impact on the breeding of domestic animals. This seems to have been for two reasons: First, scientific understanding of how the individual genes interact with the animal's phenotypic traits is at present limited. Secondly, the technology of gene transfer is still in its infancy.

Viewed in the context of these developments, the high profile unveiling of the cloned sheep "Dolly", by scientists at the Roslin Institute in Scotland in February 1997, was merely one further step in our efforts to interfere with the processes of life. Cloning somatic cells may turn out to be a useful way of disseminating the genes of female animals which possess desirable genetic potential; and the technique used to create Dolly may help in creating genetically modified animals from modified cells and thereby boost the development of transgenic animals.

However, viewed from another perspective Dolly made a big difference. This single sheep brought to many people's attention the fact that scientists had made a major breakthrough in their attempt manage and control life. It also gave rise to a widespread call for ethical limits to the interference with life to be established and enforced. Until recently the main limits to interference with life were of a technical kind: of what it is possible to do. Now, and increasingly, scientists are faced with ethical limits: of what it is *acceptable* to do.

The increase in power, and the potential increase of speed and efficiency that modern breeding and biotechnology presents, force us to recognise our moral responsibility and to discuss the limits of acceptability. In such discussion ethics provides a way of ensuring systematic and rational reflection on the moral issues involved within a framework of values and principles guiding behaviour.

The aim of this paper is to present an overview of various ethical considerations to which the applications of modern biotechnology in breeding of domestic animals gives rise. Furthermore, these considerations are to be subjected to critical reflection.

The first part of the paper will give an outline of how the use of biotechnology in animal production is perceived by the European public. The point of departure for this part will be the results from the Eurobarometer surveys on biotechnology, showing a European population sceptical towards the manipulation of animals. In the 1999 survey, the cloning of animals is grouped together with food biotechnology, as the least accepted applications of new biotechnology. This is so even though the assessed application of cloning is medical, a use that generally has higher public acceptance. On the basis of focus group interviews conducted by one of the authors, the findings of the surveys will be elaborated and explained in more detail.

The second part of the paper will provide a more systematic discussion of the considerations about animal biotechnology, partly drawing on the positions identified in the qualitative and quantitative analysis. Apart from well-known ethical categories and concerns like utility, risk, animal welfare, animal integrity, environmental concerns and human health, this will also include the fear that there is a "slippery slope" from the use of biotechnology on farm animals to uses on humans.

The final part of the paper will discuss possible regulatory reactions paying respect to the public scepticism, as expressed in the ethical categories identified earlier. Among other issues we will address the regulatory dilemma of constructing a consensus based regulation, when parts of the population are fundamentally opposed to genetically manipulated domestic animals.

The ethical basis of intensive livestock production systems

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Many of the ethical issues regarding the livestock production systems of the late twentieth century have concentrated on the process of intensification. The extent of public disquiet at the welfare and ethical implications of intensification has been reflected in the burgeoning membership of animal welfare and animal rights organisations. The reasons for this increase as well as the impact that it has had on the livestock industry can be traced back to the 1960s, to factors such as the growing urbanisation of the population and especially the emergence of the animal rights movement which focused attention on a wide range of issues including the human exploitation of other animal species. This in turn led to a demarcation between those who supported the animal welfare cause and those who argued for animal rights.

The human understanding of the welfare needs of livestock has been greatly enhanced by developments in the fields of ethology and biochemistry. These developments have provided fresh insights into the experiences and perceptions of animals kept in intensive systems. The overall result of these developments is heightened public awareness of animal welfare issues, which has had an impact on both diet and consumerism. Thus there is evidence to indicate that some members of the public will only purchase animal products which they believe to have been produced in welfare-friendly systems. As a consequence of consumer pressure, food retailers have either initiated their own welfare assurance schemes or have linked with one or more of the schemes established from within the livestock industry.

The fact that the livestock industry has initiated assurance schemes illustrates the fact that many livestock producers have taken very seriously ethical concerns over intensive systems. Most livestock producers maintain that any proficient stockperson must be concerned for the well-being of their stock and that the care of animals must be their over-riding interest. Anything less would be deemed unethical and offensive. Producers often make such statements however, without reference to any ethical basis on which to substantiate their arguments in the face of growing criticism of their methods and systems of production.

This paper seeks to provide that ethical basis by arguing that:

1. The principle of respect for the essence of an animal is an acceptable ethical principle for the assessing of livestock production systems.
2. Respect is expressed agriculturally in stewardship and stockmanship, which consequently become the criteria for judging the ethical acceptability of livestock systems.

The implications of applying the respect principle to various methods of livestock production are considered, including the battery production of eggs, the use of farrowing crates for pigs and routine animal mutilations. It is argued that some techniques and procedures employed in intensive systems of livestock production, such as farrowing crates, are ethically acceptable, whilst others, such as current battery production systems for poultry, are not.

Factors likely to encourage or hinder acceptance of the respect principle are also identified and examined. Of these, the likelihood of increased costs for both producer and consumer is identified as one of the most negative. Factors likely to encourage the wider acceptance and adoption of the respect principle include the development of consumer/retailer/producer partnerships through Farm Assurance Schemes, an increased commitment in the livestock industry to training in stockmanship skills and greater political commitment, not only to animal welfare legislation, but to the viability of those producers affected by it.

It is concluded that the application of the respect principle must therefore result in some major modifications to certain production methods, which will lead in turn to significant improvements in the welfare of the animals concerned. It is also recognised that intensive livestock production is not by definition unethical and that there are certain practices and systems within this category which are perfectly acceptable and which actually improve the welfare of the animals concerned.

Nutrition and Production – the Scientist

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Introduction

Reducing costs for unit output, be it per kg liveweight gain, per kg carcass gain or per kg saleable meat yield will be essential for the future of the UK beef industry. Traditionally emphasis has been placed on reducing feed costs per unit output and data from MLC's Beefplan shows that between 65 – 82% of the variable cost of UK beef systems are accounted for by feed costs. However, with fixed costs being similar to total variable costs, it is also important to reduce the major components of fixed costs specifically associated with the production of feed and feeding it which are labour and machinery. As far as nutrition/feeding of beef cattle is concerned, there are a number of areas where a reduction in costs can be achieved using existing knowledge but in other areas there are obvious gaps in knowledge which require further research. However, there appears to very little research into aspects of the nutrition/production of beef cattle going on, or being published, in the UK. (Only 4 out of a total of 113 papers presented at this conference are on nutrition/production of beef cattle.)

Effective diet formulation requires good information on the nutritive value of foods, the nutrient requirements of cattle and an accurate prediction of voluntary food intake. The majority of beef systems still rely on grass silage as winter forage. The use of NIR for evaluating both the nutritive value and intake characteristics of silage, coupled with least-cost computerised ration formulation programs using a modified UK ME system, gives diets which produce performance close enough to expectations in most situations. However, improvements to such programs to give better descriptions of the substitution rates of silage by the amount and type of supplement fed are required. Also required is a better description of the composition of live weight gain at various weights for the breeds/crosses now used in the UK. The current ME system overestimates the energy requirements of late maturing continental cross bulls, especially when these are taken to heavier weights. A co-ordinated effort is needed to develop improved nutritional programs for diet formulation for beef cattle but funding such a task would be a major problem. (A 'Feed into Beef' project similar to the 'Feed into Milk' that is funded by the MDC and industry would be a way forward!)

The UK Metabolisable Protein (MP) System was developed primarily for use in dairy cows but its use in its original form overestimates the ERDP and underestimates the DUP components of the protein requirement of beef cattle. This applies to some silage based finishing systems and also intensive systems of production. In both systems feeding excessive protein levels has been shown to increase carcass fatness which is undesirable, and is costly, wasteful and environmentally unfriendly.

Intensive finishing systems are becoming more popular as a result of low cereal prices relative to the costs of silage production. Such systems involve ad-libitum feeding from hoppers/bunkers that only need filling once a week thus saving on labour costs. Methods of reducing the costs of harvesting, preserving and processing cereals for feeding need to be evaluated. Whole crop wheat/barley, urea treatment, crimping and harvesting with a forage harvester fitted with a mill are all options. The latter is a new development which allows late harvesting of crops in virtually all weather conditions, either to produce conventional whole crop silage or, by cutting only the heads, a high energy feed which can be fed as a supplement or as the sole diet in intensive systems.

Although cereals are likely to be the major ingredient in intensive diets, the roles of high energy but fibrous by-products require evaluation to ensure that feed conversion ratio (FCR), and hence production costs, are minimised.

The patterns of performance in terms of liveweight gain, FCR and feed costs/kg LWG for various breeds/crosses need to be described so that better guidelines as to optimum slaughter weights are available. Routine weighing would allow producers to monitor patterns of performance for their enterprise to ensure animals are meeting their target performance and allow the cattle to be marketed at an optimum weight.

Levels of protein in intensive diets are generally too high and savings in feed costs can be achieved by feeding lower protein levels without compromising performance. A feeding system in which low protein cereals and a protein supplement are offered free choice, allowing the cattle to choose the level of protein appropriate to their needs, may be a way forward to reduce both feed costs and the labour and machinery required to mix diets. However, more work is needed to test whether this system works in all situations.

Conclusions Improvements in methods of intake prediction and estimating energy and protein requirements are required for incorporation into more effective diet formulation procedures. Ideally these improved systems should be made generally available for all to benefit but this may not happen for commercial reasons. Routine monitoring of animal performance should be carried out to ensure that the target performance for the system is being achieved. Newer methods of cereal harvesting and storage should be evaluated as a means of reducing the cost, risk and hassle of getting the crop from 'field to trough'. However, obtaining funding to carry out this work will be a problem (or a challenge).

Genetic Improvement of Beef Cattle -the Scientist

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Introduction For thousands of years, perhaps ever since the domestication of cattle, farmers have favoured, and therefore selected, certain animals above others in an attempt to create a population of beasts best suited to their needs. Over time the sophistication with which breeding cattle have been selected has improved as technology and understanding has developed. In Great Britain today the genetic improvement of cattle makes use of the three main methods available: breed substitution, crossbreeding and within-breed selection. This paper concentrates on the last of these but recognises the importance of the first two in commercial practice. For example there has been considerable substitution of native British beef breeds with continental breeds during the 1960s and 70s and there is continued use of crossbreeding on dairy cows and in suckler beef production. Within breed selection and performance recording is valuable for the continued improvement of pedigree beef cattle and as a means for commercial producers to select suitable herd sires.

The national resource Purebred beef animals constitute only a small proportion of the beef cattle population of Great Britain but have an essential role to play in the production of high genetic merit breeding bulls for crossing. The pedigree beef population is estimated to be around 80 000 cattle across more than 30 breeds. Through natural service and AI bulls from this population sire around 2 million calves a year. About 35 000 beef cattle are performance recorded, with 8 breeds accounting for 50% of performance recording.

'Beefbreeder' is the national pedigree beef evaluation scheme (run by Signet Farm Business Consultancy). It is designed to identify pedigree beef cattle with the genetic potential to produce calves that have good conformation and grow quickly. The system is designed to overcome the two difficulties that hamper genetic progress in Great Britain, namely the small herd sizes and the influence of management on performance. Without Beefbreeder, the small herd size often found in the UK pedigree beef industry means that the number of contemporaries in a herd, between which valid comparisons can be made, is low. This restricts the choices when making selection decisions and so limits the rate of genetic progress. A performance trait, like growth rate, is an expression of an animal's genotype. Management and the environment in which the animal lives influence performance traits. Without Beefbreeder comparisons between animals can only be made with confidence within herds (and then only between animals of approximately the same age, managed together) or within performance test groups. With Beefbreeder, comparisons can be made between individuals born in any herd, year or season.

Best Linear Unbiased Predictor (BLUP) In 1991 the statistical procedure known as BLUP - which has been used in the UK dairy industry since the early 1960s - was first used on data from pedigree beef herds in Britain following extensive MLC-funded research in SAC and the Roslin Institute. Essentially BLUP attempts to separate out the genetic factors influencing an animal's performance from the non-genetic (or 'environmental') factors such as management and feeding. In order to make this separation genetic links between contemporary groups are required. For example, at least one individual in a contemporary group must be the offspring of a sire who also has offspring in another contemporary group. Because of the wide use of AI in pedigree beef cattle links of this kind within breeds are good. Hence, related animals link small herds, and comparisons between herds become possible. BLUP compares related animals with their contemporaries across many different herds using the related animals as a benchmark because they share genes and are therefore expected to perform more similarly than unrelated animals.

The calculations are carried out using an individual animal model, which is the most sophisticated method of BLUP analysis available. What this means is that for each animal information on its own performance is used as well as all available data relating to the performance of its performance recorded relatives.

All the information relating to several recorded traits is then analysed *simultaneously* taking into account any correlations between traits. For example data on 200-day growth contributes to the evaluation of 400-day growth because the two traits are correlated. The degree to which each trait is inherited by the next generation (heritability) is also taken into account.

Estimated Breeding Values Estimated Breeding Values (EBVs) for each animal for each trait are then produced which are estimates of the genetic worth of the animal. The EBVs are termed multi-trait because they are calculated from information on all the measured traits. Because the influence of the environment has been accounted for the EBVs of all the animals in the breed evaluation can be directly compared, no matter which herd they belong to, which dramatically increases the size of the genetic pool from which replacements can be chosen. EBVs can also be compared across years, which allows genetic trends and progress to be monitored.

EBVs have the same units as the measured trait (e.g. kg for liveweight) and are expressed relative to a common baseline for all animals in the same evaluation. For all breeds in the UK the baseline is currently set so that the average of the breeding values for animals born in 1980 is zero. Accuracy values are presented along with the EBVs and provide a guide

to the likelihood of an EBV changing (up or down) as more information on the animal and its relatives becomes available. High accuracy values gives the commercial buyer confidence that animals with above average EBVs will pass these desirable characteristics on to their progeny.

Table 1 EBVs for nine recorded traits are currently produced:

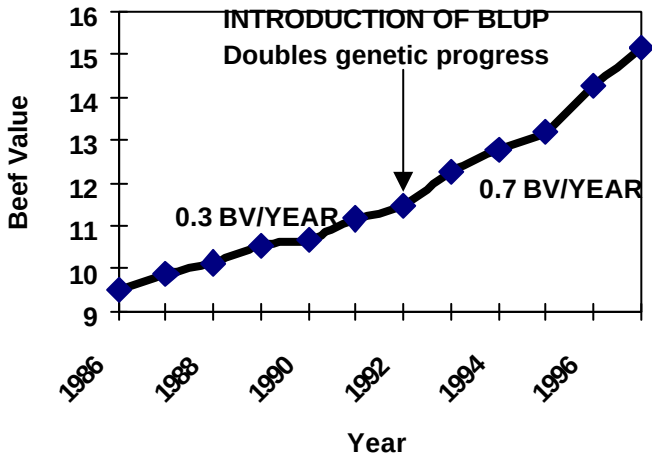
EBV	Unit of measurement
Direct	
200-day growth	kg
400-day growth	kg
muscling score	recorded at 400 days on a 1-15 point scale
muscle depth	measured in mm by ultrasonic scanning at 400 days
backfat depth	measured in mm by ultrasonic scanning at 400 days
birthweight	kg
gestation length	days
calving ease	expected change in percentage of unassisted calvings
Maternal	
200-day milk	kg

Multi-trait indexes EBVs are usually presented for each trait measured which allows breeders to decide how much emphasis they wish to place on each trait in selection. However, they can also be combined into a multi-trait selection index for a specific breeding objective, in this case for growth and carcass traits, and selection can be based on this. It is widely agreed that this is the most efficient method of improving several traits at once. Economic weightings relevant to current market conditions are used to ensure each trait is given the appropriate amount of emphasis in the index. The aim of multi-trait index selection is to maximise the change in breeding value for the overall objective. This can be expressed as the sum of the breeding values for all of the traits in the breeding objective, each weighted by its economic value.

Two indexes are currently produced for each animal in the Beefbreeder genetic evaluation service, the Beef Value and the Calving Value. The objective of the Beef Value is 'to improve financial value of the carcass by genetically improving carcass weight, fat and conformation scores in line with current commercial carcass pricing structures'. It ranks animals on the expected carcass financial merit of their offspring. The objective of the Calving Value is 'to improve profitability by reducing the costs associated with difficult calvings (e.g. veterinary costs, loss of production). It is designed to aid producers to select terminal sires that will produce calves that are born easily.

Benefits of using EBVs to aid selection If all the traits in both indexes are recorded, 80% of the variation among bulls will be due to Beef Value traits and 20% to Calving Value traits. Selection on high EBVs for all traits will result in bulls leaving progeny with heavier carcasses of better conformation at a constant age, shorter gestation lengths and which are easier to calve than average.

