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Genetic correlations between linear type traits, food intake, live weight and condition score in Holstein Friesian dairy cattle

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Abstract

Variance components were estimated from an animal model using a restricted maximum likelihood procedure which allowed for unequal design matrices and missing observations (VCE). Data sets containing: (i) 15 275 records of linear type classifications on heifers, (ii) 3399 live weight and condition scores measured at calving and (iii) 1157 records of yield, dry-matter intake, average live weight and condition score during the first 26 weeks of lactation; were analysed jointly.

Heritability estimates for dry-matter intake, live weight and condition score in the largest data set were 0·44, 0·44 and 0·35 respectively and the genetic correlation between condition score and the yield traits ranged from –0·29 to –0·46. The genetic correlation between milk yield and average live weight was negative (–0·09) but after adjusting for the genetic variation in condition score this correlation was positive (0·29). Genetic correlations between live weight and stature, chest width, body depth and rump width were consistently high (0·52 to 0·64; 0·75 to 0·86; 0·59 to 0·81; 0·56 to 0·74, respectively). Chest width and body depth were little to moderately correlated with dry-matter intake (0·25 to 0·28 and 0·20 to 0·34 respectively), and angularity (–0·47 to –0·77) and chest width (0·32 to 0·73) appeared to be good predictors of condition score. These correlations showed that (i) the relative value of live weight compared with food intake capacity determines the optimum direction of selection for stature, chest width, body depth and angularity, and consequently the optimum size of the dairy cow, and that (ii) live weight, condition score and food intake can be predicted from the type traits with little loss in accuracy. A restricted index which maintains condition score at its current level was predicted to reduce overall (economic) genetic gain by 5%.

Keywords: *dairy cattle, food intake, linear type traits, live weight.*

Introduction

Most milk producers would agree that improving the profitability of cows, consistent with their health and welfare, is the most important breeding objective for dairy cattle. Clearly food costs are important components of profitability, because food costs account proportionately for about 0·80 of the total variable costs associated with milk production (Milk Marketing Board, 1990). Generally, food is used for the separate functions of maintenance, lactation and body tissue gain, or loss, and genetic improvement of food utilization has to come from changing these components.

Accounting for increased maintenance requirements of a cow with increased live weight, the economic value of live weight is reported to be negative (Dempfle, 1986; Groen, 1989; Visscher *et al.*, 1993; Groen *et al.*, 1994). Increasing food intake, however, could have a positive economic value in the situation where additional concentrates are needed to supplement the energy from forage. In that situation, relatively more forage can be given when intake increases and the amount of concentrate in the diet at a given milk yield can be reduced (Groen and Korver, 1989). Considering that the economic weights of intake capacity and live weight are in opposing directions, whereas the genetic correlation between these two traits is positive, it seems important to consider simultaneously both intake and live weight in the breeding goal (Veerkamp, 1996).

It can be expected also that intake capacity and body condition score are likely to become more important

in the future, regardless of the feeding system. This is because selection for yield increases the gap between energy input and output during early lactation: the correlated response in food intake, from selection on yield, can cover only 40 to 48% of the extra requirements (van Arendonk *et al.*, 1991; Veerkamp, 1994). Whilst there is no evidence of a large proportion of genetic variation in partial efficiencies (Blake and Custodio, 1984; Veerkamp and Emmans, 1995), most of the remaining energy requirements for yield has to come from body tissue mobilization. Selecting for a lower live weight whilst simultaneously selecting for increased yield, or direct selection for gross efficiency, could increase the gap between the rate of progress in yield and the rate of progress in intake capacity, and hence an increase in the dependency on body tissue mobilization during early lactation. For these reasons, there is interest in including a combination of dry-matter intake, live weight and condition score in the dairy cattle breeding goal and therefore genetic parameters for these traits are required.

Measurement of individual cow's performance for live weight, food intake or condition score is not common practice for most breeding programmes and therefore there is great interest in other traits which may help to predict these potential goal traits. Linear type traits describe biological extremes for a large number of visual characteristics, are measured on a relatively large scale, international conversions are available and traits like body depth, capacity and size are perceived to be important by breeders for many reasons. Therefore it seems appropriate to estimate genetic correlations between type traits and dry-matter intake, condition score and live weight and then to evaluate the use of these traits as potential predictors in a selection index. Hence, the first objective is to estimate variance components for yield, dry-matter intake, live weight and condition score and the second objective is to estimate genetic correlations between these traits and linear type traits.

Material and methods

Data

Records were obtained from cows housed and managed at the Scottish Agricultural College/ University of Edinburgh Langhill Dairy Cattle Research Centre. In each year calving began early in September and animals joining the trial all calved between September and January. All cows involved in the study were Holstein-Friesians. Cows were kept indoors in conventional cubicle housing from calving to July and offered complete mixed diets *ad libitum*. Through the use of Calan Broadbent electronic gates the extended indoor period allowed measurement of food offered to, and refused by, individual cows for 4 days/week, from calving to a minimum of 26 weeks and up to 38 weeks after calving (depending on the calving date of each cow). The data reported here are for performance over the first 26 weeks of lactation, recorded over consecutive years from 1980/81 to 1994/95.

The complete mixed diets were dispensed into individual food bins, once daily. Weights of fresh diet offered and refused were recorded on 4 days consecutively each week. Daily samples from the different diets and refusals were analysed for dry matter. Cows on the food intake trial were weighed and condition scored once a week after milking. Condition scoring was based on the system developed by Lowman *et al.* (1976). This system scores between 0 and 5 (lean to fat), and describes each score in terms of the amount of tissue cover over the transverse processes of the lumbar vertebrae and around the tail head. Handling procedures of the weekly data were described in more detail by Veerkamp *et al.* (1994). Yield, dry-matter intake, live weight and condition score were calculated as the average of the weekly records accumulated over weeks 3 to 26 of lactation, and 1157 lactations (on 527 animals) were available for milk, fat and protein yield (milk, FY and PY, respectively), dry-matter intake (DMI), live weight (LW) and condition score (CS). Live weights and condition scores at calving or within 24 h thereof (CLW and CCS) were available for 3399 lactations (on 1046 animals).

To increase the number of records with type classification, 47 additional herds were included in the analysis. Records (no. = 15 275) were selected from the Holstein Friesian Society records on pedigree heifers from 1983 to 1994 inclusive and preference was given to herds with the largest number of sires in common with Langhill to establish genetic linkage. The pedigree file (no. = 28 547) consisted of sires, dams and all grandparents of these with links to at least two animals with a record.

For each of the two data sets, the total number of records for type and for the traits measured at Langhill (during calving or during the first 26 weeks of lactation) are given in Table 1. Of the total number of type records (15 275), 514 heifers were type classified at Langhill, and all of these animals had CLW and CCS recorded in the first lactation. However, only 314 of these type classified heifers had DMI, LW and CS recorded. Over and above all other relationships included in the relationship matrix, linkage between the 'Langhill records' and the 'national type records' was provided by 55 sires which had 3961 daughters with type records in the

Table 1 *Number of records available*

	No. of records	
	Heifers	Heifers + cows
Type	15275	15275
Langhill trial (Milk, FY, PY, DMI, LW, CS)†	410	1157
Langhill calving (CLW and CCS)‡	910	3399

† Milk, FY and PY are milk, fat and protein yield, DMI is dry-matter intake, LW and CS are live weight and condition score, all averaged over the first 26 weeks of lactation.
‡ CLW and CCS are live weight and condition score at calving.

national population (excluding Langhill) and 598 daughters with records at Langhill.

Analysis
Multivariate analyses were performed to estimate the variances and covariances. An individual animal model, with the full numerator relationship matrix included, was fitted using restricted maximum likelihood procedure (VCE) which allowed for unequal design matrices and missing observations and provided the standard errors of the estimates (Groeneveld, 1996).

The first analysis included Langhill data only, separated into (i) heifers and (ii) heifers plus cows. The model used on the latter data set included, besides the additive genetic random effect, a permanent environmental random effect to take account of the covariance between lactations on the same cow. Fixed effects included in the model for the Langhill traits were: year (1980 to 1995) by genetic line (selection or control) by diet (high or low concentrate) with 50 levels; month of calving (12 levels); lactation number (1, 2, 3 and >3); Holstein percentage of the cow as a covariate; and age at calving and age at calving squared as covariates within each lactation group.

Secondly, an analysis (on heifer and heifer plus cow data) was performed to establish the genetic correlations between the linear type traits and five of the Langhill traits (correlations between yield and type traits are available from a larger national data set (Brotherstone, 1994)). Although a complete multivariate model might have been preferable, this was not feasible computationally. For this reason, the genetic correlations between type traits and the traits recorded at Langhill were estimated by a number of bivariate analysis. A subset of the highly correlated traits (chest width (CW), body depth (BD), stature (STA), angularity (ANG), DMI, CS and LW) were fitted in a seven trait analysis simultaneously to get more consistent estimates of the genetic correlations. The effects fitted for the Langhill traits were as described above, and for the linear type traits the fixed effects included lactation stage at inspection (1 to 15), herd-year-visit (248 levels, 48 herds), month of calving (1 to 12) and age at inspection and Holstein percentage as linear covariates. In order to account for differences in the range of scoring utilized by the field officers, the type traits were adjusted by the ratio of the standard deviation of the field officer to the mean standard deviation of all field officers (Brotherstone, 1994).

Adjustment
Adjusting genetic (co-)variances for another trait makes interpretation (sometimes) easier; for example, live weight adjusted for differences in body condition score might have another (biological) interpretation than live weight in its own right. The genetic (co)variances for 'live weight adjusted for condition score' (LWA) were created using the genetic regression of live weight on condition score: $b = \sigma_{lw,cs}/\sigma^2_{cs}$, where $\sigma_{lw,cs}$ is the genetic covariance between live weight and condition score and σ^2_{cs} is the genetic variance of condition score. The genetic variance of live weight adjusted for condition score (σ^2_{lwa}) is then:

$$\sigma^2_{lwa} = \sigma^2_{lw} - b\,\sigma_{lw,cs}$$

and the covariance between adjusted live weight and a second trait (T):

$$\sigma_{lwa,T} = \sigma_{lw,T} - b\,\sigma_{T,cs}.$$

Finally, using these adjusted (co-)variances, the genetic correlations (r_g) with adjusted live weight were calculated as: $r_g = \sigma_{lwa,T}/(\sigma_T\,\sigma_{lwa})$.

Results

Overall means, phenotypic standard deviations, heritabilities and permanent environmental effects are given in Table 2. The heritabilities for the complete data set are somewhat lower than those from the data set including heifer records only. In the heifer data set, heritabilities for the yield traits agree closely with those reported by Brotherstone (1994). For CS the heritability is comparable with the heritability for yield. Measures for CLW and CCS have lower heritability than the average of several measures during early lactation. The repeatability ($h^2 + c^2 = 0{\cdot}81$) and heritability for LW are relatively high compared with these values for the other traits.

Table 2 *Means, phenotypic standard deviations (σ_p), heritabilities (h^2) and permanent environmental effects (c^2) for traits† measured at Langhill*

Heifers	Mean	σ_p	h^2	s.e.		
Milk (kg/day)	23·2	3·6	0·44	0·05		
FY (g/day)	950	153	0·50	0·05		
PY (g/day)	736	101	0·42	0·05		
DMI (kg/day)	15·0	1·4	0·43	0·06		
LW (kg)	544	44	0·55	0·06		
CS (×100)	251	27	0·43	0·06		
CLW (kg)	528	42	0·39	0·04		
CCS (×100)	271	21	0·34	0·05		
Heifers + cows	**Mean**	**σ_p**	**h^2**	**s.e.**	**c^2**	**s.e.**
Milk (kg/day)	27·2	4·0	0·27	0·02	0·26	0·02
FY (g/day)	1112	187	0·40	0·02	0·18	0·02
PY (g/day)	861	119	0·35	0·02	0·22	0·02
DMI (kg/day)	16·9	1·8	0·44	0·02	0·23	0·02
LW (kg)	597	50	0·44	0·03	0·37	0·03
CS (×100)	248	38	0·35	0·03	0·21	0·02
CLW (kg)	613	53	0·35	0·02	0·24	0·02
CCS (×100)	279	37	0·24	0·02	0·09	0·01

† Milk, FY and PY are milk, fat and protein yield, DMI is dry-matter intake, LW and CS are live weight and condition score, all averaged over the first 26 weeks of lactation; CLW and CCS are live weight and condition score at calving.

Table 3 *Phenotypic (above diagonal) and genetic (below diagonal) correlations between the traits† measured at Langhill*

Heifers	Milk	FY	PY	DMI	LW	CS	CLW	CCS
Milk		0·74	0·91	0·41	–0·19	–0·42	0·20	–0·01
FY	0·59		0·79	0·50	–0·05	–0·28	0·30	0·09
PY	0·86	0·74		0·49	–0·07	–0·27	0·25	0·07
DMI	0·34	0·62	0·49		0·32	0·05	0·32	0·12
LW	–0·30	–0·11	–0·20	0·31		0·66	0·70	0·40
CS	–0·46	–0·33	–0·29	0·23	0·66		0·31	0·58
CLW	0·08	0·20	0·07	0·26	0·80	0·27		0·40
CCS	–0·18	–0·07	–0·06	0·18	0·41	0·67	0·36	

s.e. of the genetic correlations ranges from 0·02 to 0·13.

Heifers + cows	Milk	FY	PY	DMI	LW	CS	CLW	CCS
Milk		0·74	0·90	0·43	–0·20	–0·36	0·06	–0·11
FY	0·55		0·78	0·50	–0·13	–0·27	0·12	–0·05
PY	0·80	0·75		0·50	–0·13	–0·28	0·05	–0·12
DMI	0·59	0·76	0·64		0·19	–0·12	0·11	–0·13
LW	–0·09	0·03	–0·02	0·27		0·63	0·76	0·40
CS	–0·46	–0·31	–0·35	0·00	0·67		0·48	0·72
CLW	0·04	0·06	0·05	0·18	0·85	0·41		0·56
CCS	–0·38	–0·38	–0·33	–0·08	0·64	0·88	0·51	

s.e. of the genetic correlations ranges from 0·01 to 0·05.
† Milk, FY and PY are milk, fat and protein yield, DMI is dry-matter intake, LW and CS are live weight and condition score, all averaged over the first 26 weeks of lactation; CLW and CCS are live weight and condition score at calving.

Signs agree between the phenotypic and genetic correlations for the Langhill traits (Table 3), with the exception of some of the correlations close to zero. A negative correlation was found between average LW and milk production, and a small positive genetic correlation between CLW and production. Average LW and CLW were highly correlated, 0·85 and 0·80 in the full and heifer data set respectively. Average CS was negatively correlated with yield (from –0·29 to –0·46), but highly positively correlated with weight (0·66 and 0·67 in heifer data and heifer and cow data respectively). DMI and average LW were moderately but positively correlated (0·31 and 0·27).

Heritabilities for the type traits were all close to the heritabilities in the national population (Brotherstone, 1994) (Table 4). Genetic correlations between CLW and the body measures of STA, CW, BD and rump width were high, ranging from 0·56 to 0·78. ANG was not correlated with LW at first calving but when later calvings were added this correlation was –0·31. Some of the udder traits were moderately correlated with LW at first calving. Less angular animals with a wider chest had a higher CCS, which was most strongly observed in the later lactations.

When measured during the first 26 weeks of lactation (Table 5) the same body measures appear to be useful for the prediction of LW and for the prediction of CLW. ANG was strongly negatively correlated with LW in heifers as well and CW was highly positively correlated with CS in heifers and cows. CW and BD were moderately correlated with DMI, whereas some of the udder traits (i.e. udder depth (UD) in both data sets, plus udder support (US) and teat placement side (TPS) in heifers only) also appeared to be correlated with intake. The highly negative genetic correlation of –0·69 between rear legs and DMI in heifers is somewhat surprising, especially because it was much smaller (–0·14) when later lactations were added to the data set.

Discussion

The estimated variance components between yield and intake obtained in Table 2 and 3 agree with the reported parameters in other studies (Persaud *et al.*, 1991; van Arendonk *et al.*, 1991; Svendsen *et al.*, 1994;

Table 4 *Genetic correlations between live weight and condition score at calving (CLW and CCS) measured at Langhill and linear type measurements from both Langhill and the national data set*

			Genetic correlation			
			Heifers		Heifers + cows	
Trait	Abbreviation	h^2	CLW	CCS	CLW	CCS
Total score	TSC	0·30	–0·34	–0·44	0·12	–0·07
Stature	STA	0·50	0·56	–0·09	0·61	0·19
Chest width	CW	0·29	0·78	0·32	0·75	0·67
Body depth	BD	0·38	0·59	–0·24	0·74	0·26
Angularity	ANG	0·28	–0·07	–0·47	–0·31	–0·74
Rump angle	RA	0·36	0·13	0·22	–0·09	0·02
Rump width	RW	0·23	0·56	0·05	0·62	0·21
Rear legs side	RLS	0·18	0·19	–0·11	–0·05	–0·25
Foot angle	FA	0·25	0·00	–0·10	0·18	0·11
Fore udder attachment	FUA	0·29	–0·42	–0·43	–0·15	–0·03
Udder support	US	0·15	0·10	–0·24	0·05	–0·05
Udder depth	UD	0·39	–0·41	–0·27	–0·14	0·03
Teat placement rear	TPR	0·36	–0·08	–0·03	–0·07	–0·09
Teat placement side	TPS	0·36	0·18	–0·30	0·21	–0·17
Teat length	TL	0·38	0·29	0·11	0·16	0·08
Range for s.e.			0·03-0·16	0·05-0·19	0·03-0·09	0·01-0·11

Jensen *et al.*, 1995). Heritabilities were somewhat lower in the full data set, compared with the heifer data set, the most likely explanation is the large negative sampling correlation observed between the heritability and the permanent environmental effect. This suggests that heritabilities in the full data set might be on the low side, whereas the permanent environmental effects might be on the high side.

Reports of the genetic correlation between yield and live weight have been less consistent across most of these studies. Similarly the genetic correlation between the different measures of live weight and yield varies between –0·30 and 0·20 in this study. In the heifer data primarily, the genetic correlation between yield and live weight at calving and between yield and live weight during lactation were negative and positive respectively. Hence this correlation seems to change over the lactation period as was also suggested by van Elzakker and van Arendonk (1993). One possible reason for this is that stored body fat is an important contributor to live

Table 5 *Genetic correlations between dry-matter intake (DMI), live weight (LW) and condition score (CS) measured at Langhill during first 26 weeks of lactation and linear type measurements from both Langhill and the national data set*

	Heifers			Heifers + cows		
	DMI	LW	CS	DMI	LW	CS
TSC	–0·08	–0·14	0·19	–0·05	0·05	–0·06
STA	0·18	0·64	0·32	0·13	0·52	0·13
CW	0·25	0·86	0·57	0·28	0·79	0·73
BD	0·20	0·81	0·22	0·34	0·69	0·24
ANG	–0·21	–0·56	–0·51	0·07	–0·43	–0·77
RA	–0·12	0·07	0·48	–0·14	–0·15	0·06
RW	0·11	0·74	0·14	0·24	0·70	0·29
RLS	–0·69	–0·18	–0·54	–0·14	0·04	–0·17
FA	0·21	0·19	0·42	0·06	0·08	0·10
FUA	–0·10	–0·32	–0·12	–0·25	–0·06	0·11
US	0·46	0·24	–0·02	0·06	0·12	0·09
UD	–0·46	–0·26	0·16	–0·55	–0·08	0·18
TPR	0·06	–0·04	–0·11	–0·13	–0·07	–0·07
TPS	–0·30	0·13	–0·14	0·06	0·05	–0·31
TL	0·25	0·33	0·04	0·19	0·27	0·16
Range for s.e.	0·11-0·19	0·06-0·19	0·11-0·24	0·06-0·11	0·03-0·12	0·03-0·13

Table 6 *Genetic correlation of yield, dry-matter intake, condition score and some of the linear type traits with live weight and live weight adjusted genetically for condition score (heifer plus cow data)*

	Live weight	Live weight adjusted for condition score
Milk	–0·09	0·29
Fat yield	0·03	0·32
Protein yield	–0·02	0·28
Dry-matter intake	0·27	0·37
Condition score	0·67	0·00
Chest width	0·79	0·41
Angularity	–0·43	0·12
Udder depth	–0·08	–0·27
Teat placement side	0·05	0·35

weight during some parts of the lactation and high producing cows might be expected to mobilize more body fat. This hypothesis is confirmed by the large genetic correlation between live weight and condition score (0·67) reported in this study and given that the patterns of tissue mobilization and milk yield differ across lactation, covariances between yield and live weight might also depend on the stage of lactation. The remarkable effect of adjusting live weight for condition score is shown in Table 6. The genetic correlation between milk yield and live weight (–0·09), for example, changes to 0·29 after adjustment, and hence, selection for yield will result in heavier cows when compared at the same condition score. This seems in line with the genetic correlation between measures of size (which will be one of the largest components of adjusted live weight) and yield reported by Brotherstone (1994): correlations between stature and milk, fat and protein yield were 0·22, 0·16 and 0·25 respectively. Also, the correlation between dry-matter intake and live weight is larger after adjusting live weight for condition score and some of the correlations with the type traits change considerably (e.g. angularity changes from –0·43 to 0·12). Thus, although the correlation between the two measures of live weight is consistently high in this study and in line with values reported by van Elzakker and van Arendonk (1993) and Svendsen *et al.* (1994), correlations between live weight and other traits (e.g. yield or type) might depend more on the stage of lactation that live weight is measured. Another example is the correlation between angularity and weight at first calving which is only –0·07, whereas this correlation increased to –0·43 when average weight during lactation was considered and all lactations where analysed simultaneously.

Although decreasing condition score, as a consequence of genetic selection, might not necessarily be harmful for health or welfare of the dairy cow (there is little evidence about the *genetic* relationship between condition score and health or fertility), some concern might be justified because of the 'biological buffering function' of body tissue. Therefore the high genetic correlation between the yield traits and condition score found in this study (ranging from –0·29 to –0·46) is an important finding, especially because simply increasing the ratio of concentrate to forage given does not seem an adequate solution; results from Veerkamp *et al.* (1995b) suggest that condition score decreases for a selection line on either a high or low input system. This negative correlation is not surprising however, given that a correlated increase in intake from selection for yield is expected to cover less than 50% of the requirements needed for the extra milk production (van Arendonk *et al.*, 1991; Veerkamp, 1994).

The reduction in condition score as a consequence of genetic selection is likely to be accelerated since angularity (or dairyness) is generally perceived by breeders as a trait for which a high score is favourable. Given the strong genetic correlation between condition score and angularity, even more selection pressure might be put on body tissue mobilization. Also, in some countries selection for decreasing live weight (simultaneously with increasing yield) appears to be of economic interest. In that situation condition score is expected to decrease even further because of the strong positive genetic correlation between live weight and condition score. Hence selection for higher yield, more angular cows and a lower live weight all seem to increase body tissue mobilization during lactation.