Genetic trends Until about 1990 the improvements in carcass characteristics achieved were mainly because of the positive correlations between growth and carcass traits. Since then the introduction of BLUP and the wide use of muscle scoring and ultrasound scanning has made selection more accurate and speeded up the rate of genetic progress.



In the last 3 years the Beef Value has increased at over 0.7 units per year which is worth nearly £1 million per year to the beef industry through improved efficiency. Over five years that is worth around £14 million. Genetic improvement using BLUP techniques is a potent tool that adds lasting value to the beef industry. Its effects could be improved still further if more beef animals were recorded and the EBVs used more ruthlessly. The best breeders currently progress at over 1.5 Beef Value units per year.

To a commercial breeder and finisher the difference between using an average bull and a bull from the top 1% of the breed is worth around £30 per calf finished at current prices.

Genetic improvements of beef cattle – the farmer/practioner

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Improves terminal sire line

JSR Farms at Givendale runs a commercial cross-bred suckler herd of 100 cows alongside a pedigree Charolais herd of 110 cows. The Charolais breeding programme is focused on improving the output from the cross-bred herd by using BLUP to select sires with superior genetic merit for production traits. Breeding stock with high EBV's for growth and carcase traits are selected for re-breeding and attention is also paid to 200 day milk EBV's and to controlling the level of calving difficulty. Home-bred replacement heifers are selected with Beef Values ranked in the top 10% of the National breed and the stud of reference sires used in the programme have Beef Values in the top 1% of the breed.

As the result of this fast-track genetic selection process significant progress has been made to improving growth and carcase traits as shown by graphs 1 and 2.

The combined improvement of growth and carcase traits shows an accelerated rate of Beef Value achievement when compared with the National breed averages, and also shows two Beef Value unit's improvement per year (see graph 3).

From a practical point of view what do these genetic gains mean in terms of improved output from commercial Suckler cows?

For several years at Givendale I have been progeny testing bulls with Beef Value's ranked from the top 25% to the top 1% in the breed. A picture soon started to emerge of significant difference in the performance of the progeny. Calves sired by top 1% bulls grew faster and had better conformation than the calves sired by those bulls in the top 25% range for Beef Value.

Table 1
Progeny test results Givendale suckled bulls

SIRES	*400 DAY WT KG*	*LW GAIN KG/DAY*	*CARCASE VALUE*	*DAYS TO SLAUGHTER*
CH 40	592	1.48	660	414
CH 35	570	1.42	631	410
Difference	+22	+0.06	+31	+4

In conclusion I would suggest that by selecting bulls with the highest beef values producers will significantly increase the output from their herds

Improved Suckler Dam Line

For years the dairy industry has supplied large numbers of beef-cross heifers as replacements for suckler herds. The heavily built Friesian, when crossed with our native beef breeds, produced highly fertile and efficient cows. They had the ability to maintain body condition on low-cost feeding regimes. They were robust, long-lived cows and their progeny finished to achieve high carcase specifications. The gradual expansion of Holstein bloodlines throughout dairy herds has resulted in dramatic changes to the type of animals now being used in suckler herds. These changes compromise efficiencies and therefore undermine profitability.
The disadvantages of Holstein-bred suckler cows can be summarised as follows: -

1) High-energy requirement – adding to feed costs.
2) Reduced fertility – prolonged calving periods.
3) Reduced longevity – increasing replacement costs.
4) Poorer progeny conformation – reducing income.
5) Excessive milk yields – leading to udder problems.
6) Reduced availability – UK dairy herd in decline.

Dairy-bred suckler cows are a by-product of high-yielding, large-framed, short-lived and poor conformation Holsteins. However, because of the simplicity of sourcing dairy-bred replacements there will be a continued demand for them.
It is more difficult to organise breeding programmes to produce efficient cross-bred beef cows using beef breeds with good maternal traits. The ideal cow-type should be of a medium size to control maintenance costs and should exhibit a

high level of reproductive ability, while retaining good beefing quality and longevity and adequate milk production. It is essential that these traits are consistently repeatable to maintain high levels of output.

All commercial suckler cows should be crossbreds.

Extensive trials in the USA have proved that crossbred cows and calves perform better than do pure-breds. The hybrid vigour generated by cross-breeding improves those traits with low-heritability such as fertility, calving ease, calf survival, milking ability, cow longevity and early puberty. All these traits combine to increase herd output by 22 % when compared with the output of the pure-breeds, which make up the cross-bred.

So how do we go about organising a breeding programme to produce heifers of the desired type? Members of The Beef Improvement Group (BIG) started a rotational cross-breeding programme in 1996 using South Devon and Angus bulls on existing cows. The plan was to retain the heifer calves and mate them to the two breeds alternately, i.e. rotational cross breeding. Members of BIG then went to the USA in 1997to visit the USDA Meat Animal Research Centre (MARC) in Nebraska and the Leachman Cattle Company in Montana. The outcome of that and several subsequent visits has led BIG to start a composite breeding programme in the UK using USDA MARC technology and Leachman genetics. Animal scientists at MARC have been working with 7000 cows for the last 30 years to develop more efficient methods of breeding high-output cow types. They have combined the differences of several breeds to create composite breeds with high levels of uniformity and reproductive efficiency. Their results have shown that composite breeding offers a procedure that is more effective than continuous cross-breeding for utilising genetic differences among breeds to achieve and maintain optimum performance levels for economic traits on a continuing basis, while retaining 75 % of the hybrid vigour generated by the first cross (F1). BIG has established a nucleus herd of the four-breed composite (MARC 2) known as the Leachman Stabiliser.

Table 2
Effect of cow breeding on performance

	Cont x Dairy	*Pure Cont*	*Rotation*	*Composite*
Lifetime Cow output (kg weaned calf/cow	1201	975	1620	1914
Calf Value (p/kg)	85	90	87	87
Margin per cow	15.04	17.23	57.02	65.02

Table 3
Effect of cow breeding on performance

	Cont x Dairy	*Pure Cont*	*Rotation*	*Composite*
Maintenance (MJ/d)	79	75	68	68
Calves / Cow	5	5	7	8
Fertility % (Calved/cow 12 week bulling)	89	78	89	92
Cost of calf (p/kg)	78.7	80.6	62.4	59.8

In conclusion it would appear that composite breeding offers a technique, which will provide an opportunity to increase output from specifically designed cow types. However large numbers of animals are required to build a sustainable programme to avoid in-breeding and this can only be achieved by breeders co-operating in an organised way.

Animal Health – The Scientist

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Introduction Food animal health impacts on productivity, animal welfare and human health. What happens to an animal on the farm and its resulting health status has an important influence on the quality, safety and wholesomeness of the meat and offal obtained from that animal. Our research has largely been focused on the environmental influences on beef cattle and sheep health, the farm environment and management, in particular and how it impacts on veterinary public health, i.e. food safety, and animal welfare. The main objective of the study was to examine the use of information about the health and management of cattle and lambs on the farm to predict the risk of visible lesions at slaughter. The feasibility of identifying farm-level risk factors for gross lesions detectable during post-mortem meat inspection has been investigated, from which the findings of the sheep study have been published (Edwards, *et al*. 1999).

Materials and methods Over 100 beef cattle and sheep farms were visited, the farmers interviewed and the farm and animals visually assessed. A cohort of animals was identified on each farm for subsequent follow-through to slaughter. When these animals were slaughtered the meat inspection findings were recorded. The cohort of cattle or lambs followed through to slaughter from each farm was used as the unit of analysis. Lambs and cattle in each cohort were slaughtered in batches over a period of time. The different abnormalities detected at post-mortem meat inspection comprised the outcome variables for the study. A cohort of cattle or lambs was considered positive for the abnormality if it was detected at slaughter in at least one animal in the cohort. To evaluate potential confounding factors and interactions among farm-level risk factors, unconditional multiple logistic regression analysis was performed using the statistical computer software package Egret (SERC, 1991).

Results Post-mortem meat inspection data were obtained for 7,046 lambs from 30 of the 48 farms originally enrolled in the study and 1,730 cattle from 40 of the 59 farms originally enrolled into the study. The most common lesions found in the cattle were abscesses, pneumonia/pleurisy, liver fluke, nephritis/nephrosis and trauma/bruising. In sheep the most common lesions were Pneumonia/pleurisy, lungworm, abscesses, liver fluke, nephritis, *Cysticercus tenuicollis* and *Cysticercus ovis*. Because many significant risk factors had a zero value in the univariate analysis and the sample size was small, it was not possible to construct definitive farm-level logistic regression models for the different abnormalities detected at slaughter. To illustrate how farm-level information might be used to determine the cohorts (groups of animals) that would be likely to have abnormalities at post-mortem meat inspection, the logistic regression model for any abnormality in sheep at slaughter is presented in Table 1.

Table 1: Farm-level logistic regression models for any abnormality detected during post-mortem meat inspection of 30 cohorts of slaughter lambs

Variable	β-coefficient	Odds ratio	95% CI* (odds ratio)
Constant	-3.24		
Age of cohort at slaughter (months)	0.87	2.39	1.18-4.83

* CI, confidence interval

The only risk factor significantly associated ($P<0.05$) with an increased risk of a cohort having an abnormality detected at inspection was the mean age of the cohort at slaughter. For each one-month increase in age, the odds of a cohort having at least one lamb with an abnormality detected at slaughter increased 2.39 times. For cohorts slaughtered at three months of age, there was a 35% risk of a cohort having an abnormality found at slaughter. By six months of age, the risk had risen to 88% and by nine months of age, it was 99%.

Discussion Since many risk factors analysed in the study applied to the farm, data were collected and analysed for a cohort of cattle or lambs from each farm rather than for individual animals. This approach proved problematic for those risk factors that differed among the animals in a cohort, for example, sex, disease occurrence and medication (Kabagambe, *et al*. 2000). In any system of meat inspection that uses information about the health and management of cattle or sheep on the farm, a decision will be required on whether to record data on a flock/herd basis or from individual animals. It is expensive to keep extensive records for individual animals, which must be identified. However, the power to discriminate between herds or flocks with and without abnormalities at slaughter on the basis of animal characteristics is diminished by averaging information across the herd/flock or by recording risk factors on the basis of what applies to a proportion of the herd/flock.

The farm-level risk factors associated with abnormalities at slaughter varied with the nature of the lesion. The most significant farm-level risk factor influencing post-mortem meat inspection findings for sheep was the age of lambs at slaughter ($P<0.05$). The risk of a cohort of lambs having any abnormality or lesions of pneumonia/pleurisy, abscesses and liver fluke all increased with increasing age of the cohort at slaughter. Few of the beef farmers recorded the age of their cattle on the batch cards, so the age could not be analysed as a risk factor for cattle. With the cattle the age range of animals within a single batch sent for slaughter usually varied far more than with batches of sheep. No single on-farm risk factor

was identifiable with regard to all lesions in cattle, which probably reflects the many different types of causal factors for lesions detectable at meat inspection.

With both species, environmental factors appeared to play a significant role in determining the cohorts that would have lesions at slaughter. These factors included the geographic region where the cohorts were reared, the farm terrain, vegetative cover and whether or not the animals were housed. In comparison, the health status of the animals and the disease prevention and control measures applied during rearing did not seem to be good predictors of the presence of lesions at slaughter, although coughing, runny nose, and anthelmintic treatment were significant predictors for lesions in cattle.

During the interviewing of farmers and farm managers for this study, it became apparent that not only was there poor record keeping of the medicines used, despite being legally required, other farm records relating to symptoms of sickness in cattle and lambs were rarely kept. The problem of poor record keeping on farms will have to be addressed if a valid assessment is to be made and any system of on-farm certification of livestock introduced. Farmers in the study indicated that they would have liked to receive the meat inspection findings for their lambs and a third of them said that they would improve their animal husbandry practice as a result of the information. However, none of the abattoirs in the study regularly passed the results of post-mortem meat inspection on to farmers. Therefore, it appears that the health status of cattle and sheep presented for slaughter might be improved if farmers had knowledge of the meat inspection findings from their livestock.

Conclusions This study investigated the feasibility of using a broad range of information about the health and management of cattle and sheep on farms to predict the risk of abnormalities at post-mortem meat inspection. Many farm-level factors were investigated, but, because of the small sample size, it was not possible to determine the important risk factors among the many correlated variables. If on-farm information is to be used to decide on the sheep and cattle that need to undergo a more thorough post-mortem inspection, a set of factors is required that adequately discriminates between animals with a high and low risk of abnormalities at slaughter. This study has indicated the variables that may be useful predictors of gross lesions in cohorts of cattle and lambs at slaughter, but a much larger study would be required to fully identify the definitive predictors. With cattle, the wider variation in management and environment within herds will make it more difficult to determine significant farm level risk factors than with sheep.

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Land use and the environment – the scientist

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Introduction Beef cattle can have a wide range of positive and negative impacts on the environment. Some of these impacts are general to ruminants, whilst other are specific to cattle, and, because of the type of management systems and habitats that they graze, there are some impacts which are specific to beef cattle. Impacts are often classified in terms of their potential to provide benefits and damage to the atmosphere, water, soil and vegetation. This classification will be issued below to explore the most important ways that cattle, and their land use, influence the environment and the extent to which scientists can increase understanding to influence these impacts.

Atmosphere In relation to the atmosphere, ruminants influence the amount of methane released and the amount of gaseous forms of nitrogen from the deposition of dung and urine and more importantly slurry and solid manure. Whilst a number of approaches to reducing methane emissions have been explored, it has yet to be shown that they can be of practical use. Means whereby nitrogenous emissions from slurry and manure can be minimised have been the subject of much research. Whilst the potential of a number of approaches has been demonstrated, the major ameliorating effect is through decreasing the density of stocking. Current beef cattle systems are often less intensive than many sheep and most dairy cattle systems and it can be argued that beef cattle systems could fit better than other ruminants in reducing atmospheric pollution in the future.

Water Losses of nutrients from grassland into water courses occur predominantly in the autumn and winter. The relatively low application of nitrogenous fertiliser in beef cattle systems mean that immediate losses are not as high as in high fertiliser systems but losses in the autumn and winter are similar to other ruminant systems based on grazing. Cattle, more than sheep, can cause damage to river banks whilst obtaining drinking water. This can lead to erosion and increase the amount of sediment and nutrients in water, and also lead to contamination of water through faeces and urine. These sources of damage to water quality can be reduced by provision of fences and alternative sources of drinking water. There is a need to ensure that current thinking on catchment management becomes part of planning future beef cattle systems.

Soils and vegetation The impact of cattle on soils and vegetation has important beneficial impacts on the biodiversity of flora and fauna. Because of their relatively unselective grazing, they graze plant species, e.g tussock grasses such as *Nardus stricta* and *Molinia caerulea*, that sheep will not graze readily. This helps to avoid dominance by such species and creates more plant species diversity. Moreover their avoidance of dung and urine patches, and the need to graze pastures at greater heights than sheep, creates mosaics of short and tall patches in pastures. This increase in structure of pastures increases not only the diversity of the flora but also the diversity and biomass of invertebrates, which has a beneficial effect on bird and small mammal diversity and populations.

Poaching by cattle can have deleterious effects on flora but also can benefit regeneration and colonisation by plant species by the creation of gaps in pastures. Many current beef cattle systems, particularly those that involve mixed grazing with sheep, provide important benefits to biodiversity as has been indicated above.

Conclusion The challenge for research is to demonstrate further the beneficial effects of cattle grazing and to design systems which not only provide desired increases in biodiversity but do so in such a way that minimise the other environmental costs of such systems. This should also be achieved through maximising the income to farmers by the production of high quality beef from such systems.

Mapping genes for milk and meat quality

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Background Genetic selection has proved itself to be the most powerful tool available for the improvement of livestock. Over the last 40 years the efficiency of livestock production has been increased radically by the success of animal breeding. Where animal breeders have been successful it has been through the application of relatively simple rules – selection based on traits that can be measured in a repeatable manner and where variation between individuals has both a genetic component and economic relevance. Until recently, this has meant that breeders have focussed on efficiency traits – for example growth rate or milk yield - and in some cases, certain easily measured aspects of quality, such as overall fatness as inferred from ultrasonically measured fat depth. As both the breeding industry and consumers have become more sophisticated, there has been an increasing focus on traits associated with reproduction, welfare and disease and quality. Such traits can be more difficult to improve by selection because heritabilities are low (i.e. only a small amount of variation between animals is genetic in origin, such as for some reproduction and disease associated traits). In addition, such traits may be difficult or expensive to measure (e.g. disease related traits, or quality related traits measured in the laboratory or by sensory panels) and have economic values that are difficult to quantify.

Genomics and Marker-Assisted Selection Despite the difficulties, quality traits can be included in selection indices that attempt to improve a range of traits simultaneously and such selection can be very effective. However, the tools of genomics have offered an additional route to the genetic improvement of quality traits. With the identification of single loci of major effect on variation between animals, marker-assisted selection can be applied. Molecular genetic analysis is used to identify which animals carry the desired allelic variant, either through a direct DNA test for the desired variant or by a DNA test for a linked genetic marker associated with the desired allele. Once animals have been classified according to whether they carry desired alleles, this information can be used alongside information from phenotypic information to decide which animals will be used for breeding..

Once DNA tests have been developed, they are potentially particularly useful for quality traits. A DNA test provides a direct and accurate test of the presence of a desired allele. Thus a DNA test replaces less direct and less accurate laboratory or sensory panel tests and may also be cheaper. Furthermore, a DNA test can be performed using very small samples (e.g. blood, milk sample or hair sample) that can be collected at any time. Thus an animal can be tested and selected for breeding early in its life, before other information such as meat or milk samples could possibly be available. Theoretical and computer simulation studies have been used to explore the potential increase in selection response that could come from the use of DNA marker information. For example, Meuwissen and Goddard (1996) found that, under one set of (favourable) conditions, marker-assisted selection increased the rate of progress for a production trait, where records were collected before selection (e.g. growth rate), by almost 9%. The same authors using the same assumptions found that for a carcass trait, which was recorded by slaughtering half the progeny and selecting amongst the remaining half, that marker-assisted selection increased the rate of progress by almost 64%.

Evidence for genetic effects Over and above the potential contribution of marker-assisted selection to the rate of genetic progress, the other potential advantages to using marker information are to make best use of the animal or its carcass based upon improved knowledge of its likely characteristics. This is turn will have benefits in terms of reducing variation in the product. Finding genes that influence quality also has the potential major benefit of contributing to our understanding about the processes that influence quality.

The potential combined benefits have persuaded breeders and animal geneticists to attempt identification of genes controlling variation in quality traits. The first question is whether there are likely to be genes with sufficient effect on quality to be worth seeking. The *a priori* evidence is compelling, if not conclusive. Firstly, it has been known for some time that there can be individual genes with a major effect on quality. Perhaps the best studied of these as far as meat quality is concerned is the halothane locus in pigs. This gene is associated with stress sensitivity in pigs and has been known since the 1970s that animals homozygous for the recessive allele are sensitive to stress, but also have increased carcass lean, as well as poorer meat quality, particularly an increased propensity to produce PSE (pale, soft, exudative) meat. The sensitivity of these homozygous animals to halothane gave breeders a tool to manipulate the frequency of the gene. This tool was imperfect because it was not possible to distinguish between animals carrying one copy of the allele from those carrying none. However, with the identification of the gene (the CRC locus) and underlying mutation in 1991 (Fujii *et al*., 1991), it became possible to distinguish between all three genotypes on the basis of a DNA test and hence for breeders to change the frequency of the mutation to any desired value.

Other relevant background information is that breed differences are well documented for quality traits, for example the increased levels of intramuscular fat and better eating qualities associated with the Duroc pig breed relative to white breeds, or the higher levels of fat found in milk from Jersey cattle relative to that from Holstein cattle. Finally, within breeds, there is good evidence that a proportion of the variation between animals, usually from one third to one half, is genetic in origin. It is not possible to tell *a priori* whether any individual genes make a significant contribution to this variation, but the example of the CRC locus and more recent results detailed below show that at least some of this variation can be traced to the effects of single loci.

Mapping trait genes Physiological or biochemical studies can tell us a lot about the enzymes, proteins and pathways that control variation in product quality. However, they do not necessarily indicate the importance of genes controlling variation between individuals. Just because perturbation of a particular hormone can be shown to have major effects on

a trait, it does not mean that naturally occurring genetic variation at a genetic locus controlling the hormone structure or expression play an important role in trait variation. Growth hormone, for example, has a key role in control of growth and experimental modulation of its expression has major impacts on growth. Nonetheless, studies of natural variation indicate that genetic variants of growth hormone play very little or no role in the control of genetic variation in growth.

Thus strategies are required to identify which of the genes potentially involved in trait physiology actually contribute to the natural genetic variation for a trait and also to identify additional loci that contribute to trait genetic variation but which were not *a priori* expected to be involved. The strategy adopted depends upon the assumptions made by the investigator at the outset, by available resources and by the intended application of the result. The two major strategies used are the study of candidate genes identified on the basis of their physiology (the candidate gene approach) and a scan of the entire genome using anonymous (i.e. non-functional) markers (the genome scan approach). In both approaches the aim is to identify a marker that has two or more DNA variants (i.e. alleles) present in the population of interest that are associated with variation in the trait of interest.

The candidate gene approach limits the amount of work required to the number of candidate genes available and if successful identifies a marker right in a gene of interest. The approach does not require especially designed studies in pedigreed populations to be effective, for example, it may be used to find an important association in a typical commercial population. A marker identified in a candidate gene study should generally be a robust tool in a marker-assisted selection scheme, in particular, the marker should not lose efficacy over the generations due to recombination between itself and the causative lesion. There has been debate in the literature regarding the appropriate statistical analysis and significance threshold to use when judging candidate gene effects, thus results reported as significance are often regarded with scepticism until convincingly confirmed in independent studies. The candidate gene approach also has the disadvantage that many genes do not have known functions, such genes will not be considered as potential candidates and hence important effects due to these genes will be missed in the candidate gene approach.

The genome scan approach requires that many markers be typed to cover the entire genome (usually 100+ to cover a livestock genome). Such studies also require well-structured and well-designed populations and are often initiated using crosses between genetically diverse lines, for example, although they can also be performed using carefully chosen samples from commercial populations. However, a well-designed study can provide confidence that any locus with a substantial effect on the trait has been identified without requiring prior knowledge of all potential candidate genes (it should also identify which candidate genes might potentially be involved, through an effect associated with the region of the genome in which they are located). Because genome scans are based on carefully designed populations and set high thresholds before an effect is declared significant, detected effects can generally confidently considered to be real, rather than statistical artefacts or chance events. If a QTL is identified in such a scan, the markers used for its identification will be closely linked to the causative gene, but further work is required to identify the gene itself.

Identification of the causative mutation underlying a known QTL is valuable in order to improve understanding of trait control and to provide robust tools to use in marker-assisted selection schemes. With increasing information available from mapping and sequencing in livestock, humans, mice, etc., it will often be possible to identify many or all genes mapping to the region where a QTL is located and thus to select potential candidates using information on both position and potential function. Even so, with our current very incomplete knowledge of gene function, there may be several genes or none that are putative candidates. Despite the prospect of knowing all genes in a region and their sequences, moving from a situation in which the position of a QTL effect is known to the identification of the underlying gene and the causative mutation is likely to often be a challenging and time consuming process for some years to come.

Results – Dairy cattle Much of the mapping work in dairy cattle has been associated with mapping loci associated with milk production. In addition, despite the large between breed differences in yield and milk quality reported in the literature, most of the mapping work has focused on mapping trait genes within populations, rather than generating crosses between populations to take advantage of between population differences. This focus has been dictated in part by the structure of the industry, with the ready availability in the commercial population of a mapping resource in the form of large half-sibships of recorded individuals generated by the widespread use of AI. In addition, the generation and recording of a pedigree specifically for mapping is relatively slow and costly in cattle.

Within the half-sib structure available in the industry, the recording of milk production traits and the genotyping of daughters from a number of sires (the ‘daughter design’) provide the potential to detect genes that are heterozygous in individual sires. Such genes will segregate in the daughters of the sire, with half the daughters receiving one allele and half the other. Thus if the daughters’ performance is differentiated by the allele they inherit from the sire for a particular marker, this suggests that a gene affecting performance is linked to the marker. Although this daughter design has been used to map genes, a more powerful design for the detection of such genes is the “granddaughter design” (Weller *et al.*, 1990). This design also makes use of the half-sib structure of the dairy industry, but focuses on genotyping markers in sires and their *sons.* The sons have no individual records of dairy performance, but they have records from large numbers of their daughters (granddaughters of the original sires), so the breeding value of each son is much better estimated than is the breeding value of daughters based upon their individual lactation records. Thus by genotyping sires and their sons and making use of performance records on the daughters of the sons, the power to detect a trait gene is increased. In order to detect a given gene or QTL, the granddaughter design may require the genotyping of only a quarter of the number of animals that would be required to detect the same gene in the daughter design.

The granddaughter design has been used in a number of studies to map genes associated with milk production using

animals and milk records from the national herd. Because fat and protein yields and percentages and routinely recorded along with total production, many of the mapped loci have effects on milk quality through differential effects on the components of milk. Thus, one of the largest studies reported is that of Zhang *et al.* (1998) who genotyped 206 markers on 1794 sons of 14 sires. Each son had lactation records from an average of over 1000 daughters, so the whole study was based on over 2 000 000 daughter records for some traits. The six most significant effects detected were spread over 3 chromosomes and influenced 3 traits: milk yield, and fat and protein percentage. On chromosome 3, the only significant QTL effect was on milk protein percentage. On chromosome 6 the QTL had significant effects on milk yield, and milk fat and protein percentages. On chromosome 20, the QTL had significant effects on both milk fat and protein percentages. The results of this study were typical of other studies, in that where significant effects were observed on several traits, correlated effects were in the direction expected from knowledge of the genetic correlation, i.e. fat and protein percentage increased or decreased together, and an increase in milk yield was accompanied by a decease in fat and/or protein percentage. Note, however, that the results for chromosome 3, for example, where the QTL only influenced protein percentage indicates that the associations between traits are not absolute (as expected, because the overall genetic correlations are far from either +1 or –1).

Another area of active study has been that of milk proteins themselves as candidate genes influencing milk composition and associated manufacturing qualities of milk. A large number of different variants have been identified both within and between populations in the four casein loci and in beta-lactoglobulin and alpha-lactalbumin. Convincing and replicable associations have been found between genetic variants at these loci and both the overall fat and protein content of milk and its qualities in terms of cheese yield and aspects of cheese quality. Actual associations vary from study to study, at least in part due to chance, but also perhaps due to the cattle breeds involved in each study, however, some consistent and widely observed associations are found. For example, the two major variants of beta-lactoglobulin, A and B, are associated with differences in protein content of milk. Animals homozygous for the A variant of beta-lactoglobulin have higher overall levels of protein content than those homozygous for the B variant due to higher levels of whey protein, but the latter genotype has higher casein levels than the former (Ng-Kwai-Hang, 1998). The higher casein levels in milk homozygous for the beta-lactoglobulin B variant result in higher yields of cheese. Similar consistent effects on both increased cheese yield and quality are found for the kappa-casein B variant compared to the A variant at the locus (Ng-Kwai-Hang, 1998; Buchberger and Dovc, 2000). It is interesting to note that the casein loci map in a cluster to chromosome 6 in cattle and may be associated with the variation in protein percentage identified in the genome scan results reported above. Polymorphisms in milk proteins have also been identified and been shown to be associated with both milk composition and quality in both sheep and goats.

Results – Pigs The pig has been the most thoroughly investigated species to date, but despite some notable successes in the identification of genes associated with variation in meat quality, work has only scratched the surface of what is possible. Nonetheless, the work that has been reported provide an indication of the further results that may be expected in pigs as well as an example of what may be revealed by more thorough studies of other species.

The example of the halothane locus, now known to be associated with a lesion in the CRC gene, is discussed above. Another case that followed a similar path to discovery of the underlying effect is the RN locus. In this example the initial observation was of an apparent major genetic effect (identified without genetic markers) on the yield of cured ham in synthetic populations containing a high proportion of Hampshire genes. It was subsequently also shown to be associated with lower pH and increased glycogen content in the meat**.** Using Hampshire-derived mapping populations the effect was mapped and the gene responsible identified (Milan *et al*., 2000). This required substantial effort despite a relatively precise localisation of the effect, because no obvious candidate genes were known that mapped to the region. Thus the groups involved had to specifically isolate previously unknown genes from the region and test them as potential candidates. The development of the CRC and RN genetic tests have provided powerful tools to manipulate these genes and their associated effects on quality. The CRC DNA test has been in widespread use for almost 10 years and DNA tests based on the RN gene are already being used in the breeding industry.

The CRC and RN gene are both examples where a major gene effect was identified, mapped and subsequently the gene was identified. In addition, both the candidate gene and genome scan approaches have been widely used to search for meat quality associated genes in pigs. For the most part, the two approaches have been applied to different types of populations. The candidate gene approach has been used with samples from various commercial populations whereas the genome scan approach has been used in populations derived from crosses between genetically diverse lines.

Genome scans based on crosses involving Meishan and Large White, wild boar and Yorkshire, Iberian and Landrace and Berkshire and white breeds amongst other crosses have been reported. Most of these studies have reported QTLs involved in aspects of meat quality including meat colour and pH, intramuscular fat content and androstenone levels potentially associated with boar taint. An interesting result that has generated some debate is the observation by de Koning *et al*. (2000) of significant QTL effects on both intramuscular and subcutaneous fat levels that show significant imprinting (i.e. the effect of a particular genetic variant depends upon whether it is inherited from the father or the mother). Much research is continuing in attempts to identify the genes that underlie these effects. Further work is needed to identify further QTL effects, for example, there are few convincing QTL affecting tenderness and there remains the opportunity to pin down the well-documented advantages in tenderness possessed by the Duroc breed.

Candidate gene studies have focussed on various loci including the calpain system, myogenin and fatty acid binding proteins. The latter provides the most convincing and potentially useful association of an effect of the adipocyte fatty acid binding protein on intramuscular fat that appears to be independent of overall fat levels (Gerbens *et al*., 1998).

Results – Sheep and beef cattle Some major genetic effects have been identified and reported. The most remarkable is that associated with the callipyge gene in sheep. This gene was first detected due to the highly significant effect it has on muscular hypertrophy – some individual muscles can be more than 40% heavier in older lambs expressing this trait compared to controls (Duckett *et al*., 2000). The mode of inheritance is unique in that only animals that inherit the callipyge allele from their sire and the normal allele from their dam display the trait (i.e. any animal inheriting the callipyge allele from its dam does not display the trait) (Cockett *et al*., 1996). The effect on meat quality is equally marked, animals expressing the callipyge trait have remarkably tough meat as assessed through shear-force measures or sensory panels which is associated with high calpastatin levels (Freking *et al*., 1999; Duckett *et al*., 2000). The callipyge gene has been precisely mapped on ovine chromosome 18 and it is likely that the underlying lesion will be reported soon. The effect of the callipyge locus on tenderness is perhaps the largest genetic effect known on this trait and precludes the sale of fresh meat from these animals without substantial ameliorative treatment.

Although there are no well-documented genome scans for meat quality associated QTL reported for sheep, there have been several genome scans performed in beef cattle, however, only one is well documented in the literature (Keele *et al*., 1999). This is a family derived from a *Bos taurus* x *B. indicus* (Hereford x Brahman) sire and hence focuses on genetic effects differentiating these two sub-species. A major QTL was identified for which the Brahman derived allele decreased meat tenderness substantially. However, this QTL showed significant interaction with unknown environmental effects, such that it was observed in only one of four slaughter groups. This illustrates the potential difficulties faced by studies of meat quality, but also suggests the potential advantages that might accrue from understanding and being able to control the causes of such interactions between the genotype and environment.

Conclusions The studies performed to date have shown the potential for the identification of genes making significant contributions to variation in quality traits. It is interesting to note that even in dairy cattle, where there has been intense selection for yield and significant selection for aspects of milk quality, there remain major genetic effects segregating. This suggests there are more effects to be found in under-explored species such as sheep and in populations of other species that have not yet been investigated. Both approaches to the identification of trait loci; the candidate gene and the genome scan approaches, have had confirmed success in the identification of genes with appreciable effects. As the tools of genomics become better understood, more widely disseminated and, hopefully, cheaper, these studies will become more routine. The change in the ease of applying the technology should also mean that the results of such studies will become more widely applied. Marker assisted selection is reported to be in use by some of the larger breeding organisations in pigs and dairy cattle, but its extent and success is hard to judge at present. More widespread application of the technology will spread the benefits to the wider community including the consumer, who will ultimately benefit from higher quality and more consistent products.

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International Society of Applied Ethology Programme

Open Communications session

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Early experience of ammoniated environments and subsequent avoidance behaviour in domestic fowl

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Introduction The adverse physical effects of atmospheric ammonia on stock (Charles and Payne, 1966; Wolfe et al. 1968) and stockpeople (Kirkhorn and Garry, 2000) have been documented, but broiler chickens' perception of this aspect of their own welfare has not been fully investigated. Previous studies have demonstrated a delayed avoidance of high ammonia concentrations (Jones et al., 2000) but we could not categorically claim that this was a true aversion to ammonia since the birds may simply have expressed a preference for fresh air based on their previous experience. This experiment was designed to establish whether avoidance of ammonia in broiler chickens was an intrinsic aversion, independent of previous atmospheric experience.