Alternatively, by using a restricted selection index (Brascamp, 1984) genetic selection can be used to maintain condition score at its current level, whilst milk yield is increased simultaneously. The expected reduction in genetic gain for profitability as a consequence of such an index can be illustrated by using the genetic parameters reported in this study and the economic values of the profit index (PIN) used in the United Kingdom (Veerkamp *et al.*, 1995a). Assuming one standard deviation of selection, selection on PIN alone increases milk yield by 324 kg per lactation and the margin over food costs by £64·60, but average condition score will drop by 7·0 units (units 0 to 500). When condition score was included in a restricted index, then the response in margin using the same selection intensity was reduced to £61·40, and the increase in milk yield can be expected to be 271 kg only. Therefore although the drop in condition score appears moderate as a consequence of selection for

yield only, maintaining condition score at its current level can be done at the expense of a 5% drop in genetic progress.

The genetic associations with condition score reported in this study make it even less obvious how to use the genetic variation of the complex of yield, dry-matter intake and live weight in the breeding objective. Overall, food costs can be reduced by reducing maintenance cost (i.e. lowering live weight), increased food intake capacity so that more forage can be used to supply the energy for the same amount of yield (i.e. increasing live weight), a change in partitioning of food (i.e. lowering condition score) or even improving net efficiency (e.g. Blake and Custodio, 1984; Groen and Korver, 1989; Veerkamp and Emmans, 1995; Veerkamp, 1996). Which (combination) of these options is most feasible is not obvious, but it is clear that economic and genetic arguments should be considered simultaneously. For example, it is interesting to note that the economic value of food intake and live weight could be equally important in some circumstances, but in opposite directions (Veerkamp, 1996). Given that these two traits are genetically correlated as well, including either one of the two traits needs careful consideration. This is because what might be perceived to be gained by including one of these traits, might in fact be lost because the other trait is changed as a consequence of the correlated response in the other direction.

Measurement of an individual cow's performance for live weight and food intake is not common practice for most breeding programmes, and therefore there is great interest in other traits which may help to predict these potential goal traits. Sieber *et al.* (1988) found negative correlations between estimated efficiency and seven body measurements and Gravert (1985) reported that chest circumference is an accurate predictor of food intake. Genetic correlations between live weight and the body traits were consistently high in this study, even after adjustment for condition score, and chest width and body depth were moderately correlated with dry-matter intake. When stature, chest width, body depth, angularity and rump width are combined in one index, the accuracies of selection on this index for dry-matter intake, live weight (adjusted for CS) and condition score are 0·65, 0·84 and 0·88, respectively. When milk, fat and protein yield were included in the index the accuracy with dry-matter intake increased to 0·90. Hence, selection for live weight and perhaps for food intake can be done relatively cheaply, because linear type traits are measured in most (inter-) national breeding programmes, and appear to have high genetic correlations with these traits of economic interest.

The optimal direction of selection for each of the type traits is not easy to establish until the economic values for live weight and food intake are known. Strong genetic correlations between some of the type traits make it even more difficult to anticipate what optimal index weights for the body traits might be.

Generally, whilst it is still unclear how much emphasis in the breeding goal should be placed on dry-matter intake, live weight, condition score and the related type traits it is clear that (i) there is considerable genetic variation in these traits, and that the whole complex of genetic correlations should be considered together with the economic values to determine the breeding goal, and subsequently the optimum size of the dairy cow and (ii) live weight and food intake can be predicted from the type traits with little loss in accuracy. Because linear type traits are measured in most (inter-) national breeding programmes, they appear to be a cheap alternative to measuring live weight and food intake.

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Effect of altering the non-structural: structural carbohydrate ratio in a pasture diet on milk production and ruminal metabolites in cows in early and late lactation

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Abstract

The effect on digestibility, ruminal metabolites, microbial protein synthesis and milk production of manipulating the non-structural (NSC) : structural (SC) carbohydrate ratio in a predominantly pasture diet was investigated in cows in early (trial 1) and late (trial 2) lactation. Twenty-four cows in trial 1 and 15 cows in trial 2 were offered pasture only (P), 0·85 P plus 0·15 NSC/protein mixture (PR), and P plus an additional 0·1 (trial 1) or 0·15 (trial 2) NSC (PE) in a Latin-square arrangement. All diets were isonitrogenous and P and PR were isoenergetic. PE but not PR increased microbial protein synthesis and decreased ruminal ammonia and milk urea levels, compared with P. Efficiency of microbial synthesis (g N per kg digestible organic matter intake) was not altered by treatment. Treatments had minor effects on ruminal pH and no effect on volatile fatty acid concentrations. PE and PR did not affect milk yield or protein yield and decreased fat yield compared with P in trial 1. Milk yield was increased on PE and PR compared with P and was greater on PE than PR, in trial 2. Yields of fat and protein were higher on PE than on P and yield of protein was higher on PR than on P. The results suggest that increasing the ratio of NSC : protein by increasing total carbohydrate intake was more effective in improving nitrogen utilization in the rumen than was increasing the NSC : SC ratio without increasing carbohydrate intake.

Keywords: carbohydrates, dairy cows, milk production, pasture.

Introduction

Temperate pastures based on ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) are characterized by crude protein (CP) concentrations of 200 to 300 g/kg of pasture dry matter (DM) and non-structural carbohydrate (NSC) concentrations of 50 to 250 g/kg DM. The concentration of neutral-detergent fibre (NDF) ranges from 450 to 500 g/kg DM. These CP and NDF concentrations are high, and the NSC concentration low, compared with diets recommended for high producing dairy cows (National Research Council, 1989). The CP in pasture is characterized by the high rate and extent of ruminal degradability, which can result in high concentrations of ammonia in the rumen and excessive excretion of nitrogen (N) in the urine (Ulyatt *et al.*, 1988; van Vuuren *et al.*, 1986 and 1991). As a consequence of the high rate and extent of pasture CP degradation, the amino acid supply to the small intestine in pasture-fed cows may limit animal performance. Synthesis of microbial protein from ruminally degraded CP is energy dependent and rumen fermentable energy can be first limiting. Increasing the ruminal availability of energy, and/or synchronizing the rates of fermentation of protein and carbohydrate, are considered by some to be important for optimal microbial protein synthesis (Chase, 1993; Mansfield *et al.*, 1994; Sinclair *et al.*, 1993; van Vuuren *et al.*, 1990). The relatively low NSC levels in some pastures could limit microbial efficiency and milk solids production in pasture-fed dairy cows.

Two trials are described which aimed to determine whether increasing the proportion of NSC and

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decreasing the proportion of structural carbohydrate (SC) of a predominantly pasture diet affected utilization of dietary N and microbial protein synthesis in early and late lactation. A summary of some of the results was given by Carruthers *et al.* (1996).

Material and methods

Trial 1: early lactation

Cows and treatments. Twenty-four cows (19 Friesian and five Jersey) aged 3 to 6 years and weighing 434 kg live weight (LW, range 338 to 525 kg) and 32 days in milk (DIM, 11 to 49 days), were allocated to three treatment groups balanced for DIM, LW and milk yield during the week prior to the experiment. Twelve of the Friesians were fistulated in the dorsal rumen.

Metabolizable energy (ME) intake for each cow was estimated (Holmes and Wilson, 1987), based on $LW^{0.75}$ and production in the week prior to period 1, and used to set the total ME intake of diet components for each cow on each treatment. The treatments were: P — pasture only; PR — 0·85 P plus a NSC/protein mixture to provide the remaining 0·15 of the cow's ME intake; PE — P plus NSC to provide a total ME intake of 1·10 of that on P alone. All treatments were designed to be isonitrogenous and treatments P and PR to be isocaloric. Individual ME intakes in period 1 were set at 0·9 of calculated requirements, to ensure nil refusals of the pasture component of the diet but were increased 0·02 and 0·03 in periods 2 and 3, respectively, as the cows' intakes improved. The replacement protein consisted of a 20 : 50 : 30 mixture of urea, lactic casein (New Zealand Dairy Research Institute, Palmerston North) and formaldehyde-treated casein (Brookes, 1984). The NSC mixture consisted of 50 : 50 maize cornflour and dextrose monohydrate (NZ Starch Products Ltd, Auckland). The ratio of protein to NSC in treatment PR was adjusted every 4 to 6 days based on the total N concentration of samples of herbage.

Treatments were offered during three 14-day periods in September and October 1994. Measurements were made over days 8 to 14 of each period. Cows were milked at 07.00 and 15.00 h each day. Oestrous behaviour of the cows was controlled using CIDR™ devices (InterAg, Hamilton, New Zealand).

Pasture and feeding. The pasture was divided into four areas, each area providing 7 to 10 days of food. Two of the areas were harvested twice (for periods 1 and 3) and the remaining areas once (period 2). Each area received 90 kg N per ha as urea in an equal split application 28 and 21 days before the first harvest day on that area. A second split application of 90 kg N per ha was applied to the two areas regrown for harvest in period 3. Pasture was harvested using a reel mower and pick-up wagon twice daily at 07.00 and 15.00 h and offered to the cows at 09.00 to 14.00 h (proportionately 0·45 of daily ration) and 16.00 to 22.00 h (0·55 of daily ration). The fresh weight of pasture to offer at each feeding was estimated from the microwave DM concentration of samples of harvested pasture.

Five Jerseys and 10 Friesians were penned in 2 × 10 m pens for individual feeding throughout the trial. The remaining nine rumen fistulated Friesians were penned for days 1 to 4 of each period and in metabolism stalls for days 5 to 14. Cows in pens were released on to a bare paddock between 14.00 to 15.00 and 22.00 to 07.00 h. Water was available at all times and NaCl blocks were available to cows in the bare paddock or every 2 to 3 days to cows in metabolism stalls.

The protein and NSC mixtures were mixed with water and drenched in four equal parts at 09.00, 11.00, 16.00 and 18.00 h. Cows were drenched at 09.00 and at 16.00 h with 10 g magnesium as $MgCl_2$ mixed with a pluronic-based bloat preventative.

Sampling and measurements. A sample of herbage was taken twice daily for DM determination by oven drying at 100°C for 48 h. On days 8 to 14 further subsamples were collected and bulked for determination of organic matter (OM), gross energy (GE), NDF, water-soluble carbohydrate (WSC), total N, soluble protein N, calcium, sodium, magnesium, phosphorus and potassium. A further subsample was bulked fresh over the 7 days for plant species determination. Orts were weighed and sampled for oven DM determination after each feeding.

At 4- to 6-day intervals, herbage from paddocks to be cut in the next 3 to 4 days was sampled by clipping to mower cutting height, dried at 100°C for 24 h and ground for total N determination. This was used to calculate the amount of N to administer to cows on treatment PR.

During days 8 to 14 milk weights were recorded at each milking. For penned cows a weighted aliquot of milk collected on each of days 10, 12 and 14 was analysed for fat, protein and lactose. Cows in metabolism stalls were milk sampled daily for fat, protein and lactose determination. Foremilk samples were collected from all cows at 07.00, 11.00, 15.00 and 19.00 h on days 10, 12 and 14 for urea determination. Samples were centrifuged at 1500 × **g** for 15 min and the fat free portion was stored at 4°C and bulked across days within sample times within cow.

Cows were weighed and their body condition score (BCS) assessed on a scale from 1 (very thin) to 10 (very fat) at the morning milking on 2 days consecutively at the start and end of each 14-day period.

Ruminal digesta of metabolism cows was sampled at 08.00, 12.00, 16.00 and 20.00 h on days 10, 12 and 14. Digesta from various sites within the rumen was strained through cheesecloth and subsampled. The pH was determined immediately, followed by acidification with 9 mol/l sulphuric acid. The samples were centrifuged at 1500 × **g** for 10 min and the supernatant frozen. The 3 days' samples for each cow were thawed and bulked within sampling time and analysed for ammonia-N (NH_3-N) and volatile fatty acids (VFA).

Daily faecal output from metabolism cows was weighed and an aliquot was bulked for each cow over days 8 to 14 and stored at 4°C. Nitrogen was determined on fresh homogenized samples and DM, OM, and energy determined on dried and ground material. Daily urinary outputs were weighed and aliquot samples acidified with 6 mol/l HCl, bulked over days 8 to 14 and stored at 4°C until analysed fresh for N and freeze dried for energy. A subsample of the acidified bulk was stored at –18°C prior to allantoin analysis. Uric acid was determined on a bulked unacidified sample diluted 1 : 4 with distilled water and stored at –18°C prior to analysis. In period 1 the possible loss of allantoin over 24 h in unacidified urine collections (Chen and Gomes, 1992) was investigated on each of 2 days by taking a sample of the first urine voided for each cow at about 12.00 h, acidifying half (sample 1) and leaving the remaining half open-topped on the bench beside the collection buckets (sample 2). Sample 2 was acidified the following day at the time of 24 h urine sampling (09.00 h) and both half samples were analysed for allantoin. The correlation coefficients of allantoin concentration in sample 1 *versus* sample 2 were 0·98 and 0·99 on days 1 and 2 respectively. Regression analysis for day 1 indicated a slope of 0·914 (s.e. 0·066), R^2 = 0·96. Corresponding values on day 2 were 0·923 (s.e. 0·045) and R^2 = 0·98. These results were taken to indicate that degradation of allantoin was minimal under the conditions of urine collection.

Rumen degradabilities of DM and nitrogen. *In situ* degradabilities of DM and N in pasture, casein and formaldehyde-casein were determined using three penned rumen-fistulated Friesian cows (one per treatment per period) on day 10 of each period. The procedure and results were described by Carruthers *et al.* (1996).

Trial 2: late lactation

Cows and treatments. Fifteen Friesian cows averaging 461 kg LW (399 to 508 kg), aged 3 to 5 years and 201 DIM (177 to 233 days) were allocated to three treatment groups balanced for LW and milk yield during the week prior to the experiment. All cows were fistulated in the dorsal rumen and five cows were common to trial 1. The treatments and design were the same as in trial 1 except that ME intake on PE was proportionately 1·15 of that on P. The treatments were imposed during three 14-day periods in March and April but period 3 was not completed because pastures contained unacceptably high nitrate levels (0·3 to 0·7 g/100 g DM).

Pasture and feeding. Pastures were irrigated with N fertilizer applied as in trial 1. All aspects of feeding were the same as trial 1 except that cows were not drenched with Mg or bloat preventative.

Sampling and measurements. Pasture, milk production and composition, LW, BCS, ruminal digesta, faeces and urine were measured or sampled as in trial 1.

Analytical procedures

Organic matter in foods, faeces and urine was determined according to Association of Official Analytical Chemists (1984) methods. Nitrogen concentration of foods, faeces and urine was determined on a micro-Kjeldahl digest (Model 16210, Foss Electric, Denmark) by reduction with alkaline sodium phenate. GE of pastures, NSC and protein mixtures, faeces and urine were determined using adiabatic bomb calorimetry. WSC and soluble N concentrations were determined on freeze dried material by the methods of Bailey (1958) and Krishnamoorthy *et al.* (1982), respectively.

NDF was determined by the method of Goering and Van Soest (1970). Phosphorus in dried foods was determined using micro-Kjeldahl digest and magnesium, calcium, sodium and potassium after wet oxidation and digestion with nitric and perchloric acids. Urinary purine derivatives were determined according to Chen and Gomes (1992). Milk composition was determined using a Milkoscan 133B (Foss Electric, Denmark). Milk urea and ruminal ammonia were determined using Boehringer Mannheim and Raichem Enzymatic test kits, respectively, and an Hitachi 717 auto analyser. VFA concentrations were determined according to Erwin *et al.* (1961) with an automated GLC (5890A, Hewlet Packard, Avondale, PA).

Statistical analysis

Trial 1 data were analysed as a Latin square using the MIXED procedure in SAS. One cow did not complete period 3 (PR treatment) and her data were

Table 1 *Chemical composition (g/kg dry matter (DM)) and* in vitro *organic matter (OM) digestibility (IVOMD) of the pastures in early (trial 1) and late (trial 2) lactation*

	Trial 1	Trial 2
DM (g/kg)	146·6	161·3
OM	902	896
Nitrogen (N)		
Total	37·5	29·5
Soluble non-protein N	8·2	3·4
Soluble protein N	2·0	2·5
Neutral-detergent fibre	460	489
Water-soluble carbohydrate	225	124
Phosphorus	3·9	3·1
Magnesium	1·9	2·7
Calcium	5·4	6·1
Sodium	3·2	3·5
Potassium	31·5	32·5
IVOMD	0·80	0·73

excluded for this period. Trial 2 data were analysed as an incomplete Latin square. The analyses tested for square, cow, period and treatment and their interactions. Probability values are reported for pairwise treatment comparisons and for overall treatment differences (2 d.f.).

Animal care

The trials were approved by the Ruakura Animal Ethics Committee.

Table 2 *Daily dry-matter intake (DMI), daily production of milk, fat, protein and lactose, concentrations in milk of fat, protein and lactose, and change over 14 days in live weight (LW) and body condition score (BCS) for 24 cows on pasture (P), 0·85 P + 0·15 protein/non-structural carbohydrate (NSC) mixture (PR), and P + 0·1 NSC (PE) in trial 1 (early lactation)*

	Treatment			
	P	PR	PE	s.e.d.
DMI (kg per cow)				
Pasture	14·1[a]	11·9[b]	13·9[a]	0·14
Protein mixture	0	0·5	0	
NSC mixture	0	1·2	1·1	
Total	14·1[a]	13·6[b]	15·0[c]	0·14
Yield (kg per cow)				
Milk	21·9[ab]	21·6[b]	22·4[a]	0·32
Fat	1·003[a]	0·918[b]	0·939[b]	0·0282
Protein	0·718	0·698	0·722	0·0169
Lactose	1·046	0·998	1·032	0·0241
Milk composition (g/kg)				
Fat	47·4[a]	45·1[b]	44·6[b]	0·86
Protein	33·7[a]	33·9[ab]	34·2[b]	0·22
Lactose	48·5[a]	48·1[b]	48·3[ab]	0·17
LW change (kg)	–6·5	–4·8	–2·3	4·61
BCS change	–0·17	0·00	0·15	0·094

[a,b] Means with different superscripts differ at $P < 0·05$.

Results

Trial 1: early lactation

Diet composition. Pasture concentrations (g/kg DM) of ryegrasses (*Lolium perenne* L., *L. multiflorum* Lam), grasses other than ryegrass (*Poa* sp., *Bromus willdenowii* Kunth.), white clover, weed and dead material averaged 881, 92, 14, 7 and 6, respectively. Chemical composition of the pasture is shown in Table 1. The NSC mixture contained trace amounts of N and minerals (0·0015 to 0·006 g/kg DM) and the protein mixture contained trace amounts of minerals except phosphorus (6·8 g/kg DM). The calculated proportions (g/kg DM intake) of NSC (WSC in pasture plus the NSC mixture), NDF and CP in P, PR and PE, respectively, were: NSC, 225, 287: and 282; NDF, 460, 403 and 426; CP, 234, 253 and 228.

All cows, food intake and milk production. DM intakes are shown in Table 2. Treatments PE and PR did not differ from P in milk yield (Table 2). All treatments were similar in their effects on protein yield in milk but fat concentration of milk decreased by 2·3 and 2·8 g/kg in cows when on PR and PE compared with P, resulting in a decrease in fat yield. There were no differences in change in LW or BCS over the 14-day treatment periods (Table 2).

Milk yield, milk composition and yields of components for the nine cows used in metabolism stalls were similar to those for all 24 cows, and these data are not presented.

Table 3 *Daily dry-matter intake (DMI), DM, organic matter and energy apparent digestibilities (DMD, OMD and DE), digestible organic-matter intake (DOMI), daily intake of metabolizable energy (MEI) and ME content of the diet for nine cows on pasture (P), 0·85 P + 0·15 protein/non-structural carbohydrate (NSC) mixture (PR), and P + 0·1 NSC (PE) in trial 1 (early lactation)*

	Treatment			
	P	PR	PE	s.e.d.
DMI (kg/day)				
Pasture	15·1[a]	12·4[b]	14·6[c]	0·21
Protein mixture	0	0·57	0	
NSC mixture	0	1·33	1·20	
Total	15·1[a]	14·3[b]	15·8[c]	0·23
DMD	0·80	0·81	0·81	0·004
OMD	0·82	0·83	0·82	0·004
DE	0·79	0·79	0·78	0·005
DOMI (kg per cow)	11·2[a]	10·9[a]	11·8[b]	0·17
MEI† (MJ per cow)	187[a]	178[b]	195[c]	4·0
ME (MJ/kg DM)	12·45	12·38	12·35	0·136

[a,b,c] Means with different superscripts differ at $P < 0·05$.

† MEI = gross energy intake-faecal energy-urinary energy-methane energy, where methane energy = 0·08 × gross energy intake

Table 4 *Daily nitrogen intake (NI), N apparent digestibility (ND), daily excretion of N in faeces, urine and milk and N retention (intake minus faecal, urinary and milk N) and microbial protein synthesis for nine cows on pasture (P), 0·85 P + 0·15 protein/non-structural carbohydrate (NSC) mixture (PR), and P + 0·1 NSC (PE) in trial 1 (early lactation)*

	Treatment			
	P	PR	PE	s.e.d.
NI (g per cow)				
Pasture	563	464	546	8·6
Protein mixture	0	86	0	
Total	563	550	546	9·4
ND	0·79[a]	0·78[ab]	0·77[b]	0·008
N (g per cow)				
Faeces	117	124	124	4·3
Urine	291[a]	294[a]	258[b]	8·6
Milk	117[a]	112[a]	124[b]	2·9
Retention	39	20	37	10·3
Microbial protein synthesis				
g N per day	271	299	318	28·1
g/kg DOMI	24·3	27·9	26·8	2·33

[a,b] Means with different superscripts differ at $P < 0·05$.

Metabolism cows, intakes and digestibility data. DM intakes are shown in Table 3. Relative differences among treatments in intake were reflected in faecal output (2·94, 2·73 and 3·07 kg DM per cow per day for P, PR and PE respectively, s.e.d. 0·07; $P < 0·001$). DM and OM apparent digestibilities did not differ among treatments (Table 3). GE intakes averaged 284, 269 and 294 for P, PR and PE, respectively, (s.e.d. 4·3; $P < 0·001$). Output of energy in faeces, but not in urine, was higher for cows when on PE than when on P and PR (faecal energy: 60·5, 57·6 and 64·0 MJ per cow per day for P, PR and PE, respectively (s.e.d. 1·7; $P < 0·001$); urinary energy: 13·8, 12·4 and 12·8 MJ per cow per day for P, PR and PE, respectively (s.e.d. 1·02)).

N intakes did not differ among treatments (Table 4). Apparent digestibility of N was reduced by 0·02 on PE compared with P. Concentrations of N in faeces and urine were higher for cows on PR than on P and PE (g N per kg faeces DM: 40·1, 45·8 and 41·3 for P, PR and PE, respectively (s.e.d. 1·8; $P < 0·05$); g N per kg urine: 6·6, 8·6 and 6·6 for P, PR and PE, respectively (s.e.d. 0·3; $P < 0·001$)). Microbial protein synthesis was similar for cows offered PR and either P or PE but was proportionately 0·17 higher in cows on PE than on P ($P < 0·1$; Table 4). Microbial protein synthesis per kg digestible OM intake (DOMI) was similar for all treatments.

Ruminal metabolite and milk urea data. Ruminal pH and NH_3-N concentration showed distinct but opposite daily trends (Figure 1). Treatments PR and

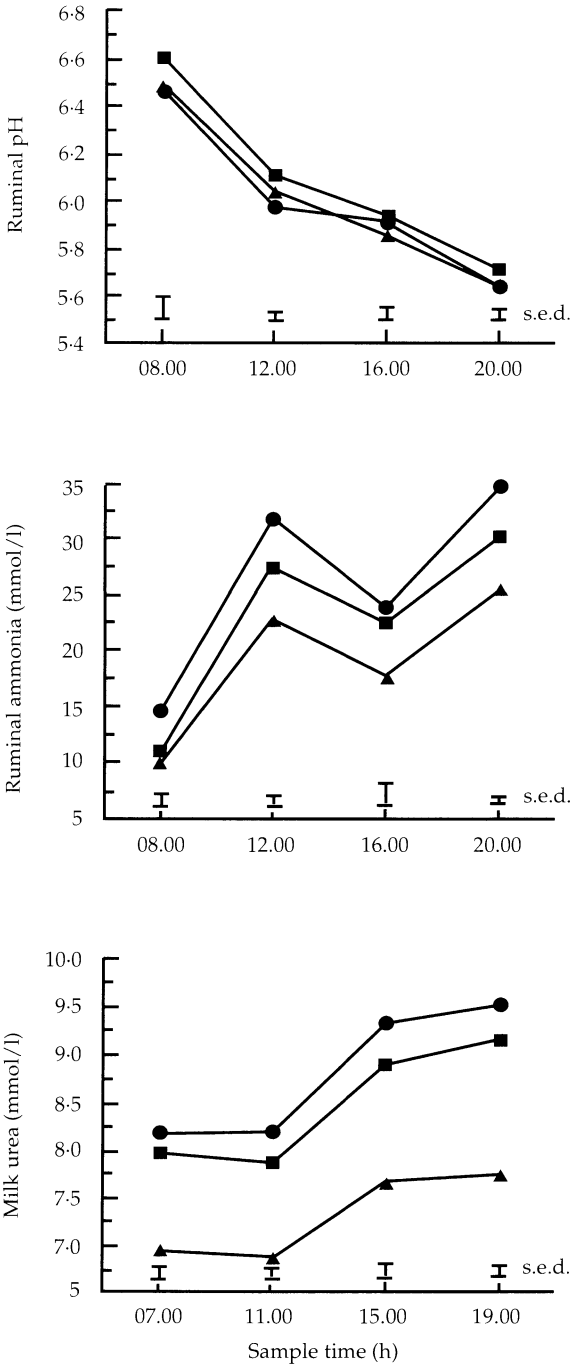

Figure 1 Ruminal pH and ammonia concentration (sampled from nine cows) and milk urea concentration (24 cows) at four sampling times in cows offered pasture (P, ■), 0·85 P plus 0·15 protein/non-structural carbohydrate (NSC) mixture (PR, ●), and P plus 0·1 NSC (PE, ▲) in trial 1 (early lactation).

Table 5 *Ruminal pH and ammonia-N (NH_3, mmol/l), milk urea (mmol/l), and total volatile fatty acid (VFA) concentration (mmol/l) and proportions (mol/100 mol) of individual acids in ruminal fluid averaged over four sampling times from cows on pasture (P), 0·85 P + 0·15 protein/non-structural carbohydrate (NSC) mixture (PR), and P + 0·1 NSC (PE) in trial 1 (early lactation)*

	Treatment			
	P	PR	PE	s.e.d.
pH	6·08[a]	5·99[b]	6·00[b]	0·02
NH_3	23[a]	26[b]	19[c]	0·9
Milk urea	8·5[a]	8·8[b]	7·3[c]	0·13
VFA				
Total	149·4[a]	163·9[b]	151·4[a]	4·28
Acetate	65·1	63·4	64·7	0·83
Propionate	18·4	18·8	19·2	0·47
Butyrate	12·5	12·8	12·1	0·53
Isobutyrate	1·1[ab]	1·2[b]	1·1[a]	0·09
Valerate	1·6[a]	1·8[b]	1·4[a]	0·06
Isovalerate	1·3[a]	2·2[b]	1·5[a]	0·37

[a,b,c] Means with different superscripts differ at $P < 0·05$.

PE decreased pH by 0·07 to 0·13 units compared with P at 12.00 h and 20.00 h ($P < 0·05$ to $P < 0·001$). Ruminal NH_3-N concentrations were lower on PE than on P at all sample times and higher on PR than on P at 08.00, 12.00 and 20.00 h. Values averaged over all sample times are shown in Table 5.

The concentration of urea in milk was lower for cows on PE compared with P and PR at all sample times (Figure 1). Concentrations were higher for cows when on PR than when on P at 11.00 h, 15.00 h and 19.00 h. Average concentrations are shown in Table 5.

Total VFA concentrations were least at 08.00 h and highest at 20.00 h, but did not differ among treatment groups at individual sample times. Total VFA concentration when averaged across all sample times was higher on PR than on P or PE (Table 5). The molar concentrations of acetate, propionate, butyrate, isobutyrate and isovalerate did not differ among treatment groups at individual sample times. Molar concentration of valerate was higher for cows when on PR than on P and PE ($P < 0·05$ to $P < 0·01$ for individual times). The proportions (mol/100 mol) of acetate, propionate and butyrate did not differ among treatment groups except for acetate at 12.00 h (67·9, 63·5 and 66·4 mol/100 mol for P, PR and PE, respectively, (s.e.d. 1·62; $P < 0·01$)). There were small differences among treatments in proportions of isobutyrate, valerate and isovalerate when averaged across all sample times (Table 5).

Table 6 *Daily dry-matter intake (DMI), daily produciton of milk, fat, protein and lactose, concentrations in milk of fat, protein and lactose, and change over 14 days in live weight (LW) and body condition score (BCS) of cows on pasture (P), 0·85 P + 0·15 protein/non-structural carbohydrate (NSC) mixture (PR), and P + 0·15 NSC (PE) in trial 2 (late lactation)*

	Treatment			
	P	PR	PE	s.e.d.
DMI (kg per cow)				
Pasture	12·0[a]	10·2[b]	11·7[c]	0·09
Protein mixture	0	0·32	0	
NSC mixture	0	0·96	1·30	
Total	12·0[a]	11·5[b]	13·0[c]	0·08
Yield (kg per cow)				
Milk	10·3[a]	10·9[b]	11·6[c]	0·28
Fat	0·517[a]	0·520[a]	0·552[b]	0·0121
Protein	0·358[a]	0·379[b]	0·408[c]	0·0086
Lactose	0·497[a]	0·530[a]	0·568[b]	0·0155
Milk composition (g/kg)				
Fat	50·8[a]	48·3[b]	48·0[b]	0·70
Protein	35·3	35·1	35·5	0·46
Lactose	48·3	48·7	48·8	0·31
LW change (kg)	–1·03[a]	–17·8[b]	9·0[a]	5·59
BCS change	–0·15	–0·25	–0·25	0·190

[a,b,c] Means with different superscripts differ at $P < 0·05$.

Trial 2: late lactation

Diet composition. Concentrations (g/kg DM) of ryegrasses, grasses other than ryegrass (*Panicum* sp., *Paspalum distichum* L., *Agrostis capillaris* L., *Bromus willdenowii* Kunth., *Digitaria sanguinalis* (L) Scop.), white clover, weed and dead material averaged 714, 103, 18, 109 and 56, respectively. Chemical composition of the pasture is shown in Table 1. The calculated proportions (g/kg DM intake) of NSC, NDF and CP in P, PR and PE, respectively, were: NSC, 124, 203 and 212; NDF, 489, 434 and 440; CP, 184, 190 and 167.

Food intake and milk production. DM intakes are shown in Table 6. Daily milk yield was 0·6 kg and 1·3 kg higher on PR and PE, respectively, than on P (Table 6). Protein and lactose concentrations of milk did not differ between treatment groups but fat concentration was 2·5 to 2·8 g/kg higher when cows were on P than on PR or PE. Fat, protein and lactose yields were greatest on PE and least on P. LW loss was greatest for cows on PR (Table 6).

Digestibility data. Apparent digestibilities of DM and OM were higher on PR and PE than on P (Table 7). GE intakes were 221, 213 and 239 MJ per cow per day (s.e.d. 1·5, $P < 0·001$). Output of energy in faeces but not in urine was lower for cows on PR than on P and PE (faecal energy: 62·5, 55·1, 64·7 MJ per cow per day for P, PR and PE, respectively (s.e.d. 1·5; $P < 0·001$); urinary energy: 14·6, 15·1 and 15·0 MJ per

Table 7 *Apparent digestibilities of dry matter, organic matter and energy (DMD, OMD, DE), daily intake of digestible organic matter (DOMI), daily intake of metabolizable energy (MEI), and ME content of the diet for cows on pasture (P), 0·85 P + 0·15 protein/non-structural carbohydrate (NSC) mixture (PR), and P + 0·15 NSC (PE) in trial 2 (late lactation)*

	Treatment			
	P	PR	PE	s.e.d.
DMD	0·73[a]	0·76[b]	0·75[b]	0·005
OMD	0·76[a]	0·78[b]	0·77[b]	0·005
DE	0·72[a]	0·74[b]	0·73[ab]	0·006
DOMI (kg per cow)	8·1[a]	8·1[a]	9·1[b]	0·05
MEI (MJ per cow)	127[a]	126[a]	140[b]	1·1
ME (MJ/kg DM)	10·60[a]	10·95[b]	10·75[ab]	0·113

[a,b] Means with different superscripts differ at $P < 0·05$.

cow per day for P, PR and PE, respectively (s.e.d. 0·6)) Apparent digestibility of energy was similar for P and PE but proportionately 0·024 higher for cows on PR than on P (Table 7).

Apparent digestibility of N was highest on PR and lowest on PE (Table 8). Concentration of N in faeces was higher on PE and PR compared with P (30·5, 32·6 and 32·4 g/kg DM for P, PR and PE, respectively (s.e.d. 0·6; $P < 0·01$)) and excretion of N in faeces was least on PR and greatest on PE (Table 8). Treatments PR and PE did not differ from P in urinary N

Table 8 *Daily nitrogen intake (NI), N apparent digestibility (ND), daily excretion of N in faeces, urine and milk and N retention (intake minus faecal, urinary and milk N) and microbial protein synthesis for cows on pasture (P), 0·85 P + 0·15 protein/non-structural carbohydrate (NSC) mixture (PR), and P + 0·15 NSC (PE) in trial 2 (late lactation)*

	Treatment			
	P	PR	PE	s.e.d.
NI (g per cow)				
Pasture	354	302	347	4·0
Protein mixture	0	48	0	
Total	354	350	348	3·7
ND	0·73[a]	0·74[b]	0·70[c]	0·004
N (g per cow)				
Faeces	97[a]	91[b]	105[c]	1·5
Urine	213[ab]	218[b]	198[a]	8·1
Milk	56[a]	59[b]	64[c]	1·4
Retention	–12	–18	–20	7·3
Microbial protein synthesis				
g N per day	139[a]	141[a]	172[b]	11·5
g/kg DOMI	17·2	17·3	19·0	1·34

[a,b,c] Means with different superscripts differ at $P < 0·05$.

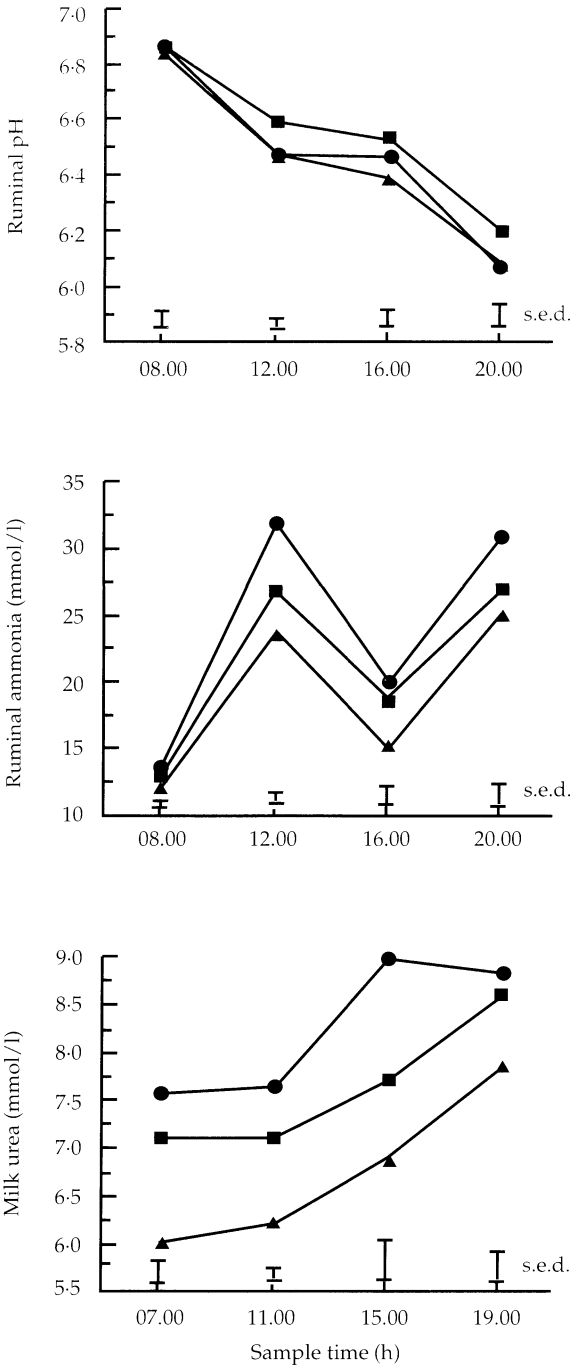

Figure 2 Ruminal pH and ammonia concentration and milk urea concentration at four sampling times in cows offered pasture (P, ■), 0·85 P plus 0·15 protein/non-structural carbohydrate (NSC) mixture (PR, ●), and P plus 0·15 NSC (PE, ▲) in trial 2 (late lactation).

Table 9 *Ruminal pH and ammonia N (NH_3, mmol/l), milk urea (mmol/l), and total volatile fatty acid (VFA) concentration (mmol/l) and proportions (mol/100 mol) of individual acids in ruminal fluid averaged over four sampling times for cows on pasture (P), 0·85 P + 0·15 protein/non-structural carbohydrate (NSC) mixture (PR), and P + 0·15 NSC (PE) in trial 2 (late lactation)*

	Treatment			
	P	PR	PE	s.e.d.
pH	6·55	6·46	6·44	0·05
NH_3	21[a]	24[b]	19[c]	0·9
Milk urea	7·6[a]	8·2[b]	6·7[c]	0·24
VFA				
Total	118·6	119·2	126·9	4·13
Acetate	71·5	70·2	70·9	0·77
Propionate	16·1[a]	17·0[b]	16·6[ab]	0·28
Butyrate	9·0	9·1	9·3	0·63
Isobutyrate	1·2[ab]	1·2[b]	1·1[a]	0·06
Valerate	1·3	1·4	1·2	0·14
Isovalerate	1·1	1·1	0·9	0·08

[a,b,c] Means with different superscripts differ at $P < 0·05$.

concentration and excretion. Microbial protein synthesis (Table 8) was similar for cows on P and PR but was proportionately 0·24 higher when cows were on PE than on P. Treatment had no effect on microbial protein synthesis per kg DOMI.

Rumen metabolite and milk urea data. Trends in pH and NH_3-N concentration were similar to trial 1, except pH values were about 0·4 units higher in trial 2 (Figure 2). Ruminal pH was 0·12 units higher for cows on P than on PR or PE at 12.00 h but similar at other times. Ruminal NH_3-N concentrations were generally least on PE and greatest on PR. Values averaged across all sample times are shown in Table 9.

The concentration of urea in milk was significantly lower for PE than for P at 07.00, 11.00 and 19.00 h (Figure 2). Concentrations of urea were higher for cows on PR than on P at 11.00 and 15.00 h. Average values are shown in Table 9.

Trends in total VFA concentration were similar to those in trial 1. Treatment groups did not differ in total concentration at any sample time or when averaged over all times (Table 9). Molar concentrations of individual acids did not differ among treatments, except for propionate when averaged over all times (24·9, 25·8 and 27·3 mmol/l for P, PR and PE, respectively (s.e.d. 0·9; $P < 0·05$)). The proportions of individual acids did not differ among treatment groups at individual sample times. When averaged over all times (Table 9) there were small differences among treatments in proportions of propionate and isobutyrate.

Discussion

Spring pasture containing WSC and NDF concentrations of 230 g/kg and 460 g/kg DM, respectively, apparently provided adequate energy for microbial protein synthesis, as increasing the proportion of NSC in PR had no effect on ruminal NH_3-N concentration, microbial protein synthesis or milk yield. Increasing the total carbohydrate : CP ratio, by increasing NSC intake in PE, increased microbial protein synthesis but not microbial efficiency. In contrast, in late lactation both PR and PE treatments increased milk and milk protein yields although only PE increased microbial protein synthesis. The greater production response for PE than for PR in late lactation suggested that on pasture with NSC as low as 124 g/kg DM, the cows responded to both increased availability of NSC and to increased microbial protein supply to the small intestine. Protein supply to the small intestine appeared to be limiting in late lactation on pasture alone. The cows were in negative N balance on all treatments in late lactation (trial 2), compared with a positive status in trial 1. In both trials the lengths of the treatment periods were insufficient adequately to assess changes in LW and BCS and the possibility that longer term feeding of the treatments might be associated with responses in either milk solids or body tissue cannot be excluded.

In both trials pasture provided an excess of ruminally degraded dietary CP, as indicated by high ruminal NH_3-N concentrations. The reduction in ruminal NH_3-N with addition of NSC has been observed on both fresh herbages (Dellow *et al.*, 1988; Robertson and Hawke, 1965) and total mixed rations (Cameron *et al.*, 1991), arising through either increased utilization of NH_3-N by microbes or decreased ruminal CP degradation (Cameron *et al.*, 1991). Increased utilization is likely to have occurred on PE because microbial protein synthesis increased and rate of degradation of N from pasture was not altered by addition of NSC (Carruthers *et al.*, 1996). The higher NH_3-N concentrations on PR compared with P and the increased rate of degradation of casein-N compared with that of pasture when incubated in the rumen (Carruthers *et al.*, 1996) indicated that the availability of N in the rumen was higher and more rapid from the protein mixture than from pasture. Cows on PR in trial 1 also showed increased N in faeces and urine. The higher ruminal N degradation may have compromised the cow's ability to respond to the increase in NSC, although in trial 2 a milk solids response was observed in PR cows despite higher ruminal NH_3-N and milk urea concentrations than on P. Cameron *et al.* (1991) found that urea supplementation increased ruminal ammonia concentration without adverse effects on milk yield and fat yield.

The lack of a production response to the PE diet compared with P in trial 1 may indicate that the combined undegraded dietary protein and microbial protein supply on pasture only was adequate for milk production, although specific amino acids may have been limiting or energy and protein co-limiting in early lactation cows. Kolver *et al.* (1995) also found that the changes in ruminal NH_3-N and pH which accompanied the synchronization of feeding concentrates with pasture were not associated with an effect on milk solids production or N retention. Cows restricted in intake and drawing on body reserves showed production responses to ruminally undegraded protein (Ørskov *et al.*, 1981) but production responses to supplementary ruminally undegraded protein in pasture-fed cows have been variable (Kellaway and Porta, 1993). Supplementation with fish meal did not increase production in early lactation cows grazing pasture similar to that in trial 1 (Carruthers and Penno, 1995). The level of production in trial 1 may have been insufficient to show a response to increased protein supply (Armentano *et al.*, 1993).

Providing additional NSC was associated with a decrease in total tract apparent digestibility of N. A portion of the starch component of the NSC may have been digested post ruminally, stimulating caecal microbial protein synthesis and excretion of N in the faeces (Ørskov, 1994), or a greater content of ruminal microbial amino acids may have appeared in the faeces. Cameron *et al.* (1991) observed a reduction in total tract N apparent digestibility with diets supplemented with maize starch, although MacGregor *et al.* (1983) observed increased ruminal protein digestibility when increasing the NSC concentration of the diet and apparent digestibility of CP was not affected. The increase in apparent digestibility of DM and OM on PR and PE in trial 2 but not in trial 1, may have reflected the addition of NSC to a pasture containing only 124 g NSC per kg DM.

Ruminal pH values in cows on spring pasture were relatively low. The optimal range of ruminal pH for cellulose digestion has been suggested to be 6·4 to 6·8 (Erdman, 1988), yet ruminal pH values in trial 1 were below 6·0 for much of the day. Similar values were observed in grazing cows run alongside trial 1 (unpublished data). Low ruminal pH may have been associated with the high VFA concentrations observed, with inadequate physically effective fibre in the diet (Allen, 1995), or with low buffering capacity of the forage (Erdman, 1988). The low values provide further support for the suggestion that spring pasture contains a high level of readily fermentable carbohydrate despite low measured WSC levels. The efficiency of protein synthesis by mixed rumen bacteria was proportionately 0·5 lower at pH 5·7 than at pH 6·7 (Strobel and Russell, 1986), suggesting microbial efficiency of pasture-fed cows should be compromised at the pH values observed. This was not supported by the estimates of microbial protein synthesis. Russell and Wilson (1995) suggest that cellulolytic bacteria do not adapt to low pH conditions although Therion *et al.* (1982) considered that pH alone was of less importance in determining shifts in bacterial population in the rumen than were availabilities of nutrients in relation to substrate preferences and affinities of the competing bacterial species.

In summary, results suggested that the measured WSC concentration of spring pasture inadequately indicated the availability of readily fermentable carbohydrate for microbial protein synthesis. The lack of response in fermentation efficiency or production in early lactation to increasing the NSC : SC ratio was consistent with poor milk solids' responses to grain supplementation where the supplement substitutes for pasture and intake is not increased. There may be more scope in late than in early lactation for increasing production through manipulating the nutrient composition of a pasture diet.

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Eating quality of beef from different sire breeds

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Abstract

Three hundred and eight cattle, comprising steers and heifers from continental and British crosses were finished on an 18-month beef production system at Warren Farm, Lambourn, Berkshire and slaughtered across a range of fatness levels. The eating quality of roasting joints (semimembranosus) *and sirloin steaks* (longissimus thoracis et lumborum) *was evaluated and the chemical composition of the lean tissue was taken on a subset of the samples. Results suggested that the greatest differences between the breeds was in the joints. There were significant* ($P < 0{\cdot}01$) *differences in the lipid, moisture and collagen contents of the lean tissue of the joints from the different sire breeds. The Belgian Blue sire progeny had significantly lower lipid content than the Charolais or Aberdeen Angus crosses, and significantly lower collagen content than the Aberdeen Angus cross. In addition joints from Belgian Blue crosses were more tender than joints from other breed crosses. Although the lipid and moisture contents of the steaks from different sire crosses were significantly different there was no evidence of differences in eating quality. Perhaps surprisingly there were no significant relationships between the eating quality and chemical composition between or within breed. Irrespective of breed, carcass fatness influenced the juiciness of both the joints and the steaks although the response was different for continental crosses and British crosses and depended on carcass sex. Over and above this response, steer meat was generally more juicy than heifer meat.*

Keywords: *beef breeds, chemical composition, organoleptic traits.*

Introduction

Major changes have taken place in the British beef industry over the past 20 years. Genes from several continental breeds, with the potential to produce larger and leaner carcasses have been introduced. Also, with almost two-thirds of clean beef coming from dairy herds, the extensive replacement of the British Friesian by the Canadian Holstein in the dairy industry has resulted in beef carcasses that have tended to be larger and have poorer conformation. The trend towards heavier and poorer conformation carcasses is evident from national carcass classification statistics collected by the Meat and Livestock Commission (MLC, 1995).