Materials and methods This study comprised two phases. In the first, four flocks of 25 broiler chickens were housed in 20 p.p.m. ammonia and a further four flocks in fresh air from 2d to 24d of age. Behaviour was observed for a total of 96hrs using continuous focal sampling. The second phase was designed to examine these birds' preferences for nominally 0, 10, 20 and 40 p.p.m. ammonia in the context of their previous experience. Twelve birds from each of the eight flocks from the first phase were placed in a preference chamber with access to four compartments. An acclimatisation period of 4 days gave birds time to become familiar with the chamber and move readily through curtains separating the compartments. The atmosphere during this period was the same as that during rearing i.e. either fresh air or 20 p.p.m. ammonia. Colour cues were provided during the test to assist birds' discrimination between environments. Videotapes were analysed and total occupancy and visit durations calculated for each ammonia concentration.

Results Statistical analysis using Generalised Linear Models identified no significant differences between treatments on broiler chicken's behaviour during the first phase of chronic exposure to ammonia both in terms of total time spent performing each behaviour ($F_{17,\ 1991}$=1.22; P=0.237) and frequency of each behavioural category ($F_{17,\ 1991}$=1.08; P=0.366). For example, birds exposed to 20 p.p.m. spent on average 172 secs with a frequency of 19.2per hour preening compared to 166 secs with a frequency of 19.1per hour by birds reared in fresh air. Data from the second phase were analysed using Analysis of Variance. A strong effect of ammonia on occupancy was identified ($F_{3,7}$=12.99, P=0.003). Median values for two eight hour observation periods per day were 24.2, 10.0, 3.4, 0.1 % at ammonia concentrations of nominally 0, 10, 20 and 40 p.p.m respectively. Furthermore there was no significant interaction between ammonia and previous exposure (P=0.949; $F_{3,12.04}$=0.11 means on a logit scale for increasing ammonia concentration, for fresh air -2.098, -2.427, -3.069, -4.486 and for 20 p.p.m. -1.716, -2.352, -3.157, -4.535; s.e.d.=0.662). Visit duration data were analysed using REML. Again there was an effect of ammonia (Wald=8.0, d.f.=3, P=0.046), medians for increasing ammonia concentration being 60, 40, 30, 30 min. with no interaction between ammonia and previous exposure (Wald=1.1, d.f.=3, P=0.777 predicted means on the natural log scale for increasing ammonia concentration, for fresh air 1.858, 1.384, 1.510, 1.197 and for 20 p.p.m. 2.277, 1.423, 1.229, 1.145; s.e.d.=0.541).

Conclusions Broiler chickens avoid ammonia concentrations commonly found on poultry units regardless of their previous atmospheric experience. Delayed avoidance behaviour of broiler chickens observed in previous experiments can be regarded as an aversion to concentrations of atmospheric ammonia greater than 0-10 p.p.m.

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The effect of demonstrator reward on social learning of operant key pecking by domestic hens

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Introduction Social learning is said to occur when social interaction facilitates the acquisition of a novel pattern of behaviour. It usually takes the form of an experienced animal (the demonstrator) performing a behaviour such that a naive animal (the observer) subsequently expresses the same novel behaviour, earlier or more completely than it would have done using individual learning. Social learning is involved in the transmission of a great variety of behaviours, e.g. tool-use, food preferences, and has also been implicated in maladaptive behaviours such as stereotypies in voles. In studies of social learning, the observers usually see the demonstrators receive a reward for performing the required behaviour. But, the role of the reward has rarely been investigated and results have been equivocal. Understanding the role of demonstrator reward on social learning is necessary to assess the cognitive abilities of individuals of different species, and aids understanding of the transmission of maladaptive behaviours.

Materials and methods The apparatus comprised a two-chamber box with a clear Perspex partition between the chambers. The 'response chamber' contained a red and a green operant key, and a food hopper, the door of which was opened by a computer whenever a hen pecked the correct key; the 'observation chamber' was featureless. Eight demonstrators were trained to peck reliably on one of the keys. Observers were randomly allocated to watch one of three types of demonstration, i.e. a demonstrator pecking a key for 20 reinforcements with each third peck being rewarded with 5 s access to food, (FR3; N=20 observers), a demonstrator pecking 60 times but receiving no reinforcement (NR; N=20 observers), or the feeder door opening 20 times (control; N=16 observers) without a demonstrator being present. Each observer bird saw the same demonstration on four consecutive days, after which she was placed into the response chamber and her behaviour recorded for 10 mins.

Results Of the 56 observers, 11 pecked on one operant key and four pecked on both; only two control birds pecked on a key. Three-way ANOVA (with demonstration, apparatus (N=4) and time period (N=4) entered as factors) shows there was a significant effect of demonstration on the total number of pecks to the front wall plus pecks to the keys ($F_{2,3,3}$=6.2, P=0.0012) and the total number of pecks to the keys ($F_{2,3,3}$=7.2, P=0.016). The NR birds pecked the operant keys most frequently (2.6 $\pm$ 1.3), control birds the least (0.2 $\pm$ 0.1), and an intermediate amount by the FR3 birds (1.2 $\pm$ 0.6).
Comparing only the FR3 and NR observers, rewarding the demonstrators reduced the number of pecks to the correct key by the observers. The mean number of pecks to the correct key by the FR3 birds was significantly less than the NR birds (FR3=0.3 $\pm$ 0.2; NR=1.8 $\pm$ 1.1; $F_{1,3,3,}$=10.2 P=0.016), although when this was expressed as a proportion of the total number of pecks to the keys, there was no significant effect of the demonstrations (FR3=0.3 $\pm$ 0.2; NR=0.6 $\pm$ 0.2; $F_{1,3,3}$=0.8, P=0.422).

Discussion and conclusions This study confirms that observing a hen pecking an operant key socially facilitates key pecking by the observer bird (Nicol and Pope 1992). Control hens seeing only the feeder door opening and closing pecked considerably less frequently at the keys than observers that had seen a demonstrator peck the keys. However, this study extends previous work by revealing that rewarding the demonstrator with food did not enhance social learning of operant key pecking. This suggests that hens are attracted to peck at the same stimulus as pecked previously by the demonstrator (stimulus enhancement) - a relatively simple form of social learning. It also suggests that socially learnt pecking in hens might have its own intrinsic reinforcement (Heyes 1994). Clearly, the NR observers could make no association between the demonstrator's pecking actions and reward with food, yet they learnt to peck the keys. It appears the observer simply pecks the key because it is attracted to peck at that location. The possibility that a similar mechanism could underlie the rapid spread of feather pecking within flocks will be discussed. The significantly higher frequency of pecking the correct key by NR hens compared to FR3 birds indicates reinforcement of the demonstrator actually hindered social learning. The mechanism of this effect remains to be elucidated.

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The chewing behaviour of growing pigs presented with tail models soaked in different fractions of blood, as a test for tail biting predisposition

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Introduction Tail biting is a widespread adverse behaviour that occurs in growing pigs but, as of yet, no one knows what initially encourages the development of this behavioural problem. It has been suggested that tail biting is linked to a behavioural predisposition, exacerbated by environmental inadequacy, or a nutritional deficiency such as inadequate protein or minerals. Using a model tail test, Fraser (1987, 1991) demonstrated an experimental link between mineral or protein dietary deficiencies and an increased attraction to blood. Using this test, Fraser demonstrated that large individual variation exists between pigs in the extent of their attraction to blood. The current experiment extended this tail test to investigate the nature of the attraction to blood, and to examine factors that may be related to tail biting predisposition.

Materials and methods 20 Duroc × Large White × Landrace pigs were selected for testing at 68.5kg (S.D. = 7.3kg) from 4 litters, each providing either 2 male and 3 female siblings or 3 male and 2 female siblings. Within each litter, pigs were randomly allocated to a 5 x 5 Latin square design that was replicated across litters. The pigs were individually housed and were fed a standard finishing diet *ad libitum*, with free access to water. They were presented with a pair of tail models, made from sash cord, for a period of 24 minutes each day for 5 days, and their chewing behaviour was directly observed and recorded every 6 seconds during these 24 minutes. The pigs had a choice of chewing either a control tail that had been soaked in de-ionised water, or a treated tail. This was soaked in either whole blood (WB), the red and white cellular fraction of blood (CF), the plasma protein fraction (PPF), the plasma ionic fraction (PIF) or a sodium chloride solution (NaCl). The pigs were weighed prior to the start of the experiment, and again each following Friday at the end of the each weekly period of the Latin Square. The chewing scores (no. of observations of chewing/total no. of observations) for each individual pig were recorded each day for both the treated and control tail models. From these chewing scores, the preference scores were determined (chewing score of the treated tail model/total chewing score for both tail models). Data on the total chewing scores and preference scores were subjected to ANOVA according to the Latin square design to test the significance of litter of origin, week and the type of treated tail offered.

Results Individual pigs varied significantly ($P<0.05$) in their preference scores for the tail models, but not in the overall chewing scores. Litter of origin had no significant effect on the chewing or preference scores of the tail models. The pigs chewed on the tail models significantly more during the first week than any other week (Figure 1; $P<0.001$). The preference scores for the treated tail models did not differ significantly between weeks. Over the five-week experimental period, the preference scores of the pigs for the tail model soaked in whole blood were significantly higher compared to any other treated tail (Table 2; $P<0.001$), with the NaCl tail having the next highest preference scores. However, only in the case of the whole blood, salty and PIF tails were the preference scores significantly different from random, (i.e. 0.5). The treated tail offered had no significant effect on the pigs' chewing scores.

Table 1 Preference and chewing scores for the pigs over the five weeks

	Preference score	Chewing score
Week	**Mean**	**Mean**
1	0.520	0.315^{d}
2	0.493	0.161^{c}
3	0.548	0.097^{a}
4	0.553	0.155^{b}
5	0.532	0.125^{a}
s.e.m.	0.010^{NS}	0.004^{***}

Table 2 Preference and chewing scores for the pigs for the five treated tails

	Preference score	Chewing score
Tail	**Mean**	**Mean**
CF	0.478^{a}	0.145
NaCl	0.534^{b}	0.182
PIF	0.472^{a}	0.163
PPF	0.492^{a}	0.169
WB	0.670^{c}	0.195
	0.010^{***}	0.004^{NS}

^{NS} Not significant; *** $P<0.001$; Means within columns with different superscripts are significantly different

Conclusions The large individual differences in the preference scores of the pigs were unrelated to litter of origin, and not seen in the overall chewing scores. The pigs preferred to chew on the tail models soaked in whole blood and the NaCl solution. In contrast to the common belief that tail biting pigs are attracted to minerals or protein, this experiment found no significant preference for any of the tail models soaked in the separate blood fractions. Further work needs to be completed before firm conclusions can be made linking either a protein or mineral deficiency and an increased predisposition to tail bite.

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Heart rate and behavioural correlates of anxiety assessment in horses

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Introduction The aims of this study were to determine behavioural and heart rate responses to isolation and a novel stimulus and to investigate the relationships with scores from a questionnaire developed to assess anxiety in horses.

Materials and Methods The subjects were 15 warm-blood geldings ranging in height between 1.5m and 1.8m and between 10 and 20 years of age. All had been subjected to the same daily management routine for the previous 3 months. When the horses were being tested they were not fed or exercised during the one hour prior to testing. When not being tested, they were kept under their usual routine. The behaviour and heart rate of each horse was recorded in three situations; during isolation in the home pen (HP), during isolation in a novel pen (IS), during presentation of novel object in a novel pen (NO). Behaviour was recorded continuously via a static camera connected to a real time video-recorder. Vocalisations were also recorded by direct observation. Heart rate (HR) was recorded using a Polar Horse Trainer (PHT) transmitter and two Polar Vantage NV HR wristwatch receivers. One receiver was set to record HR over 5-second intervals while the other was set to record interbeat intervals. During isolation in the home pen, the HR monitor was fitted and after 10 minutes acclimatisation, HR and behaviour recorded for 30 minutes. During isolation in a novel pen, the HR monitor was fitted in the home pen and the horse then moved to the novel pen, where behaviour and heart rate were recorded for 60 minutes. During exposure to a novel object, the methodology was the same as isolation in the novel pen, except that a novel object was lowered from the ceiling for 80s at 30 minutes during the 60-minute period. The novel object consisted of a piece of 1/2inch square wire mesh panelling modelled into a spherical shape, covered with red cloth and containing a Dictaphone with a 20s recording of a two-tone car alarm sandwiched between two 30s periods of silence. A questionnaire developed to assess anxiety in horses (Hoffman, personal communication) was used to provide the past behavioural histories of the individual experimental subjects, once all experimentation had been completed. Two questionnaires were distributed per horse and answered by the yard manager and her assistant, both of whom had in-depth knowledge of the horses' behavioural characteristics. These were analysed to give total anxiety scores and scores relating to the following factors; nervousness, alertness, friendliness, isolation resistance. The behaviour and heart rate data were compared across treatments using Friedman Analysis of Variance and relationships with anxiety scores were tested using Pearson correlation.

Results

Horses spent most time standing alert ($P<0.05$) and vocalising ($P<0.01$) during the NO treatment but most time engaged in locomotor ($P<0.001$) and investigatory ($P<0.001$) behaviour in the IS treatment. Horses spent more time at the stable door ($P<0.01$) in the NO treatment, i.e. furthest away from the novel object. Over the first 30-minute period, horses had the lowest mean heart rates in the HP treatment and the highest mean HRs in the IS treatment (see Figure 1). Presentation of the novel object induced a significant rise in HR from 35.3 bpm in the minute prior to presentation to 98.0 bpm ($P<0.001$). However, there was a large range in HR change between individual horses from a decrease of 3 bpm to a rise of 130 bpm, on presentation of the novel object. There was significant agreement between the anxiety scores of both observers ($r=0.72$, $P<0.001$). Total anxiety scores and nervousness scores positively correlated with behavioural and HR parameters, particularly during the NO treatment (see Table 1). Isolation resistance score was also positively correlated with the bout length of investigatory behaviour performed in the IS treatment ($r=0.60$, $P<0.05$).

Table 1: Significant correlations between anxiety scores and behavioural and heart rate measures during novel object treatment

Measures	r-value	Signif.
Total Anxiety Score		
Maximal HR response to NO	0.66	*
Time for HR to return to pre-NO levels	0.58	*
Nervousness Score		
Mean HR in response to NO	0.79	***
Time for HR to return to pre-NO levels	0.70	**
Vocalisation bout length	0.56	*

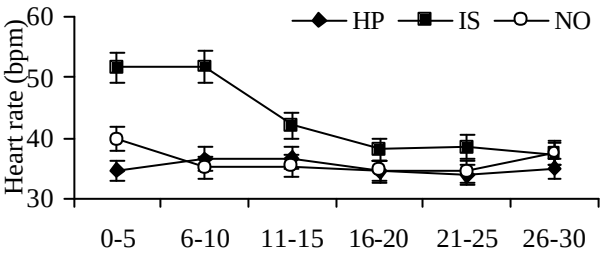

Figure 1: Mean+s.e. heart rate of horses during the first 30-minute period in the three treatments

Conclusions Horses engaged in more exploratory behaviour when placed into a novel stable and initially had higher heart rates than in the home stable. Exposure to a novel visual and auditory object resulted in a large heart rate rise and an increase in vocalisation and standing alert. These behavioural and physiological changes showed a large variation between individuals and were related to the total anxiety scores and nervousness scores obtained from a questionnaire developed to assess horse temperament.

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Can we predict which hens will feather peck?

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Introduction Selective breeding, based on feather pecking bouts per hen, can be an effective method of reducing feather pecking in a controlled research environment (Kjaer 2000). For selective breeding against feather pecking to be applied on a commercial scale using similar methodology would be extremely time consuming. A simple, quick test which predicted a young bird's likelihood of developing feather pecking would eliminate the need to observe feather pecking behaviour or expose birds to damaging pecking from conspecifics as part of the selection process. A predictive test could also increase the rate of genetic progress and reduce the cost of implementing a selection programme.

In an earlier study (Albentosa & Nicol 2000) using several different layer strains, pullets that pecked most frequently at loose feather bundles (as a model for feather pecking) also strongly avoided a novel object, suggesting that high feather peckers may be more fearful. The present study aimed to find out whether: 1) tendency to avoid a novel object was *predictive* of feather pecking, and 2) pecking at the feathers of live birds and pecking at feather bundles were related.