The relationship between diet and health is a major factor in the development of production strategies for the British meat industry. Consumers demand consistently tender, flavourful meat with low fat contents. Low or variable quality in terms of major components such as tenderness is not acceptable. There are no recent published British data to confirm overseas evidence on the relationship between fatness and eating quality but there are concerns that the trend towards larger and poorer conformation carcasses, associated with breed changes, is resulting in beef of poor eating quality. Armbruster *et al.* (1983) suggested that marbling (intramuscular fat) in beef only accounts for a small amount of the variation in eating quality. Other published evidence, mainly from North America and Denmark, indicates that at low levels of intramuscular fat, tenderness and juiciness are less satisfactory than at higher levels (Dikeman *et al.*, 1979 and Buchter, 1986) and that there is a lower limit to which fatness can be reduced without detrimental effects on eating quality. In contrast Dikeman (1987) admitted that marbling only accounts for proportionately between 0·10 and 0·15 of the variation in beef palatability.

There is also evidence that breed influences the proportion of marbling in relation to subcutaneous fat cover on the carcass (Johnson, 1987; Cundiff *et al.*, 1994), although a study carried out by the MLC

(Chadwick *et al.*, 1979) found no differences in eating quality between breeds, at the same subcutaneous fat level.

Therefore, a trial was set up by the MLC, in collaboration with Genus, to evaluate the eating quality of progeny from a range of sire breeds crossed with cows from the dairy herd. The trial had two main objectives. First, to assess the eating quality of two British sire breeds and four continental sire breeds crossed with Holstein Friesian cows and, secondly to examine the effect of fatness on eating quality within and between breeds.

Material and methods

Four continental sire breeds, Limousin (LM), Charolais (CH), Belgian Blue (BB) and Piemontese (PM) representing two of the most widely used beef sire breeds and two recently imported sire breeds, that were being evaluated as part of Genus' progeny testing programme, and two British sire breeds, Aberdeen Angus (AA) and Hereford (HF), were included in the trial. All the animals were steer and heifer progeny from Holstein Friesian type dams and a cross section of sires of each breed. They were finished on an 18-month beef production system. In several instances cattle were bought specially for the trial. These were selected from six different farms to ensure that a cross section of sires were represented. All animals in the trial were housed at Warren Farm where they spent at least 3 months prior to slaughter and were given grass silage/cereal for at least 1 month leading up to slaughter.

Low, average and high fatness levels for steers and heifers of each breed were determined in terms of MLC classification (MLC, 1995, where fatness levels are on the following scale: 1, 2, 3, 4L, 4H, 5L and 5H) as below:

	Fat class		
	Low	Average	High
Continental steers	2	3	4L
British steers	3	4L	4H
All heifers	3	4L	4H

Two abattoirs were chosen for slaughtering and were used alternately every 2 weeks. Cattle were slaughtered between April 1991 and October 1992. Carcasses were selected from the total sent to slaughter on any given day if the following rules held: a minimum of two breeds were represented; one carcass of each breed had a common fatness level; and at least two fatness levels were represented. This gave a minimum of three mixed- or single-sexed carcasses from any slaughter day. In addition, breed comparisons on a given day were monitored to ensure cattle of each breed were slaughtered with every other breed by the end of the trial. Twenty-five steers and 25 heifers from each breed were required. It was anticipated that at least seven of each sex and breed would be present in each of the fatness groups (low, average and high).

Animals were stunned with a captive bolt and carcasses were dressed to a standard specification with no electrical stimulation. After slaughter carcasses were chilled by a process which avoided any part of any muscle falling below 10°C within 10 h of stunning.

Carcass measurements

The following attributes were among those recorded on the carcasses: estimated subcutaneous fat proportion (SFe); conformation (MLC 15-point scale); pH 3 and 24 h post slaughter in the *longissimus thoracis et lumborum* (LTL) to give an indication of the rate and extent of rigor development; maximum width and depth of LTL at 10/11 rib; fat depth at the 3/4 last rib.

On the day after slaughter hindquarters were brought to MLC and held in chilled storage (approx. 3°C) for 8 days. Primal cutting was then carried out with the topside joint (*m. semimembranosus*) and sirloin (LTL) being retained for detailed assessment. Measurements of colour were made on the sirloin steaks using an EEL reflectometer (Diffusion Systems Ltd, Hanwell, London) and drip loss was measured by suspending a sirloin steak in a loose plastic bag, sealed at the neck, at 4°C for 48 h. After a total of 9 days from slaughter topside joints and sirloin steaks were blast frozen and retained for sensory evaluation. Samples of the same cuts were also retained for chemical analysis.

Sensory evaluation

At the sensory panel sessions six samples of the same cut type (i.e. joint or steak) were evaluated. The six samples evaluated in each session consisted of one from each of the six breeds, three from steers and three from heifers, representing each fatness level. Joints were cooked at 180°C, in a fan-assisted domestic electric oven fitted with external temperature controllers, to an internal temperature of 70°C. Steaks which had been cut to 20 mm thickness were grilled for 5 min each side under a gas grill set at 'high'. The timing was targeted to achieve a centre temperature of 70°C.

Samples were presented to six trained panellists, from a pool of 12, taking care to balance the order of

presentation across breed, fatness and sex. Each panellist scored for a range of characteristics including: juiciness 1 (dry) to 8 (juicy); tenderness 1 (tough) to 8 (tender); beef flavour 1 (weak) to 8 (strong); abnormal flavour 1 (weak) to 8 (strong). Panellists scores were averaged for each sample so, for statistical analysis, one average score for each joint/steak per characteristic was analysed.

Chemical analysis

One hundred and eight sirloin steaks and 108 topside joints from Aberdeen Angus, Belgian Blue and Charolais sires, covering the range of fat and conformation classes selected and both sexes, were subjected to chemical analysis by the Division of Food Animal Science, University of Bristol. These breeds were chosen as representative of a traditional British sire breed, a double-muscled sire breed and a continental sire breed respectively. All LTL and *semimembranosus* muscles were carefully trimmed of all intermuscular and subcutaneous fat, epimysium and peripheral muscles so that only the completely trimmed muscle was left. Muscles were analysed for lipid and moisture contents (as described by Savell *et al.*, 1986) and for collagen (McKeith *et al.*, 1985) and soluble collagen (Csiba, 1984) contents.

Statistical analysis

Using GENSTAT 5.2 (Lawes Agricultural Trust, 1990) analysis of variance models were fitted to each of the carcass traits measured with terms for all the major factors and regressions as follows: main effects — breed (LM, CH, BB, PM, AA, HF), sex (steer, heifer), abattoir (X, Y), slaughter day within abattoir; regression — side weight (cold (kg)), SFe (estimated subcutaneous fat proportion), conformation (MLC 15-point scale).

In addition the following interactions were tested for inclusion in the model: breed × sex, breed × SFe, breed × conformation, sex × SFe, sex × conformation.

The criterion for including any of these interactions in the model was significance at the 5% level of probability. Sensory panel results for steaks and joints were analysed separately using the same model as above although panel session replaced slaughter day. Results from the chemical analysis were analysed using the same model without the abattoir and day effects. Where the F ratio from the analysis of variance tables indicated significant differences between the breeds Newman-Keuls multiple range test was used to identify where these differences occurred.

Results

In total 308 carcasses were selected. The distribution by target fat groups (low, average and high) was generally as planned, although there was some difficulty in obtaining high fatness carcasses of LM and PM cross heifers, so these were marginally leaner than might have been expected. Table 1 shows a description of the sample.

Analysis of variance showed significant breed effects for the shape and colour of the sirloin and for the tenderness and beef flavour of the topside joints. However, no significant differences were found between the breeds in the eating quality of the sirloin steaks. Table 2 shows the least-square means by breed corrected by regression to the average fatness, conformation and side weight of each breed.

There were no significant differences between the breeds in pH at 3 h or 24 h. Meat from AA carcasses tended to be darker than meat from other breeds. The sirloin of the BB and PM was significantly larger than the sirloins from the other breeds and both British breeds had less, but not significantly less, drip loss. The topsides of BB were significantly more tender than the LM, PM or AA, which were all similar. Although not significantly different, PM

Table 1 *Description of carcasses selected from steers and heifers resulting from crossing Friesian/Holstein cows with continental or British beef sires*†

	Continental				British		
	LM	CH	BB	PM	AA	HF	s.d.
No.	48	54	53	50	52	51	
Average:							
side weight (kg)	131	137	136	142	122	114	9·96
SFe‡	68	72	68	66	84	84	15·7
conformation score	6·0	5·9	6·8	5·1	3·3	3·4	1·98

† Breeds: LM = Limousin; CH = Charolais; BB = Belgian Blue; PM = Piemontese; AA = Aberdeen Angus; HF = Hereford.
‡ Estimated subcutaneous fat (g/kg).

Table 2 *Least-square means at the average conformation, side weight and fatness level of each breed*

	Breed†							
	LM	CH	BB	PM	AA	HF	s.e.	Significance
Carcass traits								
pH_3	6·50	6·52	6·54	6·42	6·58	6·53	0·04	
pH_{24}	5·77	5·75	5·72	5·78	5·84	5·78	0·04	
LTL EEL colour‡	$23·8^{ab}$	$23·5^{ab}$	$24·6^{a}$	$23·3^{ab}$	$21·6^{b}$	$23·1^{ab}$	0·72	*
3/4 rib fat depth (mm)	$5·56^{a}$	$6·35^{ac}$	$4·88^{a}$	$5·15^{a}$	$9·04^{b}$	$7·91^{bc}$	0·46	***
LTL width (mm)	132^{a}	133^{a}	136^{ab}	139^{b}	126^{c}	132^{a}	1·4	***
LTL depth (mm)	61^{a}	61^{a}	65^{b}	63^{ab}	56^{c}	52^{d}	1·0	***
Drip loss (g/kg)	14·4	14·3	14·6	14·4	11·8	12·7	1·4	
Joints								
Juiciness	4·3	4·4	4·2	4·5	4·5	4·4	0·12	
Tenderness	$3·8^{a}$	$4·0^{a}$	$4·5^{b}$	$3·8^{a}$	$3·8^{a}$	$3·9^{a}$	0·14	**
Beef flavour	4·7	4·7	4·6	4·7	4·8	4·6	0·08	
Abnormal flavour	1·7	1·7	1·6	1·7	1·7	1·7	0·08	
Steaks								
Juiciness	5·7	5·5	5·6	5·7	5·6	5·6	0·10	
Tenderness	5·2	5·0	5·2	5·5	5·0	5·0	0·16	
Beef flavour	5·3	5·1	5·2	5·3	5·1	5·1	0·09	
Abnormal flavour	1·7	1·6	1·7	1·7	1·7	1·6	0·08	

^{a,b,c} Means with different superscripts are significantly different ($P < 0·05$).
† For breeds see Table 1.
‡ Higher values indicate lighter meat.

steaks were rated as more tender and more juicy than steaks from other breed crosses.

The rated juiciness of the sirloin steaks (and to a lesser extent the joints) was dependent on fatness levels, sex and breed. Figure 1 shows the curvilinear response of juiciness to fatness by breed and sex. The juiciness of the steers generally increased with fatness, the continental steers all behaving in a similar way. The juiciness of the heifers was significantly lower than the steers overall and, with the exception of the AA, generally decreased with fatness.

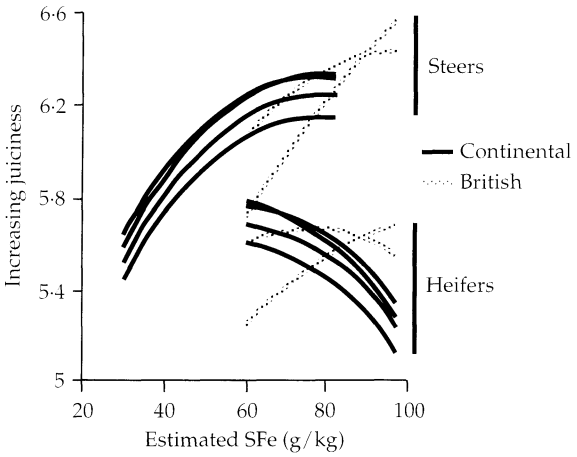

Figure 1 Response of juiciness to increasing carcass fatness (SFe) by breed and sex.

There were significant differences between the breed crosses measured for chemical content and there were significant fatness effects for the lipid and moisture contents. Neither collagen nor soluble collagen content of the steaks depended on breed but AA had significantly higher levels of total collagen than the other breed crosses in the joints. Table 3 shows the least-square means at each breed's average fatness, conformation and side weight. The AA had less moisture and more lipid in the lean tissue of both the sirloins and the joints. This appeared to be partly a breed effect and partly a carcass fatness effect — the average AA being over 10 g/kg SFe fatter than the BB or CH. However when compared at the same fatness level the AA still had significantly higher lipid contents than the other two breeds. As the fatness of the carcass increased the moisture contents of both the steaks and the joints decreased and the lipid content increased. The decrease/increase was greater for the steaks than for the joints. Across a fat class, for example from 3 to 4L, the lipid increased by 3·2 g/kg in the steaks but only 2·1 g/kg in the joints.

Table 3 *Least-square means (g/kg) at the average conformation and fatness of each breed*

	Breed†				
	CH	BB	AA	s.e.	Significance
Joints					
Moisture	737[a]	738[a]	733[b]	1·1	**
Lipid	20·2[a]	13·8[b]	24·4[c]	0·70	***
Collagen	5·5[a]	5·3[a]	6·0[b]	0·14	**
Soluble collagen	0·70	0·70	0·74	0·013	
Steaks					
Moisture	740[a]	741[a]	736[b]	1·2	*
Lipid	20·7[a]	15·6[b]	25·5[c]	0·83	***
Collagen	4·4	4·4	4·3	0·10	
Soluble collagen	0·68	0·70	0·70	0·020	

[a,b,c] Means with different superscripts are significantly different ($P < 0·05$).
† For breeds see Table 1.

Neither collagen nor soluble collagen responded significantly to changes in fatness.

Table 4 shows the Pearson correlations between eating quality and chemical composition within session. Perhaps surprisingly all the correlations were low, especially for the steaks, and not significant. Conformation or side weight contributed little to explaining the variation in eating quality.

Discussion

While some differences between sire breeds were detected, little evidence was found to support the belief that eating quality has been disadvantaged by the change from traditional British beef breeds, as crossing bulls, to imported beef breeds. In fact at typical carcass fatness levels, BB joints were significantly more tender than joints from other breeds but there were no significant differences between the British breeds and the most widely used continental breeds (CH and LM). Uytterhaegen *et al.* (1994) found highly significant differences in eating quality and collagen content between single and double-muscled BB and commented on the extreme muscularity of the double-muscled BB breed. PM were included in this trial as another double-muscled breed. Although there was no evidence of the PM having any advantage over the single-muscled breeds in terms of the eating quality of joints, they produced the most tender steaks (although the difference was not significant). Carcasses used by Uytterhaegen *et al.* (1994) were much heavier than in this trial and the difference in conformation between the double- and single-muscled breeds was much greater than in the carcasses used here. It may be that in this trial the double-muscle effects of the PM and BB crosses were reduced because of the Holstein Friesian type dams used and hence their full potential was not demonstrated. Chadwick *et al.* (1979) found little evidence of eating quality differences between breeds, all were judged satisfactory for tenderness, juiciness and flavour although double-muscled breeds were not represented.

Table 4 *Correlations between sensory and chemical analysis*

	Moisture	Lipid	Collagen	Soluble collagen
Joints				
Juiciness	0·03	0·13	–0·04	–0·03
Tenderness	0·15	–0·30	–0·09	0·17
Steaks				
Juiciness	–0·07	0·14	–0·06	0·07
Tenderness	0·14	0·0	0·0	–0·12

Although some differences were found between breeds in tenderness there was no evidence that this was due to collagen. As in other trials the relationship between eating quality characteristics and the results from the chemical analysis were very weak. There were indications that the somewhat better tenderness of the topside joints for the BB crosses might be explained by their having less collagen than the other two breeds but there was a very low correlation between collagen content and beef tenderness. Uytterhaegen *et al.* (1994) did find large significant differences between the double- and single-muscled breeds in terms of collagen.

There was evidence that, irrespective of breed, the fatness of the carcass could affect eating quality, but the picture was not clear with steers and heifers showing different responses to increasing fatness. Although the sensory panel found no relationship between increasing fatness and tenderness they did perceive a relationship for juiciness. Increasing carcass fatness in continental cross steers produced more juicy meat whereas increasing carcass fatness of the two British breeds represented, generally produced meat that was more juicy for both steers and heifers. This is somewhat contradictory to other published evidence. Berry and Bigner (1995) concluded that marbling did not affect perceived juiciness of beef loins and Cross *et al.* (1973) concluded that increasing carcass fat was not associated with changes in juiciness or tenderness.

The results confirm that there is little to choose between the breeds investigated here in terms of eating quality when selecting beef cross progeny

from the dairy herd. The positive effect of increasing fatness was relatively small and will probably not outweigh the demand for low fat products on health grounds.

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Estimation of the energy expenditure from heart rate measurements in working oxen

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Abstract

The heart rate (f_H) and the energy expenditure (EE) of seven Hinterwaelder (Bos taurus) *draught oxen and three zebu* (Bos indicus) *oxen were measured, while the animals were standing, walking and pulling different loads. Linear regressions for all animals relating EE to f_H were highly significant (P < 0·001). The standard errors of the estimate expressed as a percentage of the mean EE (PE) ranged from ±6·7% to ±10·5%. Two animals with PE ±13·7% and ±17·1% were beyond that range. One year later, f_H and EE were measured on six of the original seven Hinterwaelder oxen while the animals were standing and walking on a treadmill, on the level and at gradients of 3%, 6% and 9%. In the two experiments mean slope and mean intercept of the regressions of EE on f_H were not different (P > 0·05). Irrespective of the kind of work (draught work or lifting work), f_H allows a reliable prediction to be made of the EE of working oxen.*

Keywords: *draught animals, energy expenditure, heart rate.*

Introduction

Measuring gaseous exchange to determine the energy expenditure (EE) of working cattle under field conditions is technically difficult and can impair the animals' ability to carry out some tasks, because they have to wear a mask. Such techniques are suitable only for short-term measurements and a long time is required to train the animals. Therefore, in several experiments EE has been predicted from heart rate (f_H). Subjects include humans (Ceesay *et al.*, 1989), geese (Nolet *et al.*, 1992), sea lions (Butler *et al.*, 1992), penguins (Bevan *et al.*, 1995), pine marten (Fisher *et al.*, 1987), sheep (Brockway and McEwan, 1969; Webster, 1967), reindeer (Nilssen *et al.*, 1984), cattle (Holmes *et al.*, 1976; Yamamoto *et al.*, 1979; Richards and Lawrence, 1984; Brosh *et al.*, 1994) and buffalo (Richards and Lawrence, 1984). Richards and Lawrence (1984) were the only workers who demonstrated a relationship between EE and f_H in working oxen. However Lawrence *et al.* (1991) considered this method to be inaccurate in draught animals. In default of any other suitable method to overcome the limitations of the mask-technique for determining EE under field conditions, the aim of this project was, to test the suitability of the f_H for estimating the EE of draught oxen while doing different kinds of work.

Material and methods

Measurements of f_H and EE were carried out in 1992 with seven Hinterwaelder (*Bos taurus*) oxen (body weight 494 (s.e. 16) kg, 22 to 26 months old) in Hohenheim, Germany and three zebu (*Bos indicus*) oxen (body weight 517 (s.e. 76) kg, 84 to 132 months old) in the Republic of Niger, at the International Crop Research Centre for the Semi-Arid Tropics Sahelian Centre. Measurements were taken while the animals were standing, walking and pulling different loads (experiment 1). The Hinterwaelder oxen pulled a sledge on a tarmac circuit with a draught force between 420 and 710 N. The zebu oxen worked on a sandy circuit and exerted a draught force between 780 and 1000 N. The experiment comprised five stages, in which the work load was increased at every stage. In 1993, f_H and EE were measured again on six of the seven Hinterwaelder oxen (body weight 567 (s.e. 32) kg while the animals were standing and walking on a treadmill, both on the level and at gradients of 3%, 6% and 9% (experiment 2). Each stage in the experiment lasted 6 min. After an adaptation period, f_H, oxygen consumption and carbon dioxide production were measured for 3 min.

Gaseous exchange was determined using a mobile mask technique combined with a respiration

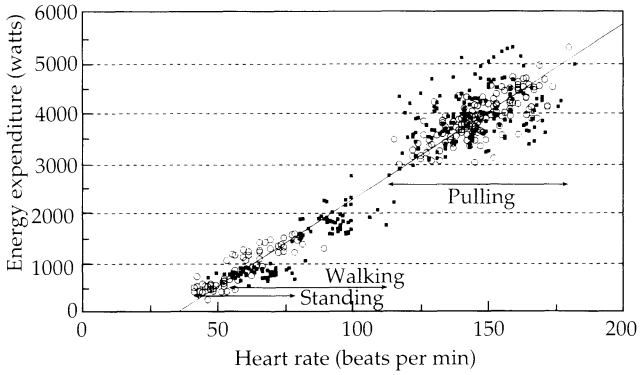

Figure 1 Plot of energy expenditure (y) *v.* heart rate (x) for seven Hinterwaelder (■) and three zebu oxen (○) while the animals were standing, walking and pulling different loads.

gasmeter followed by analysis of an aliquot sample of expired air (Clar *et al.*, 1992). The EE was calculated from the oxygen consumption (McLean, 1972). The f_H was measured with three electrodes attached to the skin and coupled to an ECG-sensor of the type described by Rometsch and Becker (1993).

Seven repetitions per Hinterwaelder ox and 10 per zebu were carried out while the animals were standing, walking and pulling loads on the tarmac circle and 10 repetitions while the animals were walking on the treadmill. The results were subjected to an analysis of normal distribution by the application of the SAS, PROC GLM, two repeated-measures factors program. Comparisons of slopes and intercepts were computed by GLM procedures.

In the regression analysis, EE was taken as the dependent and f_H as the independent variable, since the aim was to predict EE from f_H.

Results

For the first experiment carried out in 1992, Figure 1 shows a plot of EE (y) *v.* f_H (x) for seven Hinterwaelder and three zebu oxen while the animals were pulling loads.

The regression equations relating EE (y) to f_H (x), the mean y value, the coefficient of determination (r^2), the standard error of the estimate ($S_{y.x}$) and $S_{y.x}$ expressed as a percentage of the mean y value (PE) for these oxen are shown in Table 1. Separate regressions are shown for individual animals, different trials and the overall sample.

All the regressions were highly significant ($P < 0.001$). All coefficients of determination for the respective regression line were above 0·88. For eight of the oxen, PE values ranged from ±6·7% to ±10·5%. Two oxen (no. 2 and no. 6) with ±17·1% and ±13·7% respectively were beyond that range.

For the second experiment in 1993, which used six of the same Hinterwaelder oxen, Figure 2 shows a plot of EE (y) *v.* f_H (x). The oxen walked on a treadmill, both on the level and at gradients of 3%, 6% and 9%.

The regression equations relating EE (y) to f_H (x), the mean y value, the coefficient of determination (r^2), the standard error of the estimate ($S_{y.x}$) and $S_{y.x}$ expressed as a percentage of the mean y value (PE) for the 1993 experiments are shown in Table 2.

Table 1 *Regression of energy expenditure* (y) *on heart rate* (x) *in seven Hinterwaelder (animals 1 to 7) and three zebu oxen (animals 8 to 10) standing, walking horizontally and pulling loads*

Animal no.	No.	Regression	Mean y value	r^2†	$S_{y.x}$‡	PE§ (%)
1	36	$y = 39{\cdot}24x - 1472{\cdot}4$	3079·7	0·97	±273	±8·9
2	32	$y = 47{\cdot}76x - 2293{\cdot}9$	2915·9	0·88	±500	±17·1
3	42	$y = 33{\cdot}33x - 1181{\cdot}5$	2826·2	0·98	±189	±6·7
4	37	$y = 31{\cdot}52x - 1424{\cdot}6$	2750·8	0·98	±204	±7·4
5	33	$y = 44{\cdot}53x - 1929{\cdot}2$	3218·4	0·97	±293	±9·1
6	30	$y = 39{\cdot}99x - 1825{\cdot}2$	2510·7	0·94	±345	±13·7
7	41	$y = 39{\cdot}45x - 1799{\cdot}7$	3086·8	0·96	±274	±8·9
8	64	$y = 34{\cdot}76x - 1153{\cdot}9$	2766·4	0·95	±291	±10·5
9	64	$y = 31{\cdot}31x - 862$	2996·6	0·96	±306	±10·2
10	60	$y = 34{\cdot}38x - 1114{\cdot}6$	3039·7	0·98	±216	±7·1
Hinterwaelder	251	$y = 36{\cdot}52x - 1412$	2919·3	0·88	±492	±16·8
Zebu	188	$y = 33{\cdot}04x - 996$	2619·5	0·96	±287	±9·8
Total	439	$y = 34{\cdot}7x - 1195$	2924·7	0·91	±421	±14·4

† r^2 = coefficient of determination.
‡ $S_{y.x}$ = standard error of estimate.
§ PE = standard error of estimate expressed as percentage of mean y value.

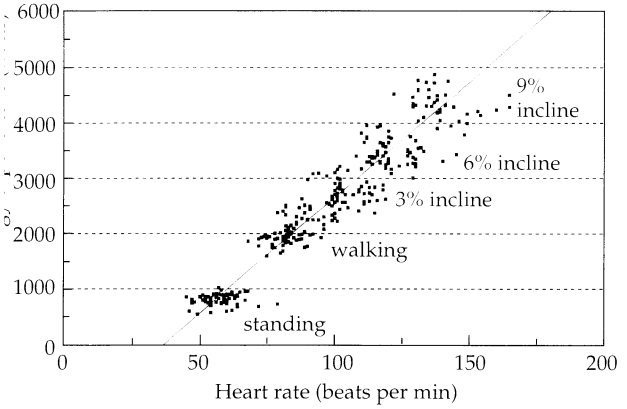

Figure 2 Plot of energy expenditure (y) *v.* heart rate (x) for six Hinterwaelder oxen while the animals were standing, walking on a treadmill, both on the level and at gradients of 3%, 6% and 9%.

All the regressions were highly significant ($P < 0{\cdot}001$). All coefficients of determination for the respective regression line were above 0·90. The PE values of individual oxen ranged from ±8·0% to ±11·4%. Between experiment 1 and experiment 2 the mean slope and mean intercept of the total regression lines did not differ significantly according to the F-test ($P > 0{\cdot}05$).

Discussion

For the regression of EE upon f_H shown in Figure 1, the $S_{y.x}$ as a percentage error of the mean EE (PE) was only 14·4%. This was despite the fact that the data were taken from two sets of oxen of different breeds working under different conditions of climate, husbandry and nutrition. The PE found in eight out of 10 draught oxen was comparable to the results of Yamamoto *et al.* (1979) and Holmes *et al.* (1976), who determined an error between 7% and 10%. The PE for these oxen is also less than the 10% suggested by Brockway (1978) as being the maximum acceptable value for nutritional studies. The regression line for the three zebu oxen is very similar to that of Richards and Lawrence (1984) for Brahman cattle (EE = 31·4 f_H – 1453, no. = 56, r^2 = 0·88). The main difference between the two regression lines is that the variability was higher in their study than in the present one, because number of observations was lower (no. = 56 and no. = 188, respectively). Webster (1967) determined an error less than 10% in three out of four sheep, when EE was increased by cold exposure and by increased levels of food intake. Webster (1967), Holmes *et al.* (1976) and Yamamoto *et al.* (1979) concluded that frequent measurements of f_H appear to offer a practical method for the estimation of EE in free-range animals.

Two oxen, no. 2 and no. 6, demonstrated PE values greater than 10%, at 17·1% and 13·7%, respectively (Table 1). The high PE of ox no. 2 can be attributed to the unusually nervous disposition of the animal. He often showed sudden increases in f_H when startled. Temperament thus seems to influence the relationship between f_H and EE. Even during hard work pulling heavy loads, the effects of which should exceed those resulting from psychogenic effects, statistical parameters of the relationship between f_H and EE were influenced by the latter. Such nervous reactions were also noted by Webster (1967) in sheep. He determined a PE of 6·8% to 8·6% in three sheep. The fourth sheep demonstrated a PE of 13·8%. After a longer adaptation period in experiment 2 ox no. 2 did calm down and this is reflected in his lower PE.

One way to reduce the PE of the overall sample was by scaling the regression lines from individual animals so that the EE of all animals at the average f_H (130 beats per min) was the same. This procedure reduced the PE by 2·5 percentage points to 11·9 in experiment 1.

Table 2 *Regression of energy expenditure* (y) *on heart rate* (x) *in six Hinterwaelder oxen standing, walking on a treadmill, both on the level and at gradients of 3%, 6% and 9%*

Animal no.	No.	Regression	Mean y value	r^2†	$S_{y.x}$‡	PE§ (%)
1	51	$y = 45{\cdot}55x - 1746{\cdot}5$	2737·6	0·97	±220	±8·0
2	50	$y = 49{\cdot}77x - 1841{\cdot}7$	2429·2	0·96	±219	±9·0
3	52	$y = 35{\cdot}02x - 1095{\cdot}0$	2150·6	0·95	±214	±9·9
4	54	$y = 34{\cdot}93x - 1211{\cdot}3$	2415·8	0·96	±232	±9·6
5	50	$y = 41{\cdot}52x - 1446{\cdot}3$	2643·9	0·94	±300	±11·4
7	54	$y = 42{\cdot}06x - 1613{\cdot}2$	2461·6	0·96	±257	±10·4
Total	311	$y = 39{\cdot}17x - 1294{\cdot}7$	2471·0	0·90	±364	±14·7

† r^2 = coefficient of determination.
‡ $S_{y.x}$ = standard error of estimate.
§ PE = standard error of estimate expressed as percentage of mean y value.

The slope and intercept in the relationship between EE and f_H of the Hinterwaelder and zebu oxen while pulling a load (Figure 1) and the slope and intercept of the relationship between EE and f_H while the oxen were lifting their own body weight (Figure 2) was not significantly different. Therefore the kind of physical work the animals did had no influence on the relationship between EE and f_H.

Heart rate measurements are very simple. They do not interfere with the animal's natural movement. The animal can graze freely. Using a logging unit, the animal can also move freely. The animals must become accustomed to wearing a harness but otherwise no adaptation to the measuring technique is necessary. Therefore, in spite of a PE of over 14% in the relationship between f_H and EE for all oxen, the general equation linking f_H to EE may be used for estimating the EE of working oxen under field conditions. If more precise estimates of EE are required, animals can be individually calibrated to reduce PE to 10% or lower in the majority of cases.

According to the data in this study, the calibration curve for any particular animal does not tend to alter over time; the mean slope and intercepts of the regression lines for all animals in the 2 years did not differ significantly according to the F test. To emphasize this point, a *T* test was performed which showed that neither the slopes nor the intercepts from the six individual oxen that were used both in 1992 and 1993 showed any significant difference between the 2 years. Average values for slopes were 39·9 and 41·5 for 1992 and 1993 respectively ($P > 0{\cdot}05$). Corresponding values for the average intercepts were −1680 and −1492 ($P > 0{\cdot}05$). These results strongly imply that once a calibration curve has been made for a particular animal it may be used with confidence under similar conditions for some time afterwards.

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Use of a stochastic model of a calving distribution for beef cows for formulating optimal natural mating strategies

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Abstract

A model of a birthdate distribution for a herd of beef cows is constructed using the probability distributions of the variables that affect reproduction in the cow — anoestrous interval, oestrous cycle length, conception to each oestrus, gestation length, period of mating and the prior calving frequency distribution. The model is general and can be reparamaterized to deal with issues such as intervention to synchronize oestrous cycles among cows in the herd by changing the form of the relevant probability distributions.

The model is applied to the question of what time to begin mating in a herd of beef cows. The average calf live weight at day 200, herd conception rate and proportion of cows calving before the planned start of calving were calculated from the model output. The model parameters given by the anoestrous period, conception rate to each oestrus and the regression between prior calving date and anoestrous period, were varied in a factorial design to investigate a range of circumstances found on a farm. Prior calving distributions were generated by random sampling from eight actual calving frequency distributions

Generally starting mating earlier produced an advantage in terms of extra calf live weight and herd conception rate. However, the proportion of the herd calving earlier than expected increased with early mating. Thus, the feasibility of early mating depends on the cost to the farmer of dealing with early calving cows as well as the advantage of heavier older calves.

Altering the fixed parameters in the model (variances and covariances, prior calving distributions, mating period) to accommodate the circumstances of herds run under different conditions may produce different results. Model structure allows easy alteration of these parameters and also the introduction of different probability distributions for some variables. This might be necessary to model oestrous synchronization and artificial insemination, issues not considered in this paper.

Keywords: *beef cows, calving, mating, stochastic models.*

Introduction

There are a number of factors which determine the date that a cow in a herd of cows will calve. If a cow has just borne a calf she will have a period of sexual inactivity (anoestrus) before resuming normal oestrous cycles. During anoestrus a cow cannot conceive. When normal sexual activity resumes the cow experiences oestrus every 21 days on average until conception occurs. Following conception there is a period of gestation which averages about 283 days in cows of the British beef breeds (Angus, Hereford, Shorthorn) before birth (Preston and Willis, 1970). A deterministic model of cow reproduction based on the relationship between these variables was constructed by Denham *et al.* (1991). However, anoestrous, length of the oestrous cycle, conception and length of gestation are random variables and the effect of these random variables on calving date should be assessed utilizing their probability distributions. Because the variables determining calf birth date are random, determining optimal mating strategies requires knowledge of the probability distributions. Calculations by Pleasants *et al.* (1991) suggested that mating strategies designed on the assumption that variables acted deterministically would not be optimal.

In a managed cow herd the times when a cow can conceive are determined by the herd manager who decides when bulls will be introduced to the herd.

Because it is desirable to time calving in the herd to coincide with requirements of marketing, work rosters, or pasture availability, a restricted mating period is usually adopted. When the average gestation is 283 days mating should begin within 283 days of the time the herd manager wishes calving to begin. If annual calving is the aim, mating should begin 82 days after the planned start of calving in an annual calendar, i.e. 82 = 365 – 283.

The structure of the calving distribution influences a number of economic issues. The earlier a cow calves, the heavier the calf at marketing and the longer the cow has to recover before the next mating. Thus, it would be desirable to design a mating strategy that ensured that the probability of a cow calving early in the designated calving period was enhanced. On the other hand cows calving too early, before the designated calving period, would be a cost if labour and food requirements were unavailable to support the cow at this time. In this context, a basic problem for a herd manager is when to begin mating, taking into account the random nature of the variables affecting reproductive performance in the cow.

In this paper a probability distribution for the calving date of a herd of naturally mated beef cows is derived from knowledge about the forms of the probability distributions of the variables affecting reproduction. This puts in analytical form the results of Azzam *et al.* (1990) obtained from Monte Carlo simulation from the appropriate probability densities. Also, these authors did not consider the relationship between prior calving date and the anoestrous interval which is included in the model constructed in this paper. Having an analytical form for a calving distribution has the advantage of deriving results much faster than through simulation and it is easier to show the relative importance of the different variables operating. Results from an analytical form are not dependent on any particular realization of simulated variables.

To illustrate the application of the model the problem of deciding when to begin mating in a herd of beef cows is investigated for a herd having a range of reproductive parameters common under New Zealand pasture grazing conditions.

The model

This section outlines the construction of a calving frequency distribution for the naturally mated beef cow. Details on the mathematical construction of a general birth-date frequency distribution (which may be used as a basis to describe artificial mating and also to derive a birth-date distribution for other species, e.g. sheep) are given in **Appendix 1a.** The specific mathematical construction of a calving frequency distribution for the naturally mated beef cow is given in **Appendix 1b.**

Let $B(t)$ be the prior birth-date frequency distribution for a herd of cows, where $t = 0$ is the time at which the first cow in the herd calves. Let $G(\tau)$ be the probability density for the length of gestation i.e. the probability that gestation takes time τ. Then the probability that a cow calves at a given time will depend on (i) the probability that the prior calving occurred at a particular time, (ii) the probability of oestrus then conception occurring at a given time after calving and during the mating period, (iii) the probability of gestation having a particular length. Thus the following birth-date frequency distribution for the herd $[H(t)]$ will be the convolution of prior calving distribution with the probability distribution of oestrous occurrence during mating with the probability distribution of the length of gestation. i.e.

$$H(t) = \iint_{0\,0} B(z)C_{iw}(z-s)G(t-s)\mathrm{d}s\ \mathrm{d}z$$

where z and s are the dummy variables of integration with units of time which are the arguments for each of the probability densities involved. Each probability density is only defined for positive numbers. $C_{iw}(\tau)$ is the probability that conception in one cow occurs at time τ during mating given by **(Appendix 1a):**

$$C_{iw}(\tau) = \sum_i c_i \Big(P[O_i = \tau] - \int_{t_b}^{\tau} (1 - c_{i-1}) P[O_i = \tau \mid O_{i-1} = t] P[O_{i-1} = t] \mathrm{d}t \Big)$$

$P[O_i]$ is the probability of occurrence of the ith oestrus after calving. The probability of conception to the ith oestrus is c_i. Mating begins at time t_b and ends at time t_f.

Suppose that a herd manager plans to begin calving at time $t_0 = 0$, and that an individual cow in the herd calves at time t. Following calving this cow will experience anoestrus before resuming sexual activity. Conditional on the calving date of the cow (relative to the mean calving date of the herd) the length of anoestrus is a normally distributed random variable (Morris, 1984; Morris and Cullen, 1988). The mean anoestrus is between 50 and 80 days which depends on factors such as the nutritional status of the herd (Pleasants and Barton, 1985). But cows which calve later in the herd may have shorter anoestrus (Morris, 1984; Montgomery, 1985) with a regression coefficient of between 0 and –0·6 days per day. If oestrus occurs during mating the cow will conceive with a probability that may depend on her nutritional status (Steenkamp *et al.*, 1975). If this is

first oestrus the probability of conception may also depend on the length of the anoestrous period. First oestrus after calving has been observed to have lower conception if anoestrus is less than 60 days with a regression of 0·0005 units of probability per day (Pleasants and McCall, 1993).

If conception does not occur the cow will have an oestrous cycle, a randomly distributed normal variable with a mean of 21 days before there is another chance to conceive (Pleasants and Barton, 1992). A correlation (–0·4 to –0·7) between the length of anoestrus and the length of the succeeding oestrous cycle has been observed (Pleasants and Barton, 1992; Pleasants and McCall, 1993). Following conception there is gestation, a randomly distributed normal variable with a mean of 283 (s.e. 3) days (Preston and Willis, 1970).

Because anoestrous and oestrous cycle lengths are normally distributed random variables the probability that a cow experiences oestrus at any given time after calving is a normal distribution conditional on the prior calving date of the cow. However, the probability distribution of the unconditional time of conception for a cow, $C_{iv}(t)$, will not be a normal distribution because the non-normal prior calving distribution is also involved in the construction of this variable. That is, when the uncertainty involved in the prior calving date of the cow is taken into account the probability of the time of conception for the cow will not be a normal probability distribution. The probability distribution $P[O_i]$ giving the probability that a cow experiences ith oestrus during a planned mating period takes the form described in **Appendix 1b.**

The model presented here can be used to investigate the effects of variation in a range of reproductive parameters and also to suggest optimum mating strategies that might be adopted by a herd manager. Strategies associated with synchronized oestrus in the herd and artificial insemination can be modelled in this format once the effect of these techniques on the probability distributions of oestrus activity and conception rate are understood.

Model application

The model of a calving distribution for a naturally mated herd of beef cows constructed in the appendices is applied to the problem of determining the best date to begin mating for a herd of beef cows managed under New Zealand pasture grazing conditions. In this example the usual practice of mating for three oestrus cycles or 63 days is followed and the aim is to maintain an annual calving period in the herd while producing as much calf live weight as possible.

If the mean gestation in a herd of cows is 283 days, then in order to maintain an annual calving period it has been the practice to target the beginning of mating at 82 days after the planned start of calving, (assuming 365 days per annum). However, the following scenario is common in beef herds and warrants consideration of other practices. If the average current calving date in the herd is day 15 (i.e. 15 days after calving is planned to begin) and the average herd anoestrus is 65 days, then cows calving close to the mean calving date of the herd will show oestrus about day 80, i.e. 2 days before mating begins. These cows will need to go through a further oestrous cycle before possible conception. That is, on average, conception will not be possible until day 101. This means that these cows may have on average two chances at conception rather than the three chances given to cows which cycle soon after mating begins on day 82. It may be that better herd productivity would be achieved by beginning mating a week earlier on day 76. This would ensure that the cows clustered around the average current calving date had an early chance at conception. However, an earlier mating date may result in an unacceptable number of cows calving before the planned start of calving. This might disrupt farm management and incur unanticipated costs if food supply was insufficient for these cows. This might occur if feeding of the newly calved cow depended on the seasonal supply of pasture. In this case cows calving early, before spring pasture growth was sufficient, would have to have supplementary feeding.

A suitable mating strategy becomes more complicated when the relationship between the length of anoestrus and the prior calving date is considered. Also the relationships between the length of anoestrus and the first cycle length and anoestrous interval and conception to first oestrus. To investigate these issues the calving distribution constructed in the appendices is generated for a range of parameter values commonly found in beef cow herds.

The following parameter values are taken to construct a specific model for the calving distribution. Clearly other parameters could be adopted that described different circumstances in other herds. For example herds of some breeds may experience a longer average gestation length, or the variation in anoestrus may be different. The following parameters are commonly found in New Zealand beef herds grazing spring pasture. (1) Mating begins on day 82 and ends on day 145, measuring from day 0 as the start of the planned calving period. (2) The standard deviation of the mean length of anoestrous is 2·25 days. (3) The mean

oestrus cycle is 21 days with standard deviation of the mean of 1 day. (4) Gestation length has a mean of 283 days and a standard deviation of the mean of 3 days. (5) Correlation between the anoestrous interval and the length of the first oestrous cycle after calving is –0·4.

To investigate the response occurring over the range of circumstances in which beef cows are run the model was simulated by changing the major parameters most likely to be effected by nutrition in a factorial design. In particular: (1) base conception rate — three levels, 0·6, 0·7 and 0·8; (2) anoestrous period — four levels, 55, 65, 75 and 85 days; (3) regression coefficient between anoestrus and the current calving time of a cow in the herd — four levels, 0, –0·2, –0·4, –0·6 days per day; (4) time at which mating begins — four levels, 69, 76, 82 and 90 days after planned start of calving; (5) constant conception rate for each oestrus during mating, or a conception rate of 0·3 on the first oestrus after calving if this oestrus occurs during mating and follows an anoestrous period of less than 55 days. After 55 days of anoestrous the conception rate to first oestrus increases at 0·03 units per day until the base conception rate is reached.

To assess these effects prior calving distributions were constructed by randomly sampling from the calving distributions of herds of Angus and Hereford × Friesian cows described in Pleasants and McCall (1993), (six herds having 68, 149, 365, 329, 219 and 267 cows) and Angus cows described in Pleasants and Barton (1992) (two herds having 87 and 121 cows). Random samples (with replacement) of calving dates were chosen from recorded calving dates for these herds where the sample size was equal to the number of cows in the herd of interest. Repeated samples were used to assess the response to changes in average herd conception rate, expected calf live weight at day 200 and the proportion of cows calving more than 5 days before the planned calving date (the outcome variables). Thus eight samples, one sequence of sampling for each herd, were used for each combination of conception rates, anoestrous periods, regression coefficients, initial mating dates and conception rates following reduced anoestrus. The expected calf live weight was calculated by assuming a calf live-weight gain of 0·9 kg/day over the period, i.e. a weighted expectation plus an expected calf birth weight of 30 kg:

$$\text{expected calf live weight at 200 days} = \int_0^{200} 0{\cdot}9(200 - t)\, H(t)\, \mathrm{d}t + 30.$$

Sampling over different herds enabled the calculation of a lower limit on variation in the response variables due to variation in the form of the prior calving distribution.

Calculations of the probabilities for the normal and conditional normal probability distributions required for the calculations was carried out using the algorithm of Schervish (1984). This algorithm calculates the quadrature of a multivariate normal distribution with given covariance between given limits.

In assessing mating strategies the situation is often that the prior calving distribution of the herd is known before decisions on mating dates are made. Thus, the known prior calving distribution can be entered into the model equations. To facilitate calculations a polynomial regression can be calculated between the outcome variables generated by the model and each date (*cp*) of the prior calving probability distribution. Changes in the outcome variables due to changes in other model parameters such as the initial mating date can be included as other independent variables in the regression. From this equation the response of a cow with a known prior calving date can be predicted.

This result is generalized to a herd with a known prior calving distribution by calculating the expected value of the outcome variables for each prior calving date over the prior calving frequency distribution.

Results

The outcome variables (calf live weight at day 200, herd conception rate and proportion of cows calving early) calculated for the model parameters (conception rate to each oestrus, anoestrous intervals, and different initial mating dates) using samples from actual prior calving distributions are shown in Table 1. As expected, better conception rates and lower anoestrous intervals give better production. There was no effect of different regression coefficients of anoestrous interval on the current calving time and neither was there any effect due to a reduced conception rate following a shorter anoestrous period. That is, variation in the prior calving distribution, which includes variation in the mean prior calving date, made these effects unimportant. Starting mating earlier than the traditional time of 82 days after the planned start of calving increases expected calf live weight and herd conception rate. It also leads to an increasing proportion of cows calving earlier in the herd.