Materials and methods Between 7-9 weeks of age 319 ISA Brown layer pullets were tested with a novel object in the centre of a circular arena. The birds were then divided into two groups (NEAR and FAR) according to whether their mean distance from the novel object was below or above the median value respectively. Within each group, birds were then systematically distributed between two pen types: pens exclusively containing birds from the same group (i.e. ALL NEAR birds or ALL FAR birds) and pens containing a 50:50 ratio of birds from the two different groups (MIXED). There were four pens (replicates) each of the ALL NEAR and ALL FAR treatments, and eight replicates of the MIXED treatment.

Between 11-13 weeks of age, birds from half the pens of all three treatments were individually removed to a test pen and provided with a loose bundle of straw and a loose bundle of feathers to peck at for 10mins (loose feather test). The remaining pens were provided with bundles of feathers fixed within the home pen for 30mins (fixed feather test). In both tests, latencies to peck at feather bundles and pecking frequencies were recorded for individual birds. Between 25-27 weeks of age, the pens that had previously been given the loose feather test were given the fixed feather test and vice-versa. In addition, the feather pecking behaviour within each pen was recorded during four separate weeks between 15-33 weeks of age, and plumage scores were recorded at 19 and 32 weeks of age.

Results Data were analysed using non-parametric statistics and mean values for each pen were treated as independent data points. The number of gentle feather pecks, number of severe feather pecks, plumage scores and number of bouts of feather pecking did not differ significantly according to treatment. However, the percentage of birds per pen that were observed feather pecking on three out of four weeks was significantly higher in the MIXED treatment ($P<0.05$). The percentage of birds per pen that feather pecked was also consistently, though not significantly, higher each week in the MIXED treatment.

Treatment did not affect loose or fixed feather test variables at either bird age. Pecking at feather bundles in the fixed feather test occurred significantly less at 25-27 weeks of age compared to 11-13 weeks of age ($P<0.05$). In the loose feather test, pecking at the straw bundle occurred sooner ($P<0.05$) and more frequently ($P<0.01$) than pecking at the feather bundle at both ages. However, pecking at the straw bundle took longer ($P<0.05$) and was less frequent ($P<0.01$) at 25-27 weeks than at 11-13 weeks of age.

Multiple logistic regression analysis using values for individual birds showed no relationship between treatment and feather pecking or between pecking at feathers in either of the feather tests and feather pecking in the home pens. Feather pecking at 15 weeks of age was not predictive of feather pecking at 33 weeks of age but, from the totals over all observation periods, gentle feather pecking was predictive of severe feather pecking ($P<0.001$) even though severe feather pecking was rarely observed throughout the study.

Conclusions Using young pullets' avoidance of a novel object we were unable to predict tendency to feather peck. This may have been due to the low levels of severe feather pecking recorded. However, the results suggest that groups of birds that vary widely in their fear of novelty may be more likely to feather peck consistently than groups of birds that are similar in their fear of novelty. Pecking at the feathers of live birds and pecking at feather bundles did not appear to be related.

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Behavioural diversity within groups of juvenile pigs

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Introduction Understanding behavioural diversity is important for commercial production, animal behaviour and welfare. One interpretation of individual differences in behaviour is that these represent different strategies for coping with a changing environment. For example, Hessing et al. (1994) categorized pigs as active/resistant or passive/non-resistant, based on each pig's reaction to restraint. However, these conclusions have been criticized. For example, Jensen et al. (1995) argued that for two such categories to exist, there must be a bimodal distribution of the scores from a population, which Hessing et al. (1994) failed to demonstrate. Determining individual behavioural characteristics and any relationship they may have with performance has been the focus of recent research. The objective of this experiment was to determine the primary characteristics that distinguish individual pigs.

Materials and methods Eighty-nine pigs (9 litters) were followed from birth until 8 weeks of age. All litters remained intact throughout the study. All pigs were cared for according to standard procedures, which included castration of males at 5-7 days of age and weaning at 3 weeks of age. The following variables were collected per pig while in the farrowing room: average daily gain, teat order consistency, injury scores, and time budgets for general activity and social behaviour. While the pigs were in the nursery, in addition to the above variables, data from the following behavioural tests were collected: restraint, ease of movement, human approach and novel arena (response to human and novel object). The restraint test was similar to that used by Hessing et al. (1994). During this test, each pig was placed on their back in a V-trough and restrained for 60 seconds. The pigs were scored based on their degree of resistance. During the movement test, each pig was scored based on their willingness to move down a novel corridor (based on Lawrence et al. 1991). The response to human approach test involved scoring each pig's response to a person appearing at the end of the corridor (based on Lawrence et al. 1991). During the novel arena test, the time to approach within 0.5 m, time to interact, total time spent within 0.5 m, and number of interactions with a human, and time taken to interact with a novel object were recorded (based on Hemsworth et al. 1986). Behavioural testing occurred when the pigs were five weeks old, and then repeated when the pigs were nine weeks old. The data were subjected to a factor analysis to determine 'personality' factors. Correlations between factors and resultant variables (injury scores and weight gain) were also determined.

Results Three primary factors were identified using factor analysis. The factors primarily distinguished pigs based on their response to humans, their willingness to walk down a novel corridor, and their involvement in social displacement activities. The three factors explained 26% of the total variance. The variables that loaded onto Factor 1 included time taken to approach, interact and remain within 0.5 m and the number of interactions with the person in the novel arena, and time taken to interact with a novel object. Activity levels during the nursery (time spent lying and standing) also loaded onto this factor. A frequency distribution of the scoring coefficients per pig on this factor revealed a skewed distribution. This factor was correlated with average daily gain (r^2=0.27; P<0.05), during the nursery phase. The variables that loaded onto Factor 2 included the timing and scoring of the pig's willingness to move down a novel corridor, their response to restraint and activity levels (time spent lying and standing) while in the farrowing room. A frequency distribution of the scores per pig revealed a normal distribution. The variables that loaded onto factor 3 included social displacement activities (pushing/biting another pig causing recipient to move). The frequency distribution of the scores per pig revealed a normal distribution.

Conclusions Based on the variable loadings for each factor, it appears that Factor 1 is primarily related to level of confidence, Factor 2 is related to level of exploration and Factor 3 is related to level of aggression. The level of confidence was correlated with weight gain. It appears that pigs showing less confidence, in response to humans, gained more weight. However, these pigs were also less active. This study will be expanded to investigate the effect different re-grouping strategies (based on weight, behaviour or random) have on 'personality' traits and form the basis of recommendations to the industry about the most appropriate grouping strategies to use.

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The effect of boar team size on reproductive behaviour in a dynamic service system

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Introduction The effect of male hierarchy on the sexual activity of individuals is unclear from the recent scientific literature and a clear distinction between the social and sexual aspects of this hierarchy is lacking. Moreover, the effect of male team size on the extent of sexual competition between individuals has not been examined in the recently developed group mating systems for pigs, such as the Dynamic Service System for gilts (DSS). The main objectives of the present study were therefore to examine the effect of boar sexual status on the sexual activity of individuals and the influence of boar team size on the reproductive output of a DSS.

Materials and Methods The mating management of the DSS studied has been described elsewhere (Grigoriadis *et al*., 2000). Briefly, gilts were introduced in groups of four at weekly intervals into the study service pens containing a resident boar team and 16 gilts at different reproductive stages, and copulations took place under no supervision. The gilts underwent synchronised puberty stimulation at 200 days and 105 kg, using exogenous gonadotrophin (PG600®, Intervet UK Ltd.), and were planned to be served at their second post pubertal heat, 4-5 days after entry into the service pens. The reproductive behaviour and performance of 72 gilts mated with a four-boar team (4B) were compared with that of 64 gilts mated with a two-boar team (2B). The 2B teams were formed from the 4B teams after removal of two boars (the sexually most superior and inferior boars within each team). The boars were 9-10 months old at the beginning of the study and had been previously used for services in the farm. The male sexual hierarchy was defined by using quantitative behavioural criteria (i.e. mating frequency and quality; see Grigoriadis et al., 2000), and the social status in a feed competition test. Each team was observed under 4B and 2B conditions for a four week period, and four different teams were used as replicates in the study. The behaviour of the sexual partners was observed continuously (24 hrs/day) using video recording equipment. All groups were floor fed once per day with 2.5 Kg/animal of a standard commercial diet. Treatments were compared using Student's T test for ordinal data or χ^2 test for nominal data.

Results Males of different sexual status differed significantly in both mating quality and frequency ($p<0.05$ and $p<0.01$ respectively), but this was unrelated to social status. The removal of two boars from each team significantly improved overall mating quality ($p<0.001$, Fig 1) and reduced the mating replacement frequency (i.e. where one boar displaced a team-mate from the back of the gilt and took his position; 26% for the 4B vs. 12% for the 2B teams, $p<0.001$). However, individual boars tended to perform more frequent services (i.e. matings of a duration where intromission and ejaculation were likely to have occurred) under 2B than 4B conditions (7.5/week vs. 5.1/week respectively, sed=1.5; $p=0.08$). Gilts mated by a 4B team exhibited oestrus two days earlier after boar contact than their 2B counterparts (4.5 vs. 6.6 days respectively, sed=0.5; $p<0.01$). The number of boars per service pen did not influence gilt oestrus duration and conception rate at first service, nor the number of piglets born alive and total litter size (Table 1).

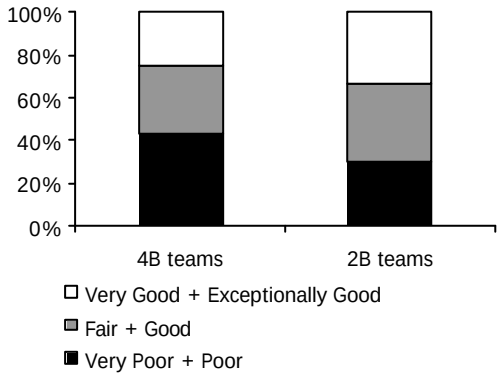

Fig. 1: Mating quality per treatment (n=1280)

Table 1: Mating and reproductive performance of 4B and 2B gilts

	4B gilts (n=72)	2B gilts (n=64)	sed	sig
Matings* per oestrus	9.8	6.6	0.9	p<0.001
Services§ per oestrus	4.2	3.5	0.5	ns
Oestrus duration (hrs)	26.0	26.5	1.8	ns
Conception rate (%)	83.1	89.1		ns
Piglets born alive	10.6	10.6	0.5	ns
Total litter size	11.3	11.2	0.5	ns

*irrespective of quality
§matings of a duration where intromission and ejaculation were likely to have occurred

Conclusion In the short term, there are no risks involved for the reproductive efficiency of a DSS by reducing the number of boars from four to two per service pen. However, longer term evaluation of this strategy is required to support the conclusion before recommendations are commercially implemented.

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The behavioural responses of mink (*Mustela vison*) to deprivation of highly valued resources.

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Introduction The application of micro-economic theory to behavioural priorities has shown that captive animals place a high value on some resources that are denied in captivity and a low value on others (Mason et al. 2001). For example, mink work hard for access to swimming water, but not for tunnels or toys. An alternative measure of value are the animal's physiological and behavioural responses to denial of resources. In this study, mink were denied access to three resources (food, swimming water and tunnels) and their behaviour recorded for symptoms of deprivation.

Materials and Methods Eight mink (4 males and 4 females) were used as experimental subjects. Each was housed for five days in a closed economy test arena, which consisted of eight compartments joined by wire tunnels (Cooper and Mason 2000). Each compartment contained a different resource. These were: 1) nest box; 2) dry ferret food (James Wellbeloved Ltd.); 3) water-bath; 4) novel objects; 5) bowl of water; 6) pliable objects such as cat-toys; 7) tunnels; and 8) opportunity for visual interactions with other mink. Each compartment had one-way lockable entrance and exit doors. On the first pre-treatment day no compartments were locked. On each subsequent treatment day, two mink were exposed to each of the four treatments. These were no compartments locked, locked Food, locked Bath and locked Tunnels. The minks' behaviour in each treatment was recorded on video for a period of eight hours. Data collection focussed on three types of measure. These were the number of attempts to gain access to locked compartments, locomotor and stereotypic activity and the use of other resource compartments. Data were analysed by repeated measure ANOVA with Tukey's t test to compare treatments.

Results When all eight compartments were accessible mink spent the majority of the observation period in the nest compartment (Table 1) and on general locomotion and little time on stereotypy. Locking the food compartment increased stereotypy ($P<0.05$), which mainly consisted of pacing in front of the locked compartment door. General locomotion was, however, not affected. Mink made more attempts to open the doors for both the locked Bath and locked Food than for locked Tunnels ($P<0.05$). There were no changes in use of any other compartments except for increased use of the toy compartment when locked out of Food ($P<0.01$).

Measure	No Lock	Lock Tunnels	Lock Bath	Lock Food	F
Push Front	N/A	4.7	12.5	25.8	23.0***
Push Back	N/A	2.0	4.7	9.2	5.98**
Stereotypy	0.30	0.70	0.30	3.63	3.31*
Locomotor	15.5	17.5	17.6	16.1	0.11
Time in Food	5.36	4.24	4.68	N/A	0.95
Time in Bath	2.39	1.33	N/A	1.96	1.08
Time in Tunnels	0.89	N/A	1.86	0.73	2.42
Time in Nest	54.4	53.2	52.7	45.7	0.67
Time in Bowl	3.27	3.18	3.89	2.39	0.45
Time in Toys	0.67	0.24	0.56	4.07	7.81**
Time in Social	8.98	15.6	11.6	13.0	0.46
Time in Novel	1.23	2.74	1.37	1.53	0.67

Table 1: The mean number of attempts (in 8h.) to open front and back doors to locked compartments and the mean percentage of observation time spent on stereotypy, on general locomotion and in each resource compartment in the 4 treatments. F calculated from repeated measure ANOVA. *P < 0.05, **P < 0.01, ***P < 0.001.

Discussion and Conclusion The minks' primary response to locking out of a valued compartment was an increase in attempts to push open the doors. There was no change in general locomotion and only locking the food compartment increased stereotypy. In addition mink locked out of the food compartment showed some re-direction of behaviour to toys. There was no evidence of re-direction of swimming motivation with no increase in use of the bowl when denied access to the swimming bath. These results are consistent with the minks' behavioural priorities, as mink pay high costs for access to food and swimming water and further confirm that these resources are important to fur-farmed mink (Mason et al 2001).

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The effect of relative abundance on diet choice in fallow deer

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Introduction The fallow deer is a generalist herbivore that eats different plants containing secondary compounds in various amounts. From observations of foraging behaviour it is known that large herbivores tend to eat from a variety of locations and, over the short term, typically ingest small quantities of a variety of foods and sample novel foods rather than making an immediate decision to either eat large amounts or to reject the food (Freeland and Janzen 1974). The diet choice of large herbivores is influenced by the presence of nutrients and toxins (Provenza 1995), but another factor that could influence the diet choice is the relative abundance of different plants and plant types. In order to study these effects we have performed experiments on the effect of the relative abundance of different food types on diet choice in fallow deer.

Materials and methods Six adult hand-reared fallow deer hinds (*Dama dama*) were used. The animals were tested one at a time in a small experiment enclosure. In initial two-choice tests, preferences for and aversions against different compounds were investigated (acetic acid, ascorbic acid, methyl salicylate, mono sodium glutamate, sucrose and tannin). The compounds were added in varying concentrations to food (pellets) and were presented to the animals in bowls placed on the ground. To test the effect of the relative abundance of food types with different concentrations of compounds, cafeteria experiments with eight bowls were then performed. The animals were tested eight times in the cafeteria experiments. In one cafeteria experiment, three concentrations of tannin were used (29.5 g/kg, 5.9 g/kg and 1.18 g/kg; the same concentrations as in the two-choice test, where first H/M and then M/L were offered). One bowl contained the lowest concentration, three contained medium, and four contained the high concentration. In another experiment, two concentrations of sucrose were used (29.5 g/kg and 8.9 g/kg). One bowl had the high concentration and the remaining seven the low. Apart from having experienced the two-choice tests, the animals had no training prior to the cafeteria experiments.

Results In the two-choice tests tannin produced the strongest aversion. The animals consistently preferred the lowest concentration of tannin (see fig. 1: low-medium, p= 0.028; medium-high, p= 0.028). However, in the cafeteria experiment with tannins the animals ate similar amounts of the food with medium and low concentrations of tannins, but less of the food with high concentration (see fig. 2: low-medium, p= 0.249; medium-high, p= 0.028). Comparing the proportion of low tannin food eaten by each animal, this proportion was significantly smaller in the cafeteria experiment than in the two-choice test (p= 0.028). The reason for the smaller consumption of low-tannin food in the cafeteria experiment was not that the bowls with this food became empty, but must instead be related to the distribution of food types. Concerning sucrose, the high concentration was preferred in the two-choice test (p= 0.046), whereas more low than high concentration food was eaten in the cafeteria experiment (p= 0.028). In fact, the consumption per bowl was similar for both concentrations (p= 0.89).