Calculations for assessing mating strategies

Equations for the average calf live weight in the herd at day 200 (*cw*), herd conception rate (*hc*) and the proportion of cows calving in the herd *t* days before

Table 1 *Least-squares means and standard errors for the effect of conception rate, anoestrous interval and mating date on model output for average herd calf weight at day 200, conception rate and the proportion of cows calving early averaged over eight different example herds*

	Average calf weight at day 200		Proportion of cows in herd calving more than 5 days early × 100		Herd conception rate × 100	
Effect	LSM	s.e.	LSM	s.e.	LSM	s.e.
Conception rate						
0·6	137·4[a]	0·43	7·0	0·8	93·1[a]	0·1
0·7	142·7[b]	0·43	7·0	0·8	96·8[b]	0·1
0·8	145·9[c]	0·43	7·0	0·8	98·9[c]	0·1
Anoestrous interval (days)						
55	152·6[a]	0·50	23·0[a]	1·0	97·6[a]	0·1
65	147·1[b]	0·50	4·6[b]	1·0	96·9[b]	0·1
75	139·9[c]	0·50	0·3[c]	1·0	96·5[c]	0·1
85	128·4[d]	0·50	0·0[c]	1·0	94·2[d]	0·1
Initial mating date (day)						
62	145·2[a]	0·61	12·3[a]	1·2	96·9[a]	0·1
69	144·8[ab]	0·61	10·7[ab]	1·2	96·8[ab]	0·1
76	143·3[b]	0·61	5·2[c]	1·2	96·5[b]	0·1
82	139·2[c]	0·61	0·5[d]	1·2	96·0[c]	0·1
90	134·1[d]	0·61	0·0[d]	1·2	94·7[d]	0·1

[a,b,c,d] Variables with different superscripts are significantly different ($P < 0·05$) for each dependent variable within each independent variable.

the planned start of calving (early(t)), were constructed by fitting regressions to the model outputs generated by changing the initial mating date (s) as in Table 1 and the prior calving date (cp). Here s and cp are independent variables. A range of prior calving dates from 0 to 60 were used.

For this exercise a herd with anoestrus of 70 days, and a conception rate to each oestrus of 0·7 was assumed. This avoids complex regression equations but also a farmer knowing the expected performance of a herd in a familiar environment might also fix these variables with confidence. However, as many model parameters as necessary could be treated as variables at the expense of cumbersome regression equations.

With these assumptions the relationships are:

$$cw(s,cp) = 60·471 - 0·347s - 0·954cp + 0·005cp^2 + 0·011cd.s - 0·00009cp^2s$$

$$hc(s,cp) = 1·042 - 0·001s - 0·0009cp - 0·00004cp^2$$

$$\text{early}(t,s,cp) = 0·0984 - 0·0028t + 0·000046t^2 - 0·00078s - 0·002cp$$

where $cw(s,cp)$ is the expected calf live weight in the herd at day 200, $hc(s,cp)$ is the conception rate of the herd and early(t,s,cp) is the proportion of cows in the herd calving t days before the planned start of calving in the herd.

From these equations the predicted outcome for a cow having a particular initial mating date and prior calving date can be calculated. To calculate results for a herd with an initial mating date of s this outcome is multiplied by the proportion of the herd having a prior calving date of cp then summed over all prior calving dates in the herd, i.e. an expectation of the outcome variables over the (known) prior calving distribution.

The above equations show that calf live weight and herd conception rates increase (at a decreasing rate) the earlier mating begins in the herd. There are several ways such a decision upon the value of earlier mating in the herd may be made.

For example, if the initial mating date was to be chosen so that it was expected that, for example, not more than 0·052 of the herd would calve more than 5 days before the planned start of calving, then the start of mating is chosen to satisfy:

$$\sum_{cp} \text{early}(5,s,cp)B(cp) \quad 0·052$$

where $B(cp)$ is the known frequency distribution of the herd prior calving dates. This equation is solved to find the initial mating date s. Using the average prior calving frequency distribution of the eight herds above, this equation is satisfied if mating begins at least 76 days after calving for the herds described above.

Alternatively, if the goal was to maximize production in the herd where calf live weight was worth $\$p$ per kg, and cows remaining unmated at the end of the mating period cost $\$q$ each and calving t days before the planned start of mating cost $\$r(t)$, then production would be maximized by choosing the start mating time so that:

$$\sum_{cp}\left([p.cw(s,cp) - q(1 - hc(s,cp)) - \sum_{t} r(t) \text{ early}(t,s,cp)]\, B(cp)\right) \quad (1)$$

is maximized. In this equation early(t,s,cp) refers to the proportion of cows calving early between times t and t–1 before the planned start of calving in the herd. Reasonable New Zealand values are p = \$1·50 per kg live weight for calves, unmated cows cost q = \$150 each, and the cost of calving before the planned start of mating is \$5 per early calving cow per day making $r(t) = 5t$. The advantage per cow generated by mating cows earlier than 82 days after the planned start of calving is given in Table 2 for the herds described above. Under these assumptions there appears to be an economic advantage in mating cows early. Thus if mating began in the herds considered on day 76 the above constraint would be satisfied and a benefit of about \$5 per cow would ensue (Table 2).

Discussion

Under the circumstances considered in this study, the example calving distributions used suggest that under many circumstances beginning mating earlier confers an advantage. The gain of about \$5 per cow for starting mating at day 76 shown in Table 2 may be worthwhile. If the cost of unmated cows or the value of calf live weight increased the benefit of early mating would increase. Similarly if the cost of early calving cows increased the benefit would decrease. Clearly the advantages and disadvantages of different strategies will depend on the circumstances of a particular herd. This will include both financial considerations and modifications of the parameters in the model due to management or nutritional effects.

Table 2 *Means and standard errors for the response to changing the time mating begins over eight herds given herd average anoestrous of 70 days and conception rate 0·7, and the expected benefit of doing so as calculated by equation (1) (Responses are expressed as deviations from beginning mating 82 days after the planned start of calving in the herd)*

Begin mating time (days after planned start of calving in the herd)	Benefit (\$ per cow)	
	Mean	s.e.
62	6·05	1·67
69	5·85	1·67
76	5·08	1·67
82	0	1·67
90	–9·60	1·67

The parameters in the model which are fixed and those which are variable may differ depending on the application. For example, in environments which are more variable the herd manager may be less certain about the values of some parameters and would wish to know how the response changed as these parameters changed. In some circumstances the variation in response will be of interest and this can be calculated by simulating the model a number of times while sampling from appropriate probability distributions defining the parameters of interest, e.g. the standard deviations of anoestrus and oestrous cycle length.

The model may be operated iteratively to investigate mating strategies over a number of years. For example, a consistent policy of early mating will result in a different herd prior calving distribution, perhaps resulting in an unacceptable number of early calving cows after several years of operation. In this case a policy of alternating early and late mating in alternate years may be most economical and could be investigated by using the output of the model to produce a prior calving distribution for the next year. This option would require synchronization with other livestock activity on a farm dependent on seasonal pasture supply. The results presented here are intended to illustrate the potential of this model to guide decision making by herd managers, not to draw general conclusions about herd management. The most powerful use of this model is to apply it to particular circumstances as the basis of a decision support computer program.

The insignificant response to the strength of the regression of anoestrous interval on current calving date is expected. This variable has the greatest effect on cows calving late, shortening the anoestrous intervals of these cows. But even with shorter anoestrous intervals these late calving cows would probably not experience oestrus in time for early mating to have much effect.

The reduced conception rate for cows experiencing first oestrus during mating after an anoestrous interval of less than 60 days has no measurable effect

on any of the responses. This is because so few cows in the herds considered are effected.

The birth-date distribution developed in the appendices is general. By modifying the probability distributions, birth-date distributions for artificial breeding and for other species such as sheep and goats can be constructed. In this case the probability distribution of the first oestrous cycle of mating would most likely be uniform over the length of the oestrous cycle of the species of interest. There are also a number of scenarios of mating strategy which can be examined with the model developed here. Investigation of an optimum time to finish mating in the herd is an obvious extension. Using the model as a basis for investigating optimal management strategies as in DeLorenzo *et al.* (1992), or Jalvingh *et al.* (1993) is another possibility. Artificial intervention in a cow's reproductive cycle with drugs could also be examined. It would be expected that such intervention would change the nature of the probability distributions and the base model can accommodate such changes, though the computational burden may be high if other than normal probability distributions occur.

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Appendix 1a

Mathematical formulation of a general birthdate distribution

Let the time that calving is to commence within a year be $t = 0$. Let mating begin at time t_b and end at time t_f. Thus, the mating period is the interval $[t_b, t_f]$. Let O_i be the event of the ith oestrous cycle after calving. Let $P[O_i]$ be the probability that the ith oestrous cycle after calving occurs within the mating period. Then the probability that the cow shows oestrus in the mating period is:

$$P[\text{oestrus}] = P[\bigcup_{i=1} O_i].$$

Now:

$$P[\bigcup_{i=1} O_i] = P[O_1] + P[O_2] + \ldots - P[O_1 \cap O_2] - P[O_1 \cap O_2] + P[O_1 \cap O_2 \cap O_3] + \ldots$$

where $P[O_1 \cap O_2]$ is the probability that both first and second oestruses after calving occur during the defined period.

Because the occurrence of the first and third oestruses after calving in the mating period imply the existence of the

second oestrus after calving in the mating period we have:

$$O_1 \cap O_3 = O_1 \cap O_2 \cap O_3$$

and:

$$O_1 \cap O_4 = O_1 \cap O_3 \cap O_4 = O_1 \cap O_2 \cap O_3 \cap O_4$$

etc. Thus cancellation among the intersections occurs and:

$$P[\cup_{i=1} O_i] = \sum_{i=1} \left(P[O_i] - P[O_i] \cap P[O_{i-1}] \right) \quad (1)$$

where for generality we note that $P[O_0]$ is an empty set, therefore any intersection of this set will also be empty.

Since a cow must have first oestrus after mating begins before having second oestrous after mating begins, and assuming oestrous cycle lengths are independent random variables, then if conception is not possible, if for example, there is no bull present:

$$P[O_1 \cap O_2] = \int_{t_b}^{t_f} \int_{t_b}^{u} P[O_2 = u \mid O_1 = t] P[O_1 = t] \mathrm{d}t \mathrm{d}u$$

If conception can occur to the ith oestrus after birth with probability c_i ($0 \quad c_i \quad 1$), then the cow will not conceive with probability $(1 - c_i)$. Thus the probability of having first and second oestrus during the mating period is:

$$P[O_1 \cap O_2] = (1 - c_1) \int_{t_b}^{t_f} \int_{t_b}^{u} P[O_2 = u \mid O_1 = t] P[O_1 = t] \mathrm{d}t \mathrm{d}u \quad (2).$$

Combining (1) and (2) the probability that a cow will conceive during the mating period

$$\sum_i c_i \left(\int_{t_b}^{t_f} P[O_i = \tau] - \left(\int_{t_b}^{\tau} (1 - c_{i-1}) P[O_i = \tau \mid O_{i-1} = t] P[O_{i-1} = t] \mathrm{d}t \right) \mathrm{d}\tau \right).$$

For the probability that a cow will conceive at a particular time τ during the mating period this equation becomes:

$$C_{\bar{w}}(\tau) = \sum_i c_i \left(P[O_i = \tau] - \int_{t_b}^{\tau} (1 - c_{i-1}) P[O_i = \tau \mid O_{i-1} = t] P[O_{i-1} = t] \mathrm{d}t \right) \quad (3)$$

where again $P[O_0]$ is defined as a null set.

If the length of gestation is independent of the time of conception, then noting that the birth date is the sum of the conception date and the length of gestation, the birth date probability distribution for one cow will be given by the mathematical convolution of the two density functions (Freund and Walpole, 1980). Let the probability density of the length of gestation be $G(t)$. Then the probability density of the birth date for a single cow $Y(t)$ with a given prior calving date is:

$$Y(t) = \int_0 C_{\bar{w}}(s)\, G(t - s)\, \mathrm{d}s$$

where the range of the probability densities for time of conception and gestation range over the positive real numbers.

The birth date distribution for a herd of cows depends on the structure of the prior calving frequency distribution. This is because the time within the herd on which a cow calves effects the probability of expressing oestrus during mating. For example late calving cows may not return to oestrus until later in mating **(Appendix 1b)**. Thus, the birth date of a cow is the sum of the prior calving date plus the time to conception plus the length of gestation. Accounting for the dependence between prior calving date and conception date in the conception time probability distribution $C_{\bar{w}}(t)$, and with prior calving frequency distribution $B(t)$, the birth date probability distribution is:

$$H(t) = \int_0 Y(s)\, B(t - s)\, \mathrm{d}s = \int_0^t \int_0^t C_{\bar{w}}(z)\, G(s - z)\, B(t - s)\, \mathrm{d}z\, \mathrm{d}s \quad (4)$$

where s and z are dummy variables of integration over time.

Appendix 1b

Construction of calving distribution for the naturally mated cow

In beef cows suckling calves the following has been observed.

(1) Anoestrous time after calving for a cow (p) is a normally distributed random variable linearly dependent on the prior calving date with standard deviation σ_p. Given average herd anoestrous time $\bar{p}$, average prior calving date for the herd $\bar{cp}$, the cow prior calving date cp, and regression coefficient between anoestrous time and calving date b:

$$p = \bar{p} + b(cp - \bar{cp})$$

(2) The length of the oestrous cycles after anoestrus are normally distributed random variables with a mean μ_c, and standard deviation σ_c. Succeeding oestrous cycle lengths are independent, though if cows lose live weight during anoestrus a longer interval between first and second oestrus may occur (Pleasants and Barton, 1992). If necessary this can also be described by a regression equation.

(3) The probability of conception to the second and succeeding oestruses after calving is a constant. However, when the first oestrus after calving occurs during mating the longer the anoestrus the better the conception when anoestrus is greater than about 55 days (Macmillan and Clayton, 1980; Pleasants and Barton, 1992; Pleasants and McCall, 1993). For anoestrous periods of 55 days or less conception is about 0·25, with an increase of 0·005 for each extra day of anoestrus. This result may be associated with the nutritional state of the cow.

Based on these observations specific probability distributions for a model of cow reproduction can be constructed.

The probability that first oestrus occurs within the defined mating time after calving given a prior calving date cp is:

$$P[O_1] = \int_{t_b}^{t_f} \phi[\bar{p} + b(cp - \bar{cp}), \sigma_p^2] \mathrm{d}x$$

where $\varphi(\mu,\sigma^2)$ is notation for a normal probability distribution with mean, μ and variance σ^2.

$$\phi(\mu,\sigma^2;y) = \frac{1}{2\,\pi\sigma^2}\, e^{-1/2((y-\mu)/\sigma)^2} \quad (5).$$

Similarly the probability of the nth($n > 1$) oestrus after prior calving occurring during mating is:

$$P[O_{in}] = \int_{t_{lb}}^{t_{ub}} \phi[p + \mu_c(n-1), \sigma_p^2 + \sigma_c^2(n-1) + 2\sigma_{pc}; y]dy$$

where μ_c is the average length of an oestrous cycle, σ_{pc} is the covariance between anoestrus and first oestrus cycle length and y is a possible time of oestrus as given in equation (5).

Succeeding oestrous cycles lengths are assumed to be independent of each other and the post-partum anoestrus.

The probability of having both oestrus i and $i + 1$ during mating is the probability of having oestrus i multiplied by the probability of having an oestrous cycle which occurs before mating finishes.

$$P[O_i \cap O_{i-1}] = \int_{t_{lb}}^{t_{ub}} \int_{t_{lb}}^{y} (1 - c_i)\phi[x + \mu_c, \sigma_c^2; y]\phi[p + (i-1)\mu_c, \sigma_p^2 + (i-1)\sigma_c^2 + 2\sigma_{pc}; x]dxdy$$

where x is the time of occurrence of oestrus i and y is the time of occurrence of oestrus $i + 1$ i.e. y is variable in the first normal distribution and x is variable in the second normal distribution in the above equation. The factor $(1 - c_i)$ is included to account for the probability of conceiving at oestrus i and therefore not having further oestrous activity.

These equations are substituted into equations (3) and (4) above to give a calving distribution for a herd of cows.

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An evaluation of the Gompertz model in degradability studies of forage chemical components

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Abstract

The in situ *dry matter (DM) and neutral-detergent fibre (NDF) degradability kinetics of eight forages (four grass hays and four legume hays, harvested at two different dates) were compared to assess the fitting ability of a first-order and a Gompertz model.*

The Gompertz model fitted DM degradability data as well as the first-order model and differences between fitted and observed data for the two models were very small but the Gompertz model proved to be statistically superior for the NDF degradability data, especially for the early hours of incubation.

A numerical but not significant difference was observed in the estimated rapidly available fraction for DM and NDF, which was respectively lower (mean values 24·4 v. 27·8%) and higher (mean values 5·8 v. 1·8%) with the first-order model. More pronounced differences were observed for the estimates of total potential degradability of NDF, which were often significantly lower with the Gompertz model (average values for the eight forages 75·1 v. 71·3%).

The sigmoidal shape of the Gompertz model was more biologically appropriate to describe the initial phases of NDF degradation and was thus applied to the cellulose and hemicellulose degradability data.

As the harvesting date progressed through the season, a decrease of the immediately available fraction of DM and nitrogen was generally observed but the effect of harvesting date was not so evident for fibre fractions; the differences within forages were very low. Correlation coefficients between lignin content and total potential degradability of fibre were always high (for NDF, r = –0·96*; for hemicellulose* r = –0·95*; for cellulose* r = –0·79*;* P < 0·001*), while the acid-detergent fibre content influenced DM and nitrogen total potential degradability* (r = –0·91 *and* –0·82*, respectively).*

Keywords: *fibre, mathematical models, nitrogen, rumen degradability.*

Introduction

A great deal of work has been performed in the past to characterize the rumen degradability of foodstuffs for ruminant animals with an increasing interest in forages, cell walls and their associated components, frequently estimated with the Goering and Van Soest (1970) procedure.

Rumen degradability data are often fitted with the first-order model of Ørskov and McDonald (1979), a model which frequently produces negative estimates for the immediately available fraction (*a*), especially for low quality foods and fibre fractions. Negative estimates for *a*, which represent delayed availability of potentially degradable substrates at the start of incubation, can be corrected by the introduction of a discrete lag phase (McDonald, 1981). In contrast, the Gompertz model (Bidlack and Buxton, 1992) does not allow negative estimates for the immediately available fraction and is considered to be more biologically appropriate to describe the rumen degradation of foods (Sauvant *et al.*, 1985; Van Milgen and Baumont, 1995).

The first objective of the present research was to compare the fitting ability of two models for the degradability data of chemical components, i.e. the first-order model of Ørskov and McDonald (1979) and a Gompertz model (Bidlack and Buxton, 1992), considering both statistical and biological aspects. The second aim of the paper was to apply the most appropriate model to study the degradability

parameters of the chemical components within forages in relation to the harvesting date.

Material and methods

Animals and feeding

Three fistulated Italian Simmental cows, weighing on average 702 (±60) kg and fitted with permanent rumen cannulas were used. Cows were given 6 kg tall fescue hay and 2 kg compound food (Electa Udine — Italy, commercial denomination B54; 180 g crude protein (CP) per kg) in two equal meals (3 kg hay + 1 kg compound food) at 07.30 and 16.30 h, with additional minerals (50 g) once a day. The diet was calculated to cover the energy and protein requirements of maintenance (Institut National de la Recherche Agronomique, 1988) and to balance the energy-to-protein ratio in the rumen (Commissione proteine nella nutrizione e nella alimentazione dei poligastrici (CPNAP), 1994). The animals were given the diet 14 days before the start of the *in situ* studies.

Forages and rumen incubation

The forage species studied were tall fescue (TFE; *Festuca arrundinacea*), Italian ryegrass (IRG; *Lolium multiflorum*), lucerne (LUC; *Medicago satiga*) and red clover (RCL; *Trifolium pratense*). Each forage was harvested at two dates, at the end of March (III) and at the beginning of May (V), except for the last harvest of RCL which occurred at the end of April (IV). At both harvesting dates, the forages were still in vegetative stages of growth. Immediately after harvesting, forages were dried at 60°C in a forced air oven and stored in a dark room at room temperature until used.

For the *in situ* degradability study, forages were ground with a hammer mill (Retsch, Germany) with a mesh of 4 mm and weighed (5 (±0·2) g dry matter) into duplicate polyester bags (9·5 × 15·5 cm closed dimensions; pore size 40 μm) giving an average weight-to-surface ratio of 17·0 mg dry matter (DM) per cm^2 (CPNAP, 1994). Each forage was incubated for 2, 4, 8, 16, 24, 48 and 72 h, with all the bags, except those for 16-h incubations, being introduced immediately before the morning meal; the 16-h bags were introduced immediately before the afternoon meal. After rumen incubation, the bags were washed in a washing machine with a cold water cycle (15 min) and dried in an oven for 48 h at 60°C, left at room temperature to re-equilibrate and weighed. The same procedure was used to determine washing losses (a) of the food samples.

Chemical analysis

Food samples and pooled (by cow and time of incubation) food residues after incubation were ground to pass a 1-mm screen in a Cyclotec mill (Tecator, Sweden) and analysed for DM, ash, CP, neutral-detergent fibre (NDF), acid-detergent fibre (ADF) and acid-detergent lignin (ADL). DM was determined by drying the samples at 105°C for 3 h. Ash was determined by ashing at 525°C and was measured only on food samples. Nitrogen (N) was determined with the Kjeldahl procedure and CP calculated by multiplying N content by 6·25 (Association of Official Analytical Chemists, 1990). The NDF, ADF and ADL were determined sequentially according to Goering and Van Soest (1970). Hemicellulose (HCELL) and cellulose (CELL) were determined by the difference between NDF and ADF and ADF and ADL, respectively. The chemical composition of the forages is presented in Table 1.

Calculations and statistical analyses

The degradability data for each time of incubation for DM and NDF were fitted with the exponential model of Ørskov and McDonald (1979):

$$y = a + b\,(1 - e^{-ct}) \qquad (1)$$

Table 1 *Chemical composition of hays (expressed in g/kg dry matter)*

	Tall fescue		Italian ryegrass		Lucerne		Red clover	
Maturity	III†	V	III	V	III	V	III	IV
Dry matter (DM) (g/kg forage)	888	907	867	887	842	864	870	882
Organic matter	893	926	886	923	854	892	861	899
Crude protein (CP)	195	78	145	68	359	185	297	235
Neutral-detergent fibre (NDF)	571	681	330	421	291	428	247	275
Acid-detergent fibre (ADF)	286	394	171	229	202	317	172	178
Acid-detergent lignin (ADL)	14	40	5	11	38	66	30	23
Hemicellulose (HCELL)	281	283	158	192	83	112	75	98
Cellulose (CELL)	272	354	166	219	163	251	142	155

† III: forages harvested in the last 10 days of March; V: forages harvested in the first 10 days of May; IV: red clover harvested in the last 10 days of April.

where: y is the fraction degraded at time t, a is the immediately available fraction; b is the potentially degradable fraction and c is the constant rate of degradation of b. A graphical appraisal of NDF degradability data revealed the presence of a lag phase, which was calculated according to the modified equation proposed by McDonald (1981):

$$L = \frac{1}{c} \cdot \ln \frac{b}{a + b - a'} \quad (2)$$

where the parameters a, b and c are the same as in equation (1) and L is the lag phase for the beginning of the degradation. The expression $(a + b - a')$ is the potentially degradable fraction, b'. The a' is the washing loss value and was only used for the determination of the lag time of NDF degradability. The washing losses were not considered for the calculation of the DM degradability because, according to Cockburn *et al.* (1993), physical losses of food particles from the bags occur during the washing machine procedure, leading to a great overestimation of DM solubility and an inflation of the estimates of the extent of degradation.

DM, CP, NDF, CELL and HCELL degradability data for the various times of incubation for each cow and forage were also fitted with the Gompertz model (Bidlack and Buxton, 1992), without considering the washing losses:

$$y = Be^{-Ce^{-At}} \quad (3)$$

where: y is the fraction degraded at time t, B is the asymptotic value of the component (total potentially degradable fraction) and C is the relative degradation rate as affected by a constant factor of microbial efficiency (A).

Curve fitting and parameter values for both the models were estimated by the Marquardt compromise of a non-linear regression method, using PROC NLIN of the SAS software (Statistical Analysis Systems Institute (SAS), 1988)

The goodness of fit for the degradability data was evaluated in terms of coefficient of determination (r^2) and residual standard error.

The comparison of model parameters was only performed for: (1) the disappearance of chemical components at time zero, estimated by a (DM) and washing losses (NDF) in the first-order models and calculated by setting t equal to zero in the Gompertz model (immediately available fraction (IAF)); (2) total potential degradability, estimated by $a + b$ and B in the first-order and Gompertz models, respectively.

The effect of maturity on the Gompertz model degradability parameters of each chemical component within forage species was estimated with the Student T test (PROC TTEST; SAS, 1988).

Results

Mathematical evaluation of the DM and NDF curves

The values of the kinetic parameters for the first-order model (a, b, c, $a + b$ and a' and b', equations 1 and 2) and the Gompertz model (A, B, C and IAF, equation 3) are reported in Tables 2 and 3, together

Table 2 *Dry-matter degradability kinetic parameters estimated with the first-order model of Ørskov and McDonald (1979) and with the Gompertz model (Bidlack and Buxton, 1992)*

	Ørskov and McDonald model						Gompertz model					
Forage†	a	b	c	$a+b$	Residual s.e.	R^2	IAF‡	B	C	A	Residual s.e.	R^2
TFE-III	16·0	72·2	0·065	88·2	0·59	0·995	21·2	86·8	1·41	0·092	0·48	0·994
TFE-V	16·5	50·4	0·048	66·9	0·32	0·996	18·4 *	65·2 ***	1·26 **	0·070 *	0·30	0·997
IRG-III	36·1	58·9	0·127	95·0	0·44	0·993	43·0	95·2	0·80	0·125	0·40	0·989
IRG-V	31·3	57·7	0·088	89·0	0·36	0·995	33·2 *	88·3 ***	0·98	0·114	0·38	0·994
LUC-III	22·2	62·7	0·113	84·9	0·68	0·984	26·0	84·5	1·18	0·147	0·62	0·985
LUC-V	21·1	49·7	0·084	71·0	0·83	0·968	22·1	70·8 ***	1·17	0·107 *	0·77	0·985
RCL-III	27·1	61·3	0·106	88·4	0·65	0·987	30·5	88·6	1·07	0·136	0·54	0·982
RCL-IV	25·1	67·4	0·095	92·5	0·79	0·984	28·3	92·1 ***	1·18	0·126	0·71	0·981

† TFE, IRG, LUC and RCL denote tall fescue, Italian ryegrass, lucerne and red clover, respectively; III, V and IV refer to harvest date (March, May and April) — see footnote to Table 1.
‡ IAF = calculated immediately available fraction.

Table 3 *Neutral-detergent fibre degradability kinetic parameters estimated with the first-order model of Ørskov and McDonald (1979) and with the Gompertz model (Bidlack and Buxton, 1992)*

	Ørskov and McDonald model							Gompertz model					
Forage†	a'	b'	c'	$a'+b'$	lag	Residual s.e.	R^2	IAF‡	B	C	A	Residual s.e.	R^2
TFE-III	4·6	82·6	0·062	87·2	2·6	0·66	0·995	2·7	83·4	3·43	0·119	0·72	0·993
TFE-V	1·2	61·0	0·043	62·2	1·0	0·53	0·996	1·2 **	55·7 ***	3·92	0·104	0·60	0·990
IRG-III	7·7	83·6	0·111	91·3	3·3	1·48	0·981	0·7	88·7	5·49	0·204	1·02	0·993
IRG-V	2·1	80·6	0·072	82·7	2·8	0·72	0·994	2·8	80·2 **	3·57	0·124 *	1·29	0·985
LUC-III	9·9	60·8	0·059	70·7	4·8	1·39	0·965	2·0	67·6	4·17	0·138	1·17	0·967
LUC-V	1·8	47·0	0·056	48·8	3·1	1·31	0·940	0·2	44·9 **	6·20	0·128	1·13	0·992
RCL-III	8·4	63·7	0·079	72·1	4·1	1·23	0·979	3·3	69·5	3·14	0·120	1·00	0·992
RCL-IV	10·4	75·3	0·066	85·7	5·6	1·72	0·969	1·4	80·5 ***	4·10 *	0·145	1·51	0·993

†‡ See Table 2.

with the coefficients of determination (R^2) and residual standard errors.

The comparison of the statistical parameters obtained with the two models did not show consistent differences for DM degradability in the eight forages; for NDF degradability, the first-order model fitted the data better for grasses than for legumes. In both models, the coefficients of determination for DM and NDF were always higher than 0·940.

The shape of the curves produced by both models for DM were very similar (reported in Figure 1 for IRG-III) and allowed the calculation of very similar total potential degradability values for DM ($a + b$ and B for the first-order and Gompertz model, respectively, Table 2). Significant differences for total potential degradability estimated with the two models were only observed for TFE ($P < 0·05$). Higher absolute differences between models (a and IAF, for first-order and Gompertz model, respectively) were observed for the immediately available fractions of DM, although these were significant only for LUC-III ($P < 0·05$). For TFE and IRG-III, the differences were only significant at $P < 0·06$. However, the estimated soluble fractions for the Gompertz model were always numerically higher.

The NDF degradation kinetics differed more between models than DM; as an example, LUC-V NDF degradability data and the curves obtained with both models are presented in Figure 2. The calculated total potential degradability of NDF was always lower with the Gompertz model and the values were significantly lower for TFE ($P < 0·01$),

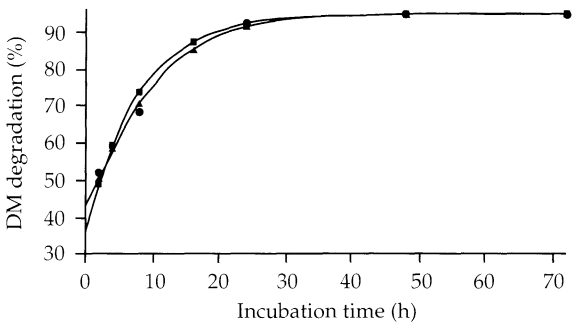

Figure 1 Observed degradability (●) and interpolated degradation curves for dry matter (DM) of Italian ryegrass cut in March fitted with the first-order model of Ørskov and McDonald (——■——) and with the Gompertz model (——▲——).

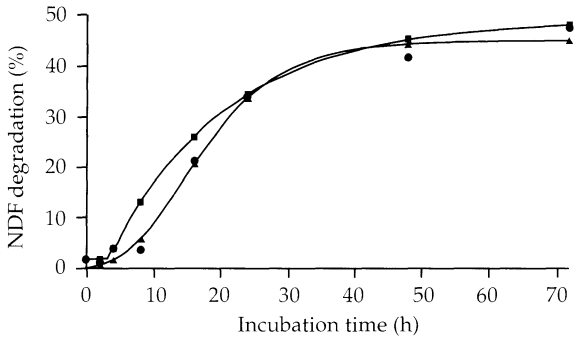

Figure 2 Observed degradability (●) and interpolated degradation curves for neutral-detergent fibre (NDF) of lucerne cut in May (LUC-V) fitted with the first-order model with a discrete lag phase of Ørskov and McDonald (——■——) and with the Gompertz model (——▲——).

IRG-V ($P < 0.05$) and RCL-IV ($P < 0.05$). Higher IAFs of NDF were estimated with the first-order model (from 1·2 to 10·4%), apart from TFE-V, but none of the differences was significant. With the Gompertz model, the variation of IAF values between forages was numerically small (0·2 to 2·8%).

In order to evaluate further the models, a regression between predicted and observed degradability values was calculated at each incubation time within each model (Table 4). The accuracy of the mathematical fitting increased with time of incubation for both models and was not very different between models for DM, but for NDF degradability the Gompertz model proved to be superior until 16 h; at 24, 48 and 72 h of incubation, the accuracy of the mathematical fit for the two models was very similar.

Degradability of chemical components with the Gompertz model

Once the suitability of the Gompertz model to fit the degradability of different chemical constituents (DM and NDF) had been demonstrated, the model was also applied to describe the degradability data for CP, CELL and HCELL. The curve fitting parameters for these chemical components are presented in Tables 5 and 6. The coefficient of determination for the CP degradability kinetics of the forages ranged from 0·985 to 0·996 and the residual standard error was always lower than 1·00, ranging from 0·36 in TFE-V to 0·75 in RCL-IV. For CELL and HCELL, the R^2 values ranged between 0·970 and 0·997 and from 0·964 and 0·993, respectively; these two fibrous fractions had slightly higher residual standard errors than those of CP, falling between 0·55 and 1·41 and 0·59 and 1·63, respectively.

The calculated IAFs for the DM (Table 2) in legumes ranged from 22·1% in LUC-V to 30·5% in RCL-III and

Table 4 *Relationship between the quality of fit of two models and the observed values*

Incubation time (h)	Model†	Dry matter R^2	Dry matter Residual s.e.	Neutral-detergent fibre R^2	Neutral-detergent fibre Residual s.e.
2	EXP	0·906	0·85	0·054	0·63
	GOM	0·893	0·91	0·495	0·50
4	EXP	0·967	0·67	0·038	1·22
	GOM	0·962	0·72	0·572	0·81
8	EXP	0·980	0·69	0·862	1·32
	GOM	0·991	0·46	0·953	0·78
16	EXP	0·994	0·42	0·965	1·09
	GOM	0·994	0·41	0·993	0·48
24	EXP	0·999	0·08	0·983	0·83
	GOM	0·998	0·23	0·991	0·58
48	EXP	0·999	0·15	0·996	0·37
	GOM	0·999	0·11	0·998	0·29
72	EXP	0·998	0·18	0·995	0·40
	GOM	0·998	0·18	0·997	0·33

† EXP = exponential (first-order) model; GOM = Gompertz model.

Table 5 *Crude protein degradability kinetic parameters and effect of harvesting date*

Forage†	IAF‡	*B*	*C*	*A*	Residual s.e.	R^2
TFE-III	30·0	89·1	0·96	0·109	0·53	0·991
TFE-V	32·4	63·6	0·67	0·088	0·36	0·987
		***	***	*		
IRG-III	31·2	95·3	1·12	0·153	0·40	0·996
IRG-V	6·0	79·6	2·64	0·230	0·68	0·992
	***	***	**	**		
LUC-III	40·3	93·4	0·84	0·153	0·45	0·991
LUC-V	31·0	88·0	1·04	0·122	0·67	0·985
	**	***	*	*		
RCL-III	37·6	93·5	0·91	0·153	0·56	0·989
RCL-IV	28·6	94·3	1·19	0·133	0·75	0·986
	**	*	**			

†‡ See Table 2.

Table 6 *Cellulose and hemicellulose degradability kinetic parameters and effect of harvesting date*

Forage†	IAF‡	*B*	*C*	*A*	Residual s.e.	R^2
Cellulose						
TFE-III	0·4	84·7	5·49	0·131	0·65	0·996
TFE-V	0·5	52·8	4·72	0·119	0·84	0·985
		***				
IRG-III	0·3	92·1	5·91	0·205	1·41	0·987
IRG-V	1·0	83·8	4·50	0·138	1·12	0·987
	*	***	**	***		
LUC-III	4·0	79·5	3·01	0·127	0·98	0·989
LUC-V	0·6	59·5	4·69	0·129	1·10	0·970
	**	***	**			
RCL-III	4·2	82·5	2·97	0·147	0·55	0·997
RCL-IV	1·0	89·7	4·51	0·169	1·23	0·987
	***	***	**	*		
Hemicellulose						
TFE-III	4·5	83·7	2·92	0·113	0·70	0·993
TFE-V	4·0	58·7	2·70	0·094	0·59	0·991
		***		*		
IRG-III	2·9	89·9	3·51	0·185	1·14	0·986
IRG-V	4·2	78·9	2·96	0·130	1·63	0·966
		***		**		
LUC-III	6·6	69·5	2·37	0·109	1·26	0·970
LUC-V	0·9	49·1	4·04	0·094	1·03	0·964
	*	***	***	**		
RCL-III	2·1	71·1	3·52	0·123	1·17	0·978
RCL-IV	2·5	82·2	3·52	0·140	1·53	0·972
		***		*		

†‡ See Table 2.

were between those of grasses (18·4% TFE-III and 43·0% in IRG-III, respectively). High IAFs (always higher than 28%) were also observed for CP (Table 5), except for the IRG-V, where the IAF for CP was much lower (6·0%). The IAFs for NDF, CELL and HCELL (Tables 3 and 6) were numerically low and exceeded 5·0% only for the HCELL in LUC-III.

With advancing harvesting date, the IAFs for DM (Table 2) significantly decreased only in grasses ($P < 0·05$), while in legumes the decrease was not significant. Similarly, the IAFs for CP (Table 5) decreased significantly in IRG ($P < 0·001$), LUC ($P < 0·01$) and RCL ($P < 0·01$) samples. In contrast, a small and non-significant increase with maturity in the IAF of CP was observed for the TFE samples.

The IAFs for NDF, CELL and HCELL did not always decrease with advancing harvesting date. The IAF for NDF decreased in legume and TFE samples, but the decrease was only significant for the TFE samples; for IRG samples, the increase in IAF of NDF was not significant. The CELL IAF of legume samples decreased significantly ($P < 0·01$ and $P < 0·001$, for LUC and RCL samples, respectively) with advancing harvesting date. In contrast, in grasses the CELL IAF increased slightly; the only significant difference was observed in IRG samples ($P < 0·05$). The HCELL IAFs of LUC samples decreased significantly ($P < 0·05$) with advancing harvesting date.

The total potential degradability (B) of DM and CP varied between 65·2 and 95·2% and between 63·6% and 95·3%, respectively (Tables 2 and 5). In all forages, total potential degradability of fibre fractions (Tables 3 and 6) were lower than their respective values for DM and CP (Tables 2 and 5).

Total potential degradability of DM and chemical components significantly decreased with advancing harvesting date ($P < 0·01$) in all forages except RCL, which had smaller differences for CP (0·8%; $P < 0·05$) and higher differences for HCELL (11·1%; $P < 0·001$). The strongest effect of harvesting date was observed for TFE, with absolute differences of 21·6% for DM and 31·9% for CELL.

Relative degradation rates (C) of fibre fractions were higher than those of DM and CP in all samples. The relative degradation rates of DM varied between 0·80 for IRG-III and 1·26 for TFE-V (Table 2) and were generally slightly higher than those of CP, which ranged from 0·67 for TFE-V to 2·64 for IRG-V (Table 5).

With advancing harvesting date, relative degradation rates of DM and CP significantly decreased only in TFE ($P < 0·01$), while in other samples these generally increased. In most samples, the relative degradation rate of NDF increased with advancing harvesting date, with the difference only being significant for RCL. The relative degradation rate of IRG CELL decreased ($P < 0·01$) and in legumes increased ($P < 0·01$); for HCELL, the relative degradation rates increased significantly only in LUC ($P < 0·001$).

The constant factors of microbial efficiency (A) of DM and CP were generally lower in grasses than in legumes (Tables 2 and 5), with the only exception being CP for IRG-V. High values of A were also observed for NDF (0·204) and CELL (0·205) of IRG-III. The differences between A for NDF, CELL and HCELL in the other food samples were not very high.

With advancing harvesting date the A values of DM and CP (Tables 2 and 5) decreased significantly in TFE and LUC ($P < 0·05$), while the microbial efficiency factor of CP increased ($P < 0·01$) in IRG. A decrease in this factor with maturity was also observed for NDF in IRG ($P < 0·05$), while a numerical increase was recorded in RCL. A significant decrease of A with maturity was recorded for HCELL (Table 6) and IRG CELL; for RCL, the A of both CELL and HCELL increased ($P < 0·05$).

A correlation analysis between fibre fractions and degradability parameters was conducted and, for the sake of simplicity, data are reported only for the B parameter (Table 7). Significant correlations between fibre fractions and B were always negative: ADF content was highly correlated with DM and CELL, and ADL content had high correlations with NDF and HCELL. Slightly lower correlations were also observed between CELL content and CP. The NDF and HCELL contents were only weakly correlated with NDF, CELL and HCELL total potential degradability.

Table 7 *Correlation coefficients between food fibre fractions and potential degradability of chemical components*†

	Potential degradability (B) of				
	DM	CP	NDF	CELL	HCELL
NDF	–0·68***	–0·82***	–0·28	–0·63**	–0·30
ADF	–0·91***	–0·82***	–0·64***	–0·88***	–0·65***
ADL	–0·77***	–0·20	–0·96***	–0·79***	–0·95***
CELL	–0·80***	–0·86***	–0·45*	–0·76***	–0·47*
HCELL	–0·35	–0·69***	0·10	–0·30	0·08

† For abbreviations see Table 1.

The coefficients of correlation between fibre component contents and IAFs (data not shown) revealed only moderate relationships, which were only significant for DM (r = –0·59, –0·74, –0·62 and –0·66 for NDF, ADF, ADL and CELL, respectively) and CELL IAFs (–0·57, –0·45, –0·54 and –0·61 for NDF, ADF, CELL and HCELL, respectively).

The C values were poorly correlated with the fibre fractions and significant correlations were only obtained between the HCELL content and C for CELL (r = 0·57, $P < 0·01$), ADF or CELL contents and C for DM (r = 0·53, $P < 0·01$ and r = 0·50, $P < 0·05$, respectively). Fibre contents (except ADL) of foods were highly correlated with A for DM (r = –0·78 to –0·86, $P < 0·01$). Significant correlations for the A parameters were found between ADL content and HCELL (r = –0·74, $P < 0·001$) and between ADF content and CELL (r = –0·65, $P < 0·001$), CP (r = –0·54, $P < 0·01$) and NDF (r = –0·51, $P < 0·05$).

Discussion

The study of rumen degradability with growth (sigmoidal) models are not very common and published reports are limited. Moreover, Sauvant *et al.* (1985) used the Gompertz model to fit the degradability data of 24 foods, only one of which was a forage, and Van Milgen and Baumont (1995) reported a statistical comparison only on the fitting ability of a modified Gompertz model (Gompertz-like model) with a logistic model and a first-order model with a discrete lag phase. Thus a comparison with published data obtained with the Gompertz model for forages is limited. In the present paper, degradability data of DM and NDF were fitted with Gompertz and first-order models in order to compare them from statistical (i.e. R^2 and residual s.e.) and biological (i.e. parameters) points of view.

The Gompertz model fitted the DM degradability data as well as the first-order model (Table 2 and Figure 1) of Ørskov and McDonald (1979) and differences between fitted and observed data for the two models were very small (Table 4), the only difference being observed in the rapidly available fraction estimates of DM, which could be a consequence of the model 'structure'. A good fit of DM degradability data with the Gompertz model was also reported by Sauvant *et al.* (1985) and Van Milgen and Baumont (1995).

The use of a first-order model to fit degradability data of low quality foods and fibre fractions often produces negative estimates for the rapidly soluble fraction. The negative estimates can be ascribed to the delayed availability of potentially degradable substrate at the start of incubation (Sauvant *et al.*, 1985) and are often corrected by the introduction of a discrete lag phase (McDonald, 1981), during which degradation does not occur due to limited hydration, microbial attachment and colonization of the substrate. Van Milgen and Baumont (1995) considered the lag phase to be a non-biological parameter, because it is quite unlikely that instantaneous hydration and colonization of the substrate occurs at the end of it. It is more likely that the process of degradation starts immediately after inserting the bags into the rumen and as time of incubation proceeds, the degradation becomes faster until the time that the substrate becomes a limiting factor. This pattern of degradation is typical of fibre fractions and can be followed better with a model which has a sigmoidal shape, such as the Gompertz model (Table 3 and Figure 2). The superiority of a sigmoidal shaped model over a first-order model for NDF is also clear from the better fitting at the beginning of incubation observed with the Gompertz model (Table 4). Even though the differences between models for the generated parameters were statistically relevant only for the total potential degradability of NDF, the use of the Gompertz model for the DM resulted in numerically higher estimates for the immediately soluble fractions and similar total potential degradability. The IAF and B values for NDF were lower than the respective a' and $a' + b'$ coefficients; considering that NDF does not have soluble fractions, the higher a' estimates obtained with the first-order model could have been due to the physical losses of particles from the bags with the washing machine, a procedure required to avoid negative estimates of the a parameter (McDonald, 1981; Cockburn *et al.*, 1993).

The IAFs of DM and CP generally decreased with increasing harvesting date (Tables 2 and 5), and this was also observed by Balde *et al.* (1993), Hoffman *et al.* (1993) and Babnik (1995), even though they used a first-order model. The effect of maturity on IAFs for the fibre fractions was not as constant as for DM and CP (Tables 3 and 6) but the absolute differences within forage were very low. Therefore it can be assumed that these differences were not nutritionally important and that they derive more from the bias due to curve fitting than from a true variation of solubility.

The poor correlations between fibre fraction contents and IAFs of chemical components (data reported in the **Results** section) suggest that the concentration of fibre components did not affect solubility.

When potential degradability of individual chemical components were compared between harvesting dates, a significant decrease was observed in all

forages, except in RCL, where the potential degradability of individual chemical components increased; this different effect of maturity in grasses and legumes agrees with published data (Nocek and Russell, 1988; Buxton, 1989; Buxton and Brasche, 1991; Hoffman *et al.*, 1993). The increased total potential degradability with maturity observed in RCL was probably due to a decrease in ADL content (Table 1), a major factor affecting cell wall degradability (Akin and Chesson, 1989). It is generally accepted that ADL content increases with advancing maturity, but the present results (Table 1) and those reported by Bidlack and Buxton (1992) indicate that the variation of lignin content in RCL can be small. Moreover, Brink and Fairbrother (1994) reported cyclical changes in ADL content during spring growth, even under well controlled environmental conditions. The importance of ADL in determining the fermentation of fibre is also evident from the negative correlation coefficients reported in Table 7. Lignin links to hemicellulose by a number of covalent bonds (Iiyama *et al.*, 1994) and this is in good agreement with the high correlation of ADL content with the potential degradability of hemicellulose. Chesson (1993) reported that during rumen digestion, lignin concentration on the surface exposed to micro-organisms increases, providing physical protection from degradation for all the underlying components. However, the concentration of lignin has not always been reported to be highly correlated with the extent of NDF degradability. Ford and Elliott (1987) suggested that the structural arrangement of chemical components within plant cell walls has a greater limiting effect than the concentration of these components alone.