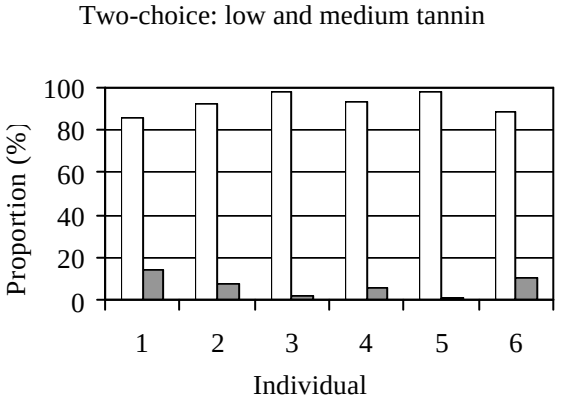

Fig. 1. Proportion eaten of each food type in the two-choice test with low and medium concentrations of tannin.

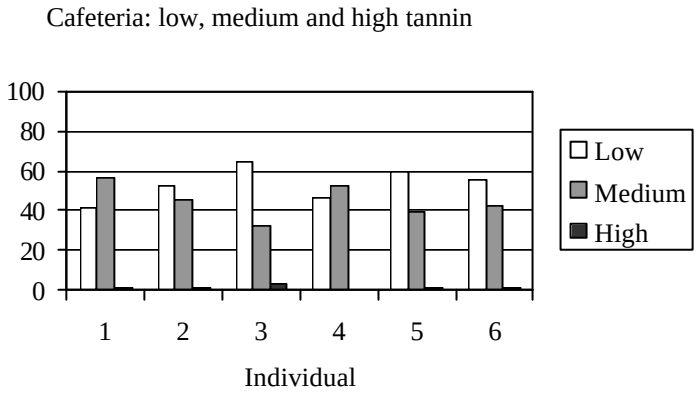

Fig. 2. Proportion eaten of each food type in the cafeteria experiment with low, medium and high concentrations of tannin.

Conclusions These results suggest that the relative abundance of food types influences the diet choice in fallow deer. The general tendency seems to be that high frequency food types are eaten to a greater extent than would be expected from preferences demonstrated in two-choice experiments.

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Long-term Psychophysiological Response of Dairy Calves to Hot-Iron Dehorning

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Introduction It is generally accepted that routine invasive husbandry procedures, such as disbudding and dehorning, cause considerable distress in farmed animals. In the case of dehorning, it has repeatedly been shown that calves exhibit many pain related symptoms both during (e.g., withdrawal from noxious stimulus) the procedure and for some hours post procedure (e.g., rearing, tripping, head rubbing, etc.). Little is known about the long-term consequences of dehorning as previous studies have only monitored responses for up to 48 hours. The objectives of this work were to examine the long-term response of calves to hot-iron dehorning and to determine the effectiveness of non-steroidal anti-inflammatory drugs (NSAID) for reducing post-operative distress.

Materials and Methods The experiment was conducted using 20 Holstein calves between 4 to 8 weeks of age housed at the University of British Columbia's Research and Education Dairy Unit. All calves were individually housed and were bucket fed whole milk at 5% of their body weight (BW) twice daily, morning and evening. They also had *ad libitum* access to hay, calf concentrate, and fresh water. Calves were assigned to one of two treatments balanced for both gender and age, dehorning with the NSAID (Trt 1) and dehorning without the NSAID (Trt 2). Trt 1 calves received both pre-emptive and post-operative pain relief (3mg/kg BW of Ketoprofen) at 8am pre-procedure and at 12:30pm and 6pm post-procedure. Calves on Trt 2 received neither pre or post operative pain relief. All calves first underwent a sham dehorning before being dehorned 72 hours later. At 7am on the morning of both the sham and actual dehorning calves on Trt 1 received 3mg/kg BW of Ketoprofen in their milk whereas those on Trt 2 just received milk. Two hours later all calves were administered 0.2mg/kg BW of Xylazine intramuscularly. Xylazine acts as a potent sedative in calves thereby removes the need for any physical restraint during subsequent procedures. Ten minutes after the delivery of Xylazine calves on both treatments received 6ml of a local anaesthetic (LA) (Lidocaine) per horn bud. Four mls were injected into cornual groove midway between the eye and the horn bud and a further 2 mls were injected as a ring block around the bud itself. The calves were then given 15 minutes to allow sufficient time for the LA to take effect before the application of the dehorning iron to the buds. For the sham procedure the iron was at room temperature when applied to the buds but was preheated to approx. 600°C for the actual dehorning. Behaviour and cardiac activity was continuously recorded for 12, 30-minute, periods over the course of both the sham and actual dehorning. The first observation period took place 1 day before sham dehorning and continued at fixed intervals until 7 days post dehorning.

Results Pre-procedure (1 day) there were no differences in activity levels across treatment groups. However, on the mornings before sham and actual dehorning calves on the ketoprofen treatment showed higher levels of locomotory behaviour and spent less time lying than the control animals ($P<0.05$). There was an effect of observation period on general behaviour ($P<0.05$). Locomotory behaviour decreased (Figure 1) and lying behaviour increased after both sham and actual dehorning. Activity levels remained depressed for up to 7 days post dehorning but this did not differ significantly. There was a consistent effect of treatment on the frequency of vocalisations that continued until 3 days post dehorning (Figure 2) There were no pre-procedural differences between treatments but Trt 2 calves were more vocal post both sham and actual dehorning. Trends in mean heart rate (HR) response (bpm) were similar to those seen in activity levels. There were no effects of treatment on HR but there was a significant decrease after both sham and actual dehorning. At 3pm, +1 day, and +3 days post dehorning there was a tendency for HR to be elevated above baseline values. By 7 days post dehorning HR had returned to near baseline levels. Time domain measures of heart rate variability indicated that Calves on Trt 2 had lower levels of overall heart rate variability (HRV) immediately post dehorning compared with those treated with ketoprofen ($P<0.05$), suggesting lower parasympathetic input into cardiac control.

Figure 1. Effects of Treatment on Locomotion

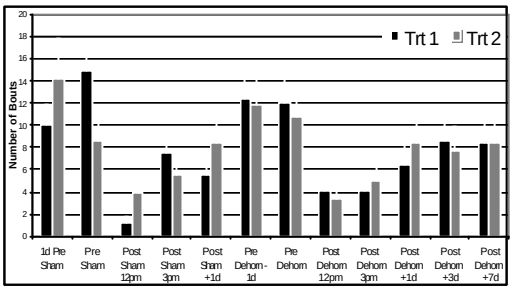
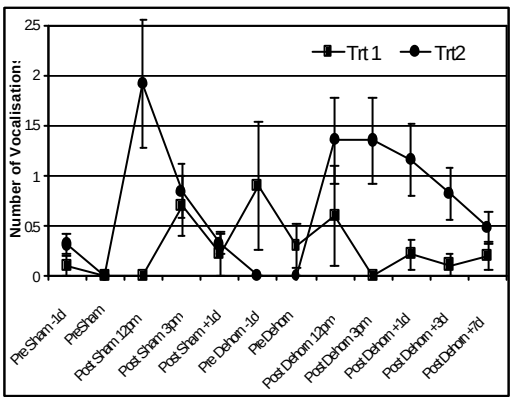


Figure 2. Vocal Response to Dehorning



Discussion There were some limited effects of treatment on pain-related behaviours and vocal response which suggest that ketoprofen may help to reduce immediate post-operative pain. However, this benefit was short lived and by 24 hrs post dehorning there were few treatment differences. The trends in cardiac activity also imply an absence of any long-term benefits. Overall, behavioural responses suggest that some post-operative distress may still be evident at 7 days post dehorning whereas cardiac activity returns to near normal levels on day 3 post-procedure. In conclusion, ketoprofen helps to reduce some immediate post dehorning distress but this distress may persist for up to 7 day after dehorning.

Relationship between rooting behaviour and foraging in growing pigs

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Introduction Evidence suggests that rooting behaviour in growing pigs involves elements of both foraging for food (Day et al., 1995) and exploration (Wood-Gush and Beilharz, 1983). The objective of this experiment was to investigate the relationship between rooting behaviour and foraging in growing pigs. This involved assessing the affects of food rewards and feed restriction on the performance of rooting behaviour.

Materials and method Study 1 assessed the effect of food rewards in the rooting substrate on the performance of rooting behaviour. Ninety six 11-week-old pigs were allocated in a randomised block design to one of two treatments: (1) access to a rooting substrate containing feed pellets mixed through it at a ratio of 1:8 (pellets:substrate), or (2) access to a rooting substrate without feed pellets. In both treatments, the same feed pellets which were mixed with substrate in treatment 1 were offered *ad libitum* to the pigs. Study 2 assessed the effect of feed restriction on the performance of rooting behaviour. In this study, one hundred and ninety two 11-week-old pigs were allocated in a randomised block design to one of four levels of feeding: (1) 100 percent of appetite, (2) 90 percent of appetite, (3) 80 percent of appetite, or (4) 70 percent of appetite. Feed restriction levels were based on *ad libitum* intake. All pigs had access to a rooting substrate. Landrace x Large White pigs (¾ bred) were used in studies 1 and 2 and were housed in groups of six animals which were balanced for gender and weight. The pigs were housed in rooms (3.3 x 1.8 m) with solid walls and floors and one single-space feeder supplying both feed and water. The rooting substrate used in both studies was partially-dried spent mushroom compost (46 percent moisture content), and this was supplied at a rate of 4 kg/group/day. Each group was videotaped for a continuous 72 hour period at the end of the first and second week of treatment. The tapes were scanned at 10 minute intervals and the number of pigs involved in active rooting behaviour (rooting while standing up) was recorded. Data were analysed using Genstat 5. The number of pigs rooting in the substrate was expressed as a percentage of the group and treatment means were compared by analysis of variance. In study 2, an analysis of variance was used to assess whether there was a linear response in rooting behaviour to feed restriction.

Results There were no significant differences between treatments in the average percentage of pigs performing rooting behaviour over a 24 hour period in study 1 (Food rewards: 3.6 percent; no food rewards: 4.4 percent of group; SEM 0.63) or study 2 (100 percent appetite: 2.7 percent; 90 percent appetite: 2.3 percent; 80 percent appetite: 3.4 percent; 70 percent appetite, 3.9 percent of group; SEM 0.57). However, in study 2 there were significantly more pigs performing active rooting behaviour between 0800 and 1200 hours, when pigs were fed at 70 and 80 percent appetite than at 90 and 100 percent appetite ($P<0.05$) (Figure 1). The percentage of pigs rooting in the substrate over a 24 hour period did not show a linear response to feed restriction ($P>0.05$).

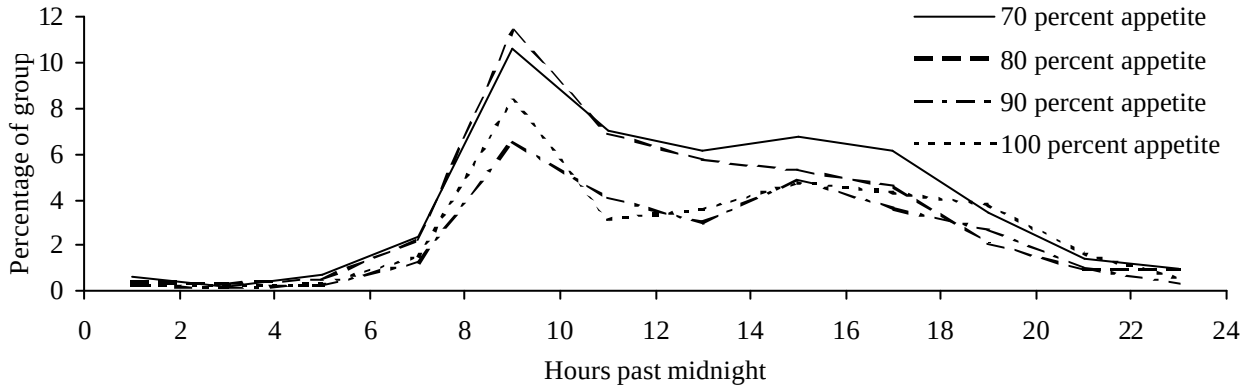

Figure 1 *Influence of feed restriction on active rooting behaviour*

Conclusions The fact that the pigs did not show an increase in rooting behaviour when offered food rewards may have been because the same type of food was freely available from feeders. This would suggest that the rooting behaviour which was shown in study 1 was motivated more by exploration than by foraging. It is possible rooting behaviour only became associated with foraging when feed was restricted to 80 percent in study 2. This may have implications for sow behaviour, as feed levels for gestating sows may be restricted to as little as 40 percent of appetite (Walker and Beattie, 1994).

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The effect of salt deficiency on the behaviour of finishing pigs in a tail chew test

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Introduction Tail-biting is an adverse behaviour which can lead to injury in the recipient pig, reducing welfare and causing abscesses in the carcass. A survey in abattoirs in the UK found that 5 percent of pigs at slaughter have their tails bitten (Guise & Penny, 1998). Work by Fraser (1987) suggested that tail-biting is linked to a deficiency in dietary minerals. This study investigated whether finishing pigs were more attracted to salt after being offered a diet deficient in salt for two weeks.

Materials and methods 271 pigs from 41 first cross Large White x Landrace dams mated with 25 different sires were tested in a tail chew test at 16 (tail chew test 1) and 18 (tail chew test 2) weeks of age. All pigs were housed in fully slatted finishing pens and were offered a standard commercial diet *ad libitum* up to 16 weeks of age. When the pigs were 16 weeks old all salt was removed from the diet and the pigs remained on this salt deficient diet until after the second tail chew test at 18 weeks of age. Both tail chew tests entailed placing each pig individually in a unfamiliar pen for 10 minutes. Two ropes, one soaked in 5 percent sodium chloride solution and the other plain were suspended in the pen approximately 0.6m above floor level. The duration and frequency of all behaviours were recorded and the data were subjected to ANOVA to identify if the behaviour of the pigs differed between the two tail chew tests.

Results Overall behaviour directed towards the ropes did not differ between tail chew test 1 and 2 however both the time spent and frequency of sniffing both the plain and salty ropes increased between tests ($P<0.001$) (Table 1). The frequency of activity ($P<0.05$), rooting ($P<0.01$), walking ($P<0.01$) and time spent nosing the ground ($P<0.05$) all were greater in tail chew test 2 compared with tail chew test 1 (Table 1).

Table 1 Time spent and frequency of behaviour in tail chew tests before and after salt deficiency

Behaviour	Before salt deficiency (Tail chew test 1)	After salt deficiency (Tail chew test 2)	s.e.m.	P
Rope directed (% dur)	5.9	6.3	0.61	NS
Chew plain rope (% dur)	2.4	2.7	0.40	NS
Chew salty rope (% dur)	2.0	2.3	0.30	NS
Sniff plain rope (% dur)	0.1	0.2	0.04	<0.05
Sniff plain rope (min-1)	0.03	0.06	0.007	<0.001
Sniff salty rope (% dur)	0.1	0.3	0.04	<0.001
Sniff salty rope (min-1)	0.03	0.07	0.007	<0.001
Activity (min-1)	4.71	4.98	0.135	<0.05
Rooting (min-1)	0.26	0.35	0.030	<0.01
Walk (min-1)	3.16	3.42	0.085	<0.01
Nose ground (% dur)	16.0	14.3	0.64	<0.05

Conclusions Removing salt from the diet of finishing pigs increased the time spent sniffing at ropes but did not differentiate between plain and salty ropes or increase the time spent or incidence of chewing or manipulating ropes. Work by Breuer et. al. (2001) has shown that chewing ropes in a tail chew test is linked with tail-biting in the home pen. Therefore it would appear from this study that salt deficiency in the diet of finishing pigs may not be linked to tail biting as previously proposed by Fraser (1987). However salt deficiency did increase the general activity of pigs especially rooting and nosing ground supporting the hypothesis that rooting behaviour may be linked with foraging (Day *et. al*., 1995).

Acknowledgements The authors gratefully acknowledge funding from ministry of Agriculture, Fisheries and Food.

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Does aggressiveness of individuals affect the feeding behaviour of group-housed growing pigs?

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Introduction The feeding behaviour of group-housed pigs differs from that of pigs housed individually in that they make fewer visits to the feeder of a longer duration (Bornett *et al*., 2000). Possible reasons for this difference in feeding behaviour include competition and group cohesion and these may be influenced by group composition in terms of aggressiveness. Indeed, Erhard *et al*. (1997) found that groups comprising all low aggressive pigs integrated into a group quicker than groups of all high aggressive pigs. The aim of this study was to investigate the effect of aggressiveness of individuals on the feeding behaviour of group-housed growing pigs.