For DM and CP degradability, the highest correlations were observed with ADF and cellulose (Table 7) and the possible utilization of ADF content as a predictor of DM degradability was also proposed by Giger-Reverdin (1995). This negative correlation confirms that cell wall components form a considerable physical barrier, which limits rumen degradation of cell contents.

According to Beuvink and Kogut (1993) and France and Thornley (1984) the *C* and *A* parameters produced by the Gompertz model describe the relative degradation rate and the constant factor of microbial efficiency. If the biological meaning of these parameters can be accepted, the limited use of this model to fit degradability data does not permit definite conclusions to be drawn on the effect of maturity. It is not clear if the low variation of these parameters can be ascribed to the limited variation of maturity within forages or if the values can be viewed as characteristics of the chemical components under consideration within plant species. Further applications of the Gompertz model to a larger number of forages, differing in chemical composition and age, are thus required.

Conclusions

The use of the Gompertz model was statistically satisfactory for describing the degradability of DM and other chemical components in forages. Moreover the model described the initial phases of degradability better for slowly fermenting substrates, such as fibre fractions. Furthermore, the model partitioned the soluble and potentially degradable pools of nutritionally relevant compounds differently to the first-order model and so was conceptually more correct from a biological point of view.

The estimates of the amount and the efficiency of rumen microbial growth obtained with the Gompertz model need to be evaluated and considered with care.

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Effect of ammoniation or protein supplementation of barley straw on digestion and purine derivative excretion in sheep

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Abstract

An experiment was conducted to examine the effect of ammonia treatment or soya-bean meal supplementation on rumen and lower tract digestion and urinary purine-derivative excretion in sheep given barley straw-based diets. Four wethers each fitted with simple rumen and duodenal cannulae were randomly allocated to four diets in a 4 × 4 Latin-square design. The diets were, untreated barley straw + molasses meal (diet 1), ammonia-treated barley straw + molasses meal (diet 2), untreated barley straw + soya-bean meal + molasses meal (diet 3) and ammonia-treated barley straw + soya-bean meal + molasses meal (diet 4). Dry-matter (DM) intake, total tract neutral- and acid-detergent fibre apparent digestibility and faecal nitrogen (N) output were higher for diets 2 and 4. Microbial protein yield, determined from total urinary purine-derivative excretion, was also high in sheep on diets 2 and 4. However, supplementation with soya-bean meal in diet 4 reduced DM and fibre digestion but increased intake and microbial synthesis. Despite the lower microbial protein synthesis for diet 3, total tract N digestion was high for the diet, an indication of higher nutrient absorption in the lower tract. The above results indicated that there was a substantial increase in rumen microbial N yield from ammoniated straw-based diets. However, not all the N was made available to the animals. Some of the microbial N absorbed in the lower tract may have been overestimated by the use of the purine derivative-microbial N evaluation method. On the other hand, when protein supplements were given the amount of microbial protein available to the ruminant through absorption in the lower tract may have been underestimated by urinary purine derivatives.

Keywords: *ammonia treatment, protein supplements, purines, sheep, straw.*

Introduction

Ammoniation of cereal straws, apart from being a source of nitrogen (N), also improves the digestibility and intake. The chemical action on fibre by alkali treatment has been widely reported (Sundstøl *et al.*, 1978; Fahmy and Ørskov, 1984). Direct supplementation of straw with protein-rich food and non-protein nitrogen (NPN) (including soya-bean meal and urea respectively) also increase intake and digestibility of straw (Church and Santos, 1981). At rumen level, energy and N substrates made available through digestion result in increased microbial biomass yield and thus microbial N supply for absorption in the lower tract (McAllan and Smith, 1973). Determination of microbial yield from both ammoniated and protein supplemented straw can be achieved indirectly from purine derivative (PD) measurement in urine as there is a strong relationship between microbial nucleic acid-concentration in the rumen and PD excreted into the urine of sheep and other ruminants (Topps and Elliot, 1965; Fujihara *et al.*, 1987; Chen *et al.*, 1990). Balcells *et al.* (1993b) reported that PD excretion can provide a reliable estimate of microbial yield and that it is sensitive to variations in rumen fermentation induced by changes in the basal diet and by the source of supplementation. However, Puchala and Kulasek (1992) reported a long delay between the absorption of microbial purines and excretion of urinary PD and that this could affect microbial protein estimates from PD. Such delay could mainly be due to digestion and absorption of purines in the lower tract. Microbial protein estimates from PD could also be affected by differences in metabolism of NPN and conventional protein sources. However, there is little information on the metabolism of microbial N produced from diets of varying N sources in the lower tract. The

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objective of this study was therefore to investigate the effect of rumen fermentation of ammonia-treated and protein-supplemented straw on urinary PD excretion and the effect on the availability of microbial N in the lower tract of sheep.

Material and methods

Ammonia treatment

Straw was chopped to 3 cm using an electric chopper and then treated with 770 ml solution containing 250 g/kg of aqueous ammonia. Treatment was done in airtight polythene bags (130 × 170 cm in size) with a capacity of 5 kg straw. A vacuum pump was used to evacuate air from the bags before mixing the ammonia solution with the straw to provide 35 g of ammonia per kg dry matter (DM) of straw. The bags were then securely tied with a cord and stored. During storage the minimum and maximum ambient temperatures were 9 and 15°C respectively. After 8 weeks the bags were opened and straw aerated to disperse the excess ammonia before the start of the experiment.

Diets

Four experimental diets were formulated based on ammonia-treated and untreated straw. They were: untreated barley straw + 180 g molasses meal (diet 1), ammonia-treated barley straw + 140 g molasses meal (diet 2), untreated barley straw + 100 g soya-bean meal + 66 g molasses meal (diet 3) and ammonia treated barley straw + 30 g soya-bean meal + 70 g molasses meal (diet 4). Diets were formulated to meet the maintenance requirements of sheep (Agricultural and Food Research Council (AFRC), 1993) and contained equal amounts of metabolizable energy and N. Table 1 shows the chemical composition of ammonia-treated, untreated straw and other food ingredients used in the formulation of the diets.

Animals and their management

Four Japanese Corriedale wethers with an average body weight of 41 kg, fitted with simple rumen and duodenal cannulae as described by Smith and McAllan (1970), were used. The cannulae used were of a wide mouth-type designed to cause total digesta diversion through the cannula, allowing collection of truly representative samples. Each animal was placed in a metabolism cage housed in a well ventilated building. During the 10-day adaptation period, foods were offered to the animals in exactly the same routine as during subsequent experimental periods. Straw was offered each day after adjusting the amount to proportionately 1·1 times that of the previous day. Fresh foods were offered in equal amounts for the morning (09.00 h) and evening (21.00 h) feeding, allowing proportional refusals of about 0·20 of that offered. Supplements were offered on a restricted basis and animals had free access to water. Animals were also allowed free access to a multi-mineral salt block (Nippon Zenyaku Kogyo Co. Ltd, Fukushima, Japan) which was placed in each food box. Required vitamins (AFRC, 1993) were provided together with supplements. Food refusals were collected before each feeding, weighed, bulked (per animal) and stored for subsequent chemical analyses.

Experimental design

The four sheep were randomly allocated to the four diets in a 4 × 4 Latin-square design. The experiment was carried out in four periods each comprising a 10-day adaptation and a 7-day experimental period.

Marker administration

An aqueous solution of ytterbium chloride (YbCl) was dosed directly into the rumen as a particulate marker, at a rate sufficient to maintain an estimated 0·05 g/kg DM. Polyethylene glycol (PEG 4000) was made into pellets with the molasses meal and offered at a rate of 6 g/day (i.e. 3 g at each feeding) as a liquid marker. Before marker administration began, each animal was given a prime dose of 5 g PEG and 1 g YbCl on the 10th day. Both markers were administered before each feeding from the 10th to the 15th day of each experimental period.

Rumen and duodenal sampling

A single duodenal sample of about 30 ml per animal per day was collected for a period of 4 days (from the 11th to 15th days) for marker stabilization assessment. Actual sampling commenced on the 15th day. About 50 ml duodenal digesta was collected via the 'T' cannula at intervals of 3 h, i.e. 0, 3, 6, 9, and 12 h after the morning feeding. This was repeated on the 16th day at 0, 2, 5, 8 and 11 h after the morning feeding. On the 17th day, the same amount of rumen samples was also collected at 0, 1, 2, 3, 5 and 7 h after the morning feeding. Duodenal samples from each animal were bulked, subsampled and centrifuged at 15 000 **g**, for 10 min. After separation, the liquid portion was stored at −10 °C while the particulate matter was lyophilized and stored at 4 °C for subsequent analyses (Faichney, 1975). Before freezing, rumen fluid from each animal was immediately strained through six layers of surgical gauze and a drop of mercury II chloride added to prevent further bacterial fermentation.

In vivo *apparent digestibility*

Apparent digestibility was measured for each food within the same experimental period. During the latter part of the preliminary period, diets were offered at proportionately about 0·90 of the *ad libitum* level of intake for 4 days, after which total collection of urine and faeces was done beginning from the

11th to the 15th day of each experimental period. Faeces were immediately dried in a forced air oven at 60°C for 48 h after which they were thoroughly mixed and bulked for each animal and a representative sample obtained. The samples were then ground through a 1-mm mesh for determination of apparent digestibility of DM (DMD). Urine was collected and preserved in containers containing about 100 ml sulphuric acid (0·1 mol/l) to prevent N loss. Urine volume was measured each morning and a sample taken which was later bulked with other samples taken during the same period and a subsample taken per animal and stored at –10°C for further analysis.

Analyses

DM and ash for all samples (except urine) were determined according to the Association of Official Analytical Chemists (AOAC, 1984). Crude ash and crude fibre for foods and refusals were also determined according to AOAC (1984). N in all samples was determined by the Kjeldahl method. Neutral- and acid-detergent fibre (NDF and ADF) were determined according to the method of Goering and Van Soest (1970). Ammonia-N in rumen fluid and the liquid portion of duodenal digesta were analysed according to the procedure of Oser (1965). Volatile fatty acids (VFAs) in rumen fluid were determined by gas chromatography (Erwin *et al.*, 1961). Ytterbium (Yb) concentration in the particulate portion of duodenal digesta was determined using an Inductively Coupled Plasma Emission Spectrophotometer (ICPS 1000, Shimadzu Co., Japan) and PEG concentration by the procedure of Morimoto (1971). Allantoin was determined by the method of Young and Conway (1942) while xanthine, hypoxanthine and uric acid were determined according to Fujihara *et al.* (1987).

Calculations

Marker stabilization was ensured by monitoring its concentration in duodenal digesta samples taken between the 11th and 15th days of each experimental period and was found to have reached a steady state by the 13th day. DMD and DM intake were both calculated by difference. DM flow (g/day) at the duodenum was calculated by dividing the daily Yb dosage (g/day) by Yb concentration in duodenal digesta for each animal. Flow of each nutrient (g/day) was then calculated by multiplying DM flow at the duodenum by the concentration of the given nutrient in duodenal digesta. Duodenal fluid flow (l/day) was similarly determined from PEG concentration. N in duodenal fluid and ammonia-N flow at the duodenum (g/day) were then calculated by multiplying their concentration (g/l) by fluid flow (l/day). Total N concentration in duodenal digesta was calculated as the sum of its concentration in fluid and solid portions of digesta.

Microbial purines absorbed (X) were determined from total urinary PD (Y) using the equation proposed by Chen *et al.* (1990) thus:

$$Y = 0{\cdot}84X + 0{\cdot}150\ M^{0{\cdot}75}e^{-0{\cdot}25X}$$

where 0·84 represents the slope (PD recovery in the urine) and $M^{0{\cdot}75}$ the metabolic body weight. Microbial N supplied to the animal was calculated as 0·73 X, assuming an 830 g/kg DM digestibility coefficient for microbial purines (Chen *et al.*, 1992).

Statistical analysis

Data were analysed with analyses of variance using the general linear models (GLM) procedure of Statistical Analysis Systems Institute (SAS, 1985). Where necessary, differences between means were

Table 1 *Chemical composition of food ingredients and of the experimental diets (g/kg dry matter)*

Constituent	Untreated barley straw	Ammonia-treated barley straw	Soya-bean meal	Molasses meal†	Diets‡ 1	2	3	4
Dry matter	913	898	836	783	908	891	904	892
Organic matter	926	925	931	876	957	947	938	936
Nitrogen	15	75	443	105	40	91	92	95
Crude fibre	384	412	49	116				
Neutral-detergent fibre	746	704	126	236	631	634	601	639
Acid-detergent fibre	518	530	84	144	432	462	415	469
Cellulose	436	458	62	111				
Hemicellulose	228	174	42	92	199	172	186	170
Lignin	82	72		33				

† Molasses meal constituents (g/kg DM): molasses 450, sheanut cake 80, copracake 300, rice bran 70, corn jam meal 40, calcium cabonate 60.
‡ See text for details.

compared using Duncan's multiple range test (Duncan 1955).

Results

Chemical composition of the experimental food ingredients and diets are given in Table 1. The DM of the food ingredients ranged from 892 g/kg DM in diet 4 to 908 g/kg DM in diet 1. Ammoniation increased N concentration in the untreated straw from 15 to 75 g/kg DM. The N concentration in the diets ranged from 40 to 95 g/kg DM in diet 1 and diet 4 respectively. NDF ranged from 601 to 639 g/kg DM and ADF from 415 to 469 g/kg DM in diets 3 and 4 respectively.

Table 2 shows that there were significant differences in mean daily DM and fibre intake between diets ($P < 0.05$). Ammonia treatment significantly enhanced DM intake of animals on diet 2 ($P < 0.05$). DMD and fibre digestion was significantly improved by ammoniation, both in the rumen and in the total tract ($P < 0.05$).

Table 3 shows that total tract N digestion was significantly higher ($P < 0.05$) for diet 3 (696 g/kg N) than for all other diets. Total N, ammonia-N and non-ammonia N flow at the duodenum were significantly higher on diet 4 ($P < 0.05$). Net gain in recycled N in the rumen was observed only on diet 1. Ammoniation of straw resulted in higher N retention and total urinary purine derivative excretion by sheep on diets 2 and 4 ($P < 0.01$) and efficiency of microbial N synthesis was significantly higher in diet 4 compared with the rest of the diets ($P < 0.05$).

Table 4 shows that total VFA concentration was significantly higher in animals given diets 3 and 4 (56·0 and 74·0 mmol/l respectively) compared with the other two diets ($P < 0.01$). Unlike acetic and valeric acids, propionic and butyric acid concentrations were not significantly affected by ammoniation ($P < 0.05$).

Discussion

Treatment of straw with ammonia increased DM intake and DMD, both in the rumen and in the whole tract, as has been reported by Sundstøl *et al.* (1978), Van Soest *et al.* (1984) and Fahmy and Ørskov (1984). Soya-bean meal supplementation in diet 3 improved intake and apparent digestibility of untreated straw. However, DM intake and total tract DM and fibre digestion were not affected in comparison with diet 2. This is in agreement with the previous report of Mawuenyegah *et al.* (1994). This could possibly have been due to the excess N and low energy made available from ammoniated straw. Satter and Roffler (1981) reported NPN to be poorly utilized in high protein and low energy rations due to excess ammonia which is not efficiently converted into microbial protein. The oversupply of N in diet 4 was reflected in high rumen ammonia-N concentration

Table 2 *Voluntary intake, duodenal flow and apparent digestibility of dry matter (DM), neutral- and acid-detergent fibre (NDF and ADF) in sheep given ammonia-treated and untreated barley straw*

	Diet					
	1	2	3	4	s.e.d.	Significance
Intake (g/day)						
DM	588	716	632	699	36·95	*
NDF	370	451	379	447	29·97	*
ADF	254	331	262	328	25·61	*
Duodenal flow (g/day)						
DM	337	400	390	420	26·83	*
NDF	219	237	252	252	16·41	
ADF	151	185	173	195	12·04	*
Apparent rumen digestibility (g/kg)						
DM	325	380	347	368	9·50	*
NDF	317	420	394	414	5·53	*
ADF	309	417	310	381	2·96	**
Apparent lower tract digestibility (g/kg)						
DM	182	210	248	201	8·90	**
NDF	189	206	161	186	2·50	
ADF	85	86	82	83	2·98	
Apparent total tract digestibility (g/kg)						
DM	507	590	595	569	2·35	**
NDF	500	626	555	600	2·38	**
ADF	394	503	392	464	2·07	**

Table 3 *Nitrogen (N) intake, apparent digestibility, flow at duodenum, balance, and purine derivative (PD) excretion in sheep given ammoniated or protein-supplemented baley straw*

	Diet					
	1	2	3	4	s.e.d.	Significance
Intake (g/day)	5·40	9·12	9·18	10·49	1·47	*
Total tract N apparent digestibility (g/kg)	430	640	700	650	4·31	*
Total N flow (g/day)	5·83	8·74	8·21	9·10	0·56	*
Ammonia-N flow (g/day)	1·28	3·93	3·23	4·01	1·31	**
Non-ammonia N flow (g/day)	4·54	4·81	5·03	5·10	0·54	
Rumen ammonia-N concentration (mmol/l)	3·60	11·90	14·80	14·10	1·90	*
N recycled to rumen (g/day)	1·80	–0·40	–1·00	–1·40	0·51	*
Faecal N excretion (g/day)	2·31	3·33	2·82	3·69	0·50	**
Urinary N excretion (g/day)	1·81	3·62	4·60	4·10	1·00	**
Retained N (g/day)	–0·10	2·20	1·82	2·72	1·01	**
Allantoin (mmol/l)	2·50	5·25	4·59	6·03	0·95	*
Xanthine + hypoxanthine (mmol/l)	0·26	0·35	0·13	0·15	0·16	
Uric acid (mmol/l)	0·94	1·20	0·90	1·05	0·45	
Total PD (mmol/l)	3·70	6·80	5·62	7·23	1·87	**
Microbial protein (g/day)	2·19	5·56	4·39	5·94	1·43	**
DOMI† (g/day)	283	395	344	356	34	*
DOMR‡ (g/day)	178	251	201	227	19	*
EMNS§	12·90	22·34	22·50	26·0	2·56	*

† DOMI: Digestible organic matter intake (g/day).
‡ DOMR: Digestible organic matter fermented in rumen = 0·65 × DOMI.
§ EMNS: Efficiency of microbial nitrogen synthesis = g microbial N per kg DOMR.

(Table 3) and the high levels of N recycled through the rumen wall into urine.

Although the level of total purine-derivative excretion reported in this experiment was low compared with those reported by Abdulrazak *et al.* (1992), similar levels were reported by Chen *et al.* (1992). The PD excretion values reported for ammoniated straw-based diets (Table 3) were also similar to those of Balcells *et al.* (1993a). In their study, the latter authors reported that ammoniation of barley straw resulted in higher urinary PD values compared with urea supplementation.

Microbial protein synthesis and yield were significantly influenced by DM and fibre intake,

Table 4 *Volatile fatty acid (VFA) concentration in sheep given ammonia-treated and untreated barley straw as basal diet (mmol/l)*

	Diet					
	1	2	3	4	s.e.d.	Significance
Acetate	33	35	38	52	5·10	*
Propionate	11	12	12	15	1·4	**
Butyrate	4·0	5·0	5·0	6·0	0·9	
Valerate	0·2	0·3	1·0	1·0	0·2	*
Total VFA	48·2	52·3	56·0	74·0	5·1	*

ruminal and total tract apparent digestibility for diets 2 and 4 ($P < 0·05$). Lower tract DM and total tract N digestion were higher for diet 3, although its urinary PD excretion and DM digestion in the rumen were lower than those for diets 2 and 4. This indicates that for ruminants given ammoniated-straw diets, a certain proportion of the synthesized microbial N is not absorbed in the lower tract. As shown in Table 3, a relatively high amount of N was excreted in the faeces for ammonia-treated straw-based diets. According to the report of Cann *et al.* (1991) a high portion of faecal N from ammoniated straw diets is of microbial origin. Under such situations there could be an overestimation of the microbial protein available to the ruminant when PD excretion values are utilized in its calculation. On the other hand, urinary N content was high in diet 3 and faecal N excretion was low for the same diet. This is in agreement with a previous report on faecal N excretion (Warly *et al.*, 1994). The latter authors reported no change in faecal N excretion with increased protein supply.

The high digestible organic matter (OM) apparently fermented in the rumen (DOMR) indicates an improved microbial protein synthesis. At higher DM intake, there is a tendency for DOMR to be low (Chen *et al.*, 1990). Therefore, OM content in duodenal digesta was used to calculate DOMR in this experiment. This gave similar values for

efficiency of microbial nitrogen synthesis (EMNS) for diets 2 and 3 but a higher value for diet 4, an indication that ammoniation and protein supplementation result in microbial protein yield with similar efficiency. However, when ammoniated straw diets are supplemented, EMNS values further emphasize the effect of overestimation of microbial protein by PDs.

Lack of any appreciable increase in total VFA concentration by ammoniation in diet 2 (Table 4), conflicts with the higher microbial protein synthesis associated with this diet, but this is in agreement with other reports (Fondevila *et al.*, 1994). These authors attributed this situation to a reduction in the OM fermented in the rumen and the high ruminal fluid dilution rate due to ammoniation.

N added by supplementation had a higher apparent digestibility than that added by ammoniation and nutrient absorption in the lower tract was significantly higher ($P < 0{\cdot}05$) for untreated straw supplemented with protein. This could be partially attributed to the amount of N loss in faeces of animals on ammoniated straw-based diets (reported to be mostly of microbial origin) being fairly high compared with that from supplemented straw diets. From the above discussion, it is therefore concluded that part of microbial N (estimated from urinary PD) in the rumen of animals given ammoniated straw-based diets, is not utilized by the ruminant. Both ammoniation and protein supplementation of the straw had similar efficiency in as far as microbial protein yield was concerned. However, exogenous supply of N to sheep given ammoniated straw resulted in the excess N being efficiently converted into microbial protein. There is therefore a need to supplement high-producing ruminants given ammoniated straw with protein rich foods to take advantage of this metabolic activity. Although direct measurements of digestibility were not made in this study, microbial N was calculated using a digestibility coefficient of 830 g/kg for microbial purines (Chen *et al.*, 1992). It may also be necessary to include a correction factor in the calculations to account for faecal microbial N losses when ammoniated straw diets are offered. This needs to be confirmed experimentally by measuring microbial mass at both duodenum and in the faeces of animals consuming ammoniated straw diets.

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Effect of ammonia treatment or protein supplementation on rumination behaviour in sheep given barley straw

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Abstract

A study was conducted to compare the effects of ammoniation and protein supplementation of barley straw on rumination behaviour of sheep. Four wethers were allocated to four diets offered ad libitum *in a 4 × 4 Latin-square design. The diets were, untreated barley straw + molasses meal (diet 1), untreated barley straw + soya-bean meal + molasses meal (diet 2), ammonia-treated barley straw + molasses meal (diet 3) and ammonia-treated barley straw + soya-bean meal + molasses meal (diet 4). Animals were kept in metabolism crates throughout each 16-day experimental period and allowed free access to water and a mineralized salt lick. The first 11 days of each period were for adaptation to the harnesses and diets while the last 5 days were used for rumination studies. Animals given diets 3 and 4 had slower eating rates compared with those given diets 1 and 2. Rumination index and duration