Materials and methods Large White X Landrace, male and female pigs from 10 litters were used in an experiment consisting of 3 replicates, each replicate comprised 3 periods. In Period 1 (3 weeks), all pigs were housed with their littermates after weaning at 4-5 weeks of age. Each pig's aggressiveness was assessed using an attack latency test. The 8 fastest attacking pigs were classified as being High (H) aggressive and the 8 slowest attacking or non-attacking pigs as Low (L) aggressive. In Period 2 (2 weeks), the 8 H pigs and 8 L pigs were individually housed for 2 weeks and their feeding patterns and food intake were recorded. In Period 3 (3 weeks), the 16 pigs were combined into 2 groups, one group consisted of 8 H pigs, the other of 8 L pigs. In total there were 3 replicates of each group composition. Throughout Period 3 feeding patterns, food intake and social behaviour were recorded. Feeding behaviour was analysed in two ways; firstly in terms of feeder visits and secondly, visits were collapsed into meals using the 2-log normal model (Tolkamp *et al*., 1998). Data were analysed on an individual pig basis using analysis of variance so that results from the pigs when they were individually housed and group housed could be compared.

Results Frequency and duration of fighting decreased over time from mixing in Period 3 ($P<0.001$, and $P<0.001$ respectively). Pigs in H groups had a higher frequency and duration of fights on the day of mixing than pigs in L groups ($P<0.001$ and $P<0.001$ respectively). As expected feeding behaviour changed between individual (Period 2) and group (Period 3) housing (see Table 1). There were no effects of aggressiveness (A) of individuals within a group on feeding behaviour described in terms of feeder visits.

Table 1 Feeding behaviour of pigs in Period 2 (housed individually) and Period 3 (housed in groups of 8 H or 8 L pigs).

	Period (P)	H	L	P s.e.d.	P sig.	A s.e.d.	A sig.	P X A s.e.d.	PXA sig.
Visits/day	2	127	115						
	3	32	28						
	Mean	79	71	5.8	***	6.8	NS	8.2	NS
Visit duration (s)	2	30	43						
	3	115	111						
	Mean	73	77	7.2	***	8.3	NS	11.0	NS
Food intake per visit (g)	2	11	15						
	3	107	156						
	Mean	59	86	28.4	***	25.8	NS	40.1	NS
Food intake (g/d)	2	1533	1471						
	3	1757	1697						
	Mean	1645	1584	41.9	***	73.3	NS	59.2	NS

***$P<0.001$, NS=$P>0.05$

There were no effects of aggressiveness on meal criterion or between meal length. However, H pigs had more meals per day than L pigs in both in Periods 2 and 3 (means across periods: 24.2 vs. 20.0 meals per day respectively, $P<0.05$).

Conclusions Attack latency successfully predicted aggressiveness on Day 1 of grouping; pigs in H groups had a higher frequency and duration of fighting than pigs in L groups. When group housed, pigs altered their feeding behaviour in the direction of fewer feeder visits of a longer duration and ate more per visit at a faster rate than when individually housed. The aggressiveness of individuals within a group did not affect feeding behaviour in terms of visits but H pigs had more meals per day than L pigs. It is possible that this link between feeding behaviour and aggression could be used as a tool for identifying high aggressive pigs within a population.

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Responses to ACTH challenge of previously stall-housed sows, housed in groups with free-access stalls

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Introduction There is some concern among Irish producers converting from stall to loose housing for pregnant sows that sows will be unable to adapt to these systems having spent several parities in stalls. The objective of the 2 experiments referred to in the current paper was to assess the welfare of sows previously housed in stalls when changed to group housing. In the 1st experiment housing constraints meant that it was not possible to measure sow welfare in the loose housing treatment for more than one month. With the advent of additional loose pens it was decided to conduct a second experiment whereby sow welfare was measured up to the end of the gestation-housing period. The current paper presents the findings from both experiments on responses of sows to an ACTH challenge at different stages.

Materials and methods *Experiment 1*- At day 30 of pregnancy, 48 multiparous sows were randomly assigned in batches of four to stalls [S] (n=4 [16 sows]) or to loose [L] pens in groups of four (n=8 [32 sows]) for one month. On days 29 and 56 of pregnancy, 5 two-hourly saliva sample collections were made beginning at 0900h to determine circulating corticosteroid concentrations. *Experiment 2* - At day 30 of pregnancy, 52 multiparous were randomly assigned in batches of four to stalls [S] (n=7 [28 sows]) or to loose pens [L] in groups of four (n=6 [24 sows]) until 5 days pre-partum (i.e. day 110 of pregnancy). At days 29 and 88, 9 hourly saliva sample collections were made beginning at 0900h to establish circulating corticosteroid concentrations. Between 1000h and 1100h on day 57 (Exp.1) and day 89 (Exp.2), 200i.u. ACTH (Synacthen, CIBA-Geigy) was administered by intra-muscular injection following collection of a baseline sample. Further saliva collections were made 1.0 and 2.0 hours post-injection. In both experiments, saliva sampling on day 29 was conducted while all sows were housed in stalls. The loose housing treatment consisted of pens with a fully slatted roaming area and 4 full-length, free-access, feeding stalls. All sows were wet fed twice per day. Data were analysed by ANOVA for repeated measures using PROC GLM of SAS.

Results Salivary cortisol concentrations did not differ significantly between treatments in both experiments prior to the start of the experiments (i.e. day 29) (Table 1). At both days 56 (exp. 1) and 88 (exp. 2) of pregnancy, [L] sows had significantly higher circulating salivary cortisol concentrations than [S] sows. In exp. 2, [L] sows also had significantly higher baseline concentrations of cortisol prior to challenge with ACTH on day 89 (3.3 ± 0.66 *vs* 1.6 ± 0.12, [L] *vs* [S] respectively; P<0.01). Therefore, baseline cortisol concentrations were included as a covariate in the analysis of the data from the ACTH challenge test.

Table 1 Salivary cortisol concentrations (lsmean $nMol^{-1}$ ± s.e.) in loose and stall housed sows prior to entry to the housing treatments (day 29) and during pregnancy (day 56 – Exp.1 and day 88 – Exp.2)

		Loose	Stall	P
Experiment 1	Day 29	1.8 ± 0.18	1.5 ± 0.26	NS
	Day 56	3.4 ± 0.59	1.5 ± 0.26	0.05
Experiment 2	Day 29	1.5 ± 0.07	1.6 ± 0.09	NS
	Day 88	4.3 ± 0.54	2.0 ± 0.19	<0.01

There was no significant difference between housing treatments in the responses shown to ACTH challenge in either experiment 1 (Figure 1) or experiment 2 (Figure 2).

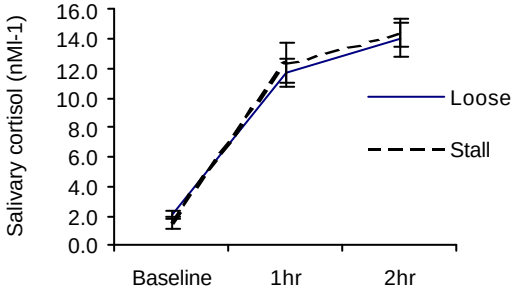

Figure 1 Effect of treatment at day 57 of pregnancy on cortisol concentrations (lsmean nMl^{-1} ± s.e.) post ACTH

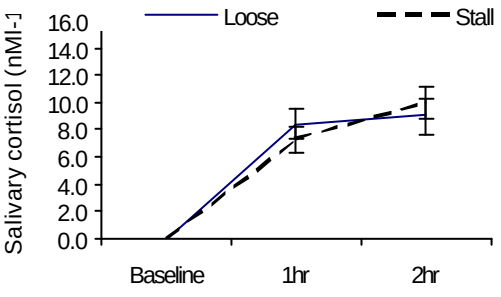

Figure 2 Effect of treatment at day 89 of pregnancy on cortisol concentrations (lsmean nMl^{-1} ± s.e.) post ACTH

Conclusions Circulating levels of cortisol were higher in [L] sows compared to [S] sows after one and two months in the housing treatments. However, the findings from the ACTH challenge tests suggest that [L] sows were no more chronically stressed than [S] sows in either experiment. Thus, it appears that [L] sows may have been more 'stimulated' by the saliva sampling procedure (Spoolder et al., 1995) which was conducted while the sows remained unrestrained in the loose pens. In conclusion, the loose housing system investigated in the current experiments appeared to pose no problems for previously stall-housed sows in terms of physiological adaptation.

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The effect of paddock rotation management on pasture damage by organic dry sows

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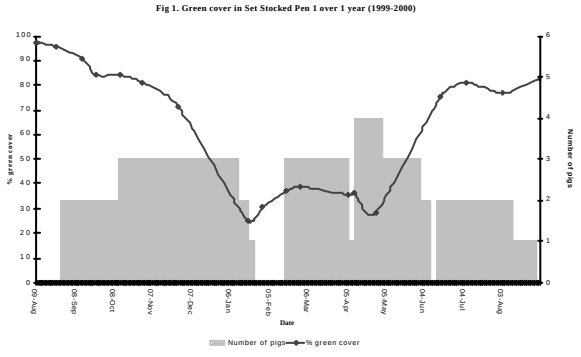
Introduction Nose ringing is widely used in conventional outdoor pig production as the only reliable method of preventing sows destroying pasture by rooting (Edwards *et al.*, 1998), but is prohibited by some organic sector bodies as it inhibits the sows' behaviour. Some organic producers use a rotation policy in which the sows are moved to fresh pasture about three times a year, after green cover has been destroyed. As well as limiting nutrient leaching, frequent movement also limits parasite build-up in a system which prohibits the routine use of anthelmintics. However, it has a high labour demand. An alternative strategy is to maintain the sows on a larger area for the whole year. This abstract presents initial data on comparison of the two systems regarding annual pattern of pasture damage by sows.

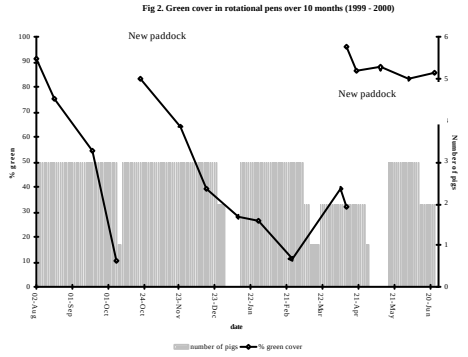
Materials and Methods Two alternative dry sow systems were established on a second year grass-clover ley on the same organic pig unit. In the "Rotational" system, up to 6 sows occupied a 40m x 40m paddock which was moved approximately every four months, thus utilising 120m x 40m over the whole year. In the "Set Stocked" system, up to 6 sows occupied a 120m x 40m paddock for the whole year. In 3 replicates of each system, sward quality was assessed at regular intervals of 2-4 weeks by scoring % green cover using a regular pattern of 0.5m x 0.5m quadrats in a W-formation across each paddock. In calculating mean % green cover, it was assumed that change between observations was linear. The number of pigs in the paddocks varied throughout the year; therefore pig occupancy over the whole year was expressed as % of maximum possible pig days (maximum of 6 sows x days of paddock life).

Results Data from the two paddock types are summarised in Table 1. The mean level of vegetation cover of the two paddock types was broadly similar, but the rotational system gave greater variation over time and lower minimum values. These paddocks were characterised by loss of green cover, which was then restored as the pigs were moved to a fresh 40m x 40m paddock. In the set-stocked pens, green cover was reduced in the autumn and early winter, then remained at a lower level (< 40%) until spring. In the summer, regrowth to levels of greater than 80% was recorded. Typical data for the Set Stocked paddocks are shown in Figure 1, and for the Rotational paddock in Figure 2.

Table 1. Vegetation cover in rotational and set stocked paddock management systems

Paddock	Date		Days in	%	Green cover			
	Start	End	use	occupancy	mean %	max %	min %	% days <50%
Set stock 1	09Aug99	01Sep00	389	37	62.0	97	25	38
Set stock 2	03Nov99	20Oct00	352	40	57.8	91	17	42
Set stock 3	07Jan00	20Oct00	287	47	69.3	81	66	0
mean			***343***	***39***	***63.0***	***90***	***36***	***27***
Rotational 1	02Aug99	23Jun00	326	42	53.1	98	10	46
Rotational 2	04Oct99	31Oct00	385	40	57.8	96	11	36
Rotational 3	13Oct99	23Oct00	376	49	56.8	97	13	27
mean			***320***	***41***	***55.9***	***97***	***11***	***36***





Conclusions There was little overall difference between the two systems in terms of vegetation cover, however, the patterns of change in cover differed markedly. Use of Rotational paddocks after the pigs have been moved has major implications for nutrient losses, i.e. if a crop is planted immediately or the site left "fallow" for some months before further cropping. Foraging and excretory behaviour in the different systems is being analysed.

Reference

Edwards S A, Jamieson W, Riddoch I, Watson C A. 1998. Effect of nose ringing and dietary modification in outdoor pig production on temporal changes in soil nitrogen status. *Proceedings of the British Society of Animal Science*. p42.

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The effect of rearing environment upon behaviour and the rate of 5-HT synthesis and hypothalamic 5-HT levels in pigs

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Introduction Enriching the environment of young pigs reduces aggressive behaviour and increases exploratory behaviour. This difference in behaviour is maintained throughout life, even in the absence of enriching stimuli (Beattie *et al*., 1995). Although the exact neurochemical and neurophysiological mechanisms underlying aggression are not well understood there is evidence which indicates that low brain 5-hydroxytryptamine (5-HT) may be involved in the etiology of aggression (Burrows, 1999). This study examined the possibility that behavioural changes induced by environmental enrichment may be associated with subtle alterations in brain 5-HT chemistry.

Materials and methods In the enriched treatment four sows and their litters were housed in large bedded pens from three days post-partum. In the barren treatment four sows and their litters were housed in farrowing pens. Animals were weaned at 4 weeks of age and one group of eight, one boar and one gilt from each of the four litters, was formed per treatment. The group on the barren treatment remained in their farrowing pen after weaning and remixing. In the enriched environment pigs remained in their pens after weaning and remixing and were provided with additional enrichment in the form of shredded paper and mushroom compost on the floor, and mushroom compost suspended by a rack. At five weeks of age behaviour was assessed in a social confrontation test (SCT) (Burrows, 1999). At six weeks of age behaviour was assessed in an open field test (OFT) (Burrows, 1999).
Pigs received an intraperitoneal injection of 600mg NSD-1015 (3-hydroxybenzylhydrazine) (50mg/ml saline) at six weeks of age, followed 30 minutes later by a lethal injection of Dolethal. Systematic administration of the amino acid dehydrogenase inhibitor, NSD-1015, enabled the determination of the rate of 5-HT synthesis in pigs (Burrows, 1999). Immediately after the lethal injection each brain was removed and the hypothalamus dissected and stored in liquid nitrogen until returning to the laboratory, where it was stored at -80ºC. Hypothalamic tissue was analysed for 5-Hydroxytryptophan (5-HTP) and 5-HT using HPLC with Electro-Chemical Detection (Burrows, 1999).
Analysis of Variance (ANOVA) was used to determine statistically significant differences between experimental treatments. In the SCT behaviour is expressed as the number of occurrences per minute.

Results In the SCT pigs from enriched (E) environments sniffed their penmates more (No. of Sniffs: E 4.39, B 2.09, sem 0.456; $P<0.05$) than pigs from barren (B) environments, whereas barren pigs fought more than their enriched counterparts (No. of fights: E 0.57, B 1.97, sem 0.513; $P=0.059$). In the OFT barren pigs were slower to investigate the novel object than their enriched counterparts (Time (s) E 21, B 165, sem 29.5; $P<0.05$).
No significant differences were observed in hypothalamic 5-HTP concentrations between animals raised in either barren or enriched environments (Figure 1).

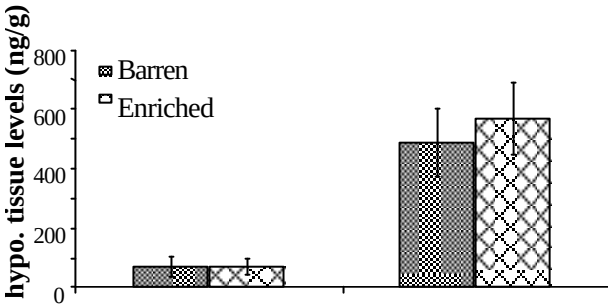

Figure 1 Effect of environment on brain chemistry

No significant differences were observed in brain 5-HT levels between pigs raised in either barren or enriched environments (Figure1).

Discussion Behaviour results in the present study support previous findings which report that enriching the environment of young pigs reduces aggressive behaviour and increases exploratory behaviour (Beattie *et al*., 1995). However in the present study no significant differences were observed in the rate of 5-HT synthesis or in hypothalmic 5-HT levels between pigs raised in barren or enriched environments. It is possible that killing pigs at six weeks of age was too early for them to have developed significant differences in brain chemistry. Work by O'Connell and Beattie (1999), which indicates the strengthening of a behavioural effect over time, supports this hypothesis.

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Technology versus ethics in the animal experimentation debate

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Historically, animal experimentation had been debated utilising an ethical standard. More recently ethics has been largely displaced by an assessment using a technological standard. Technological innovation has, it has been argued, the potential to remove most of the moral issues by producing subjects who suffer less, who are genetically destined to develop certain conditions (e.g. oncomouse) or environmental innovations that allow individuals to experience less pain and distress while the experiment(s) are carried out.

When pressed into a justification of animal experimentation the scientific community present the issues involved as revolving around technological innovation. Russell and Burch's 3Rs are the paradigm example of this. Reduction, replacement and refinement assume the ethical validity of conducting animal experiments albeit within a welfarist framework with abolition as a potential, but probably unreachable, goal.

A relative newcomer to the critique of animal experimentation is scientific anti-vivisectionism. Scientific anti-vivisectionists have argued that the results of animal experiments cannot reliably be extrapolated across species barriers; this illustrates a fundamental and fatal methodological weakness. Alternatives using, for example, human tissues are presented as offering properly "scientific" alternatives to primitive animal experimentation methods.

Neither approach questions the central issue that is the legitimacy of making a sentient being suffer for the benefit, or perceived benefit, of another. Ethics is overtaken by conflicting epistemological claims to scientific truth. Technological judgement may offer means of assessing differing techniques of experimentation but they do not address the central ethical dilemma.

No amount of technical innovation eradicates the need to decide on public ethical matters utilising ethical standards. It is by these standards that the debate around animal experimentation must be resolved and relentless technological innovation and claims to epistemological supremacy simply muddy the waters.

A survey to investigate the level of commercial human-animal interaction during rearing and fear of humans in commercial dairy heifers

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Introduction Recent increases in mechanisation, larger dairy units and financial pressure on dairy farmers result in a reduction in labour and time available to spend with stock. Cattle are innately fearful of humans and this fear has been found to have a substantial impact on their productivity. This survey compliments experimental work conducted at Newcastle University on approach behaviour (see companion paper). The objective was to gather information from dairy farmers on commercial heifer rearing systems; establish the different levels of human interaction and familiarity with stockpersons, explore on-farm indicators of fear and solicit farmers' views on the subject.

Materials and methods The survey was distributed to 1000 dairy holdings across England and Wales taken at random from the sample set published by National Milk Records for England and Wales (1998). Questioning based around indicators of approach behaviour (to establish fear of humans in individual herds) was an attempt to adapt the approach test (see companion paper) to reflect practical situations which could be reported in a questionnaire. The preliminary analysis is based on frequencies.

Results The response generated 516 usable cases.

The trend in intensity of human attention during rearing is a decline with age. The majority of heifers experience contact with more than one person on a regular basis before entering the dairy herd. Only 19% interact with a sole person, 54% have regular contact with 2 people and an additional 27% become accustomed to more than 2 people during rearing. 30% are regularly tended to by a female stockperson during this period. 82% enter the dairy herd knowing the person who is to milk them.

From the frequencies of certain behaviours it can be concluded that in general heifers are harder to handle than older cows in the first few weeks of lactation. 48% of respondents thought that previous human interaction led to more docile cows. For problem milkers, 5% said it was either due to negative experiences as a heifer or the problem milkers were the heifers. 9% gave the reason that previous experiences with humans led to difficult behaviour and a further 20% gave the blanket reason of previous experience (which could include maltreatment from humans).

When asked to outline the close human contact or handling procedures during rearing, 3% stated that positive interactions (talking, stroking etc) were encouraged. 30% listed neutral experiences (neither positive or negative); compared to 99% stating procedures of an aversive nature.

71% of respondents thought that dairy cattle were not fearful of humans; 21% thought they were whilst 8% said that some were. A frequent substantiation was that it depends on how they were treated or had previously been treated, and often it was argued that respondents' cows were not fearful of them but would be of strangers.

Table 1: Approach behaviour towards known stockperson (KS) and unfamiliar people (UP) on commercial farms

% reponses to:	KS - Heifers	KS - Cows	UP - Heifers	UP - Cows
Overly friendly	9.7	11.8	1.0	2.8
Easy to approach	59.9	76.3	39.6	52.2
Neither easy / nervous	27.1	11.0	41.0	35.2
Nervous but approachable	2.8	.6	17.0	8.9
Difficult to approach	.6	.2	1.4	1.0

The cows were more confident than the heifers in their approach behaviour towards both familiar and unfamiliar humans although less so towards an unknown human, this was also the case with heifers who were in general more nervous in their approach of an unknown person.

A section of questions was included to gain an idea of how farmers viewed their cows and the individual attention they gave. 66% of respondents knew all the cows in the herd, some adding – "but not all the heifers". 15% knew most and 13% of the respondents indicated that they knew none. When asked whether it is important to know every individual 93% replied "Yes" it was. As indicated above only 66% did. This could be a reflection of time dedicated to stock or due to a high turnover of animals.

The respondents were asked what in their opinion were the three most important factors influencing dairy cow welfare, 53% stated the person within this response, time allocated to animals was mentioned in 13% of responses, and reducing stress from both conspecifics and other humans represented 12% of the factors.

Conclusions

This survey highlights the influential role stockpeople believe humans can play in shaping an animal's temperament. More in depth analysis will explore associations between commercial management practices with indices of welfare and production. Supported by the findings of the concurrent experimental work it is hoped this study will reveal feasible ways of implementing positive human-animal experiences on our nation's dairy farms to improve the human-animal bond and discover what benefits this will have in terms of heifer production, ease of management and improved welfare.

The influence of positive human-animal interaction during rearing on the approach behaviour of young dairy heifers

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Introduction Modern management of dairy heifers leads to lack of familiarisation with humans. Consequently when the dairy heifer calves and enters the milking herd in close contact with humans whom she innately fears, her productivity and welfare are at risk. The rearing period has potential for shaping the heifers experiences of people prior to the regular contact of milking where productivity can suffer and behaviour be disruptive. This experiment was to determine if positive treatment reduced fear of humans and if so if heifers generalise this response to other humans. The results will be used in further analysis relating to subsequent measures of behaviour, production and welfare associated with milking.

Materials and Methods 30 6month old Holstein-Freisain heifers were allocated to one of two rearing treatments for 9 months. The control group experienced minimal human interaction (associated with routine commercial husbandry). The treatment group received additional positive human-animal interaction (the experimenter brushed the heifer's head, neck and shoulders and talked to her). The allocation to treatment group ensured an even distribution for initial fear of humans (based on approach tests). The heifers were managed in their treatment group with the positive group visited weekly, targeting each heifer for 5 minutes. At the end of the treatment, 2 sets of approach tests were conducted with the heifers, involving a known and unknown female experimenter. The heifers entered a 5 x 5m barren arena, the experimenter then entered and the range of heifers' behaviour (see below) were recorded for 5 minutes. Assumptions were that the difference between an individual's motivation to explore the test arena is minimal and the difference between animals in approach to the experimenter is predominantly determined by level of fear in humans. The flight distance was then recorded – a measure of how close the human could approach the heifer before she turned away. Analysis was by ANOVA.

Results Table 1: Differences in some behaviours studied. (Ü indicates group analysis favour)

	Treatment (1)	Treatment (2)	Mean diff. (1-2)	Std. Error	Signif.
Time to enter within 1m (secs)	Known/positive ⇐	Known/control	- 84.93	38.24	0.002**
Flight distance (m)	Known/positive ⇐	Unknown/positive	- 0.48	0.23	0.037*
	Known/positive ⇐	Known/control	- 0.40	0.23	0.083†
Time spent within 1m (secs)	Known/positive ⇐	Known/control	77.45	19.75	0.000***
		Unknown/positive	65.78	19.75	0.002**
		Unknown/control	53.23	53.23	0.008**
Time to first interaction (secs)	Known/positive ⇐	Known/control	- 124.78	39.15	0.002**
	Known/control	Unknown/positive ⇐	90.73	36.91	0.017**
Duration of interactions (secs)	Known/positive ⇐	Known/control	72.68	17.82	0.000***
		Unknown/positive	55.88	17.82	0.003**
		Unknown/control	59.40	17.35	0.001***
Number of vocalisations	Known/positive ⇐	Unknown/positive	- 16.37	3.04	0.000***
		Unknown/control	- 17.40	2.96	0.000***
	Known/control ⇐	Unknown/positive	- 13.33	2.87	0.000***
		Unknown/control	- 14.37	2.78	0.000***
Number of escape attempts	Known/positive ⇐	Unknown/control	- 1.94	0.94	0.043*
	Known/control ⇐	Unknown/control	- 1.87	0.88	0.038*

Conclusions The positive human contact group showed an improvement in behaviours associated with low fear response to human presence, especially towards the female who had imposed the treatment, they also extended this to the unknown female. On occasions the heifers in the control group showed a preference for the known experimenter with whom they had only had what was assumed to be negligible contact (This has possible implications regarding heifers' memory). Other work has shown that heifers can distinguish between people but may not display a difference in behaviour (see Rushen et al 1999). A commercial survey (see companion paper) showed that many heifers will know the milker prior to entry to the dairy herd but to differing degrees.This work shows that (assuming the treatment has not been negative) it will help lessen the heifers' fear. Where heifers have been treated well, not necessarily by the person who is to milk them, they will generalise this response to some degree to other humans. Further approach tests will now be conducted with familiar and unfamiliar males. This study shows that positive treatment reduces anthrophobia which has important implications for improved heifer welfare. The ongoing study will investigate how this lessened fear translates in behaviour, production and welfare once the heifer calves and enters the milking parlour.

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Treatment with Gonadotrophin Releasing Hormone increases male-male mounting behaviour in 8-week-old beef bull calves

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Introduction Gonadotrophin Releasing Hormone (GnRH) is secreted in a pulsatile manner by the hypothalamus. GnRH is the major hormone controlling the pituitary-testicular axis and therefore influences aggressive and sexual behaviour in bulls. In 6 to 10 week old bull calves an increase in GnRH pulse frequency is responsible for a short-lived rise in circulating levels of LH. It has been shown that bulls with a higher 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 with a premature increase in LH brought about by GnRH treatment (Chandolia *et al.,* 1997). This experiment tests the hypothesis that bull calves with increased GnRH pulsatility engage in more male-male mounting behaviour during this early period while the pattern of hormone secretion is becoming established. Studying this behaviour may give an indication of reproductive potential from as early as 8 weeks of age.

Method 12 Charolais X Hereford bull calves were treated with intra-muscular injections of GnRH (120ng/kg: n=6) or saline (n=6) twice daily between 4 and 8 weeks of age, a time period just before and during the early part of their predicted rise in LH. The GnRH dose was designed to give 2 extra pulses of LH, of an amplitude within the physiologically normal range. All calves were kept with their mothers and also had *ad libitum* access to hay and fresh water. During the last two days of the treatment the male-male mounting behaviour of all calves was observed for 2-hour periods commencing immediately after their morning injection, and again 6 hours later (prior to their evening injection). The identity of each calf exhibiting mounting behaviour and the time of each mount was recorded. Frequent blood samples were obtained from the calves 2 days after the observation period to indicate the LH response to the GnRH injections. In addition the scrotal circumference of each calf was recorded every two weeks from 2 weeks of age onwards to determine the rate of testicular development. The difference in scrotal circumference and frequency of male-male mounting behaviour was analysed using a Mann-Whitney U test.

Results There were no significant differences between days within observation periods. Therefore the mean number of mounts per animal per observation period was calculated and used in the subsequent analysis. There were a significantly greater number of mounts observed amongst the GnRH treated calves than amongst the control calves during both observation periods. However the number of mounts observed did not differ significantly between the observation periods immediately after, and 6 – 8 hours after the GnRH injection.

Table 1 Frequency of mounting behaviour.

Observation period (Hours after GnRH injection)	Mean (±sem) number of male-male mounts/calf/hour.	
	GnRH treated	Saline treated
0 - 2	$2.54^{a} \pm 0.699$	$0.42^{b} \pm 0.279$
6 – 8	$1.75^{a} \pm 0.418$	$0.42^{b} \pm 0.201$

Values with no common superscript are different at $p<0.05$.

At 24 weeks the scrotal circumference of GnRH treated calves was significantly greater than that of control calves (20.9 ± 0.77 and 18.7 ± 0.31cm respectively; $p<0.05$).

Conclusions This experiment has demonstrated that bull calves given 2 extra pulses of GnRH per day between 4 and 8 weeks of age engage in more male-male mounting. Calves exhibited similar behaviour when observed both 0 and 6 hours after the GnRH injection, despite LH concentrations returning to baseline around 2 hours after GnRH administration. The increased incidence of male-male mounting at this early age is accompanied by an increase in testicular development at a later stage. We can speculate from our data that calves which have a naturally higher rise in GnRH early in life will engage in more male-male mounting. Secondly that animals which exhibit an earlier endogenous increase in GnRH will in turn, exhibit mounting behaviour earlier in life.

Implication Since an increased incidence of mounting behaviour appears to be linked to precocious testicular development, observations of such behaviour could provide a non-invasive, early indication of animals which will mature faster, or become more fertile adult animals (as age at puberty is related to subsequent fertility).

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Does consistent choice of one side of a milking parlour by dairy cows relate to their behaviour in novel and competitive situations ?

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Introduction Cows can be classified by their consistency of entry to one side of the milking parlour, which follows a normal distribution in a large herd of cows, from consistent to random selection (da Costa and Broom, 2001). We investigated whether this characteristic relates to the coping strategies indicated by cows in a novel environment and a competitive one, where two cows have access to a bucket with food.

Materials and methods Two groups of Holstein-Friesian dairy cows were identified in relation to their consistency of visiting the same side of a two-sided milking parlour: twelve cows that regularly visited the same side (>86% of 25 milkings in one side) and twelve cows that visited both sides regularly (<72% of 25 milkings in one side) (da Costa and Broom, 2001). They were observed in an unfamiliar environment (an open pen) for 15 minutes after visiting the milking parlour. Subsequent to this test, the cows were subjected to a paired food-competition test to assess different social strategies, where they were joined in the pen by another cow (the same one in all tests) and a food bucket. This lasted for up to 15 minutes or until the two cows stopped interacting or feeding. Finally, the cows were individually observed in their normal environment, group-housed in a straw-yard, while feeding for two hours.

Results In the open pen test, the cows with a consistent parlour side selection spent longer standing motionless than 'inconsistent' cows, but the time spent sniffing the pen and the number of steps and vocalisations was similar in the two groups. In the food competition test, the 'consistent' cows took less time to start feeding and were in control of the food bucket for considerably longer than the 'inconsistent' cows. They were also more aggressive in this test and pushed the other cow more. 'Inconsistent' cows stood inactive for more time than 'consistent' cows. In the straw yard, differences in behaviour were non-significant, but across treatments the more aggressive and more successful cows in the paired food competition were those receiving more non-aggressive actions, like licking, in the straw-yard, possibly indicating a higher social status.

Table 1 The behaviour of cows with or without consistent entry to one side of the parlour, when alone in an open pen for up to 15 minutes or in the pen with a bucket of food and another cow.

	Treatment			
	Side preference	No side preference	SED	Probability
Open pen test				
Standing inactive (s)	665	521	42.6	0.02
Sniffing pen (s)	39.8	49.6	9.7	0.51
Steps (no.)	198	182	32.5	0.66
Vocalisation (no.)	17.4	15.0	5.49	0.92
Food competition test				
Latency to feeding (s)	70	137	32.4	0.04
Feeding (s)	101	7	-	0.49†
In control of bucket (s)	151	19	31.5	0.01
Aggression instigated (no.)	3.4	0.6	0.75	0.05
Push with head (no.)	2.8	0.5	0.61	0.06
Licking/sniffing cow (s)	4.6	1.4	1.36	0.11
Standing inactive (s)	222	373	43.3	0.01

† Not normally distributed, analysed by Kruskal Wallis test

Discussion The paired food-competition test revealed that the cows with a side preference in the milking parlour are either more able or have a higher motivation to reach a food goal.

Conclusion All cows might have a motivation to create a routine in the milking parlour but only some succeed due to their greater social competence in the waiting area. Thus, cows with a side preference in the milking parlour might display more successful social coping strategies.

Reference

Paranhos da Costa, M.J.R. and Broom, D.M.; Consistency of side choice in the milking parlour by Holstein-Friesian cows and its relationship with their reactivity and milk yield. *Applied Animal Behaviour Science (in press).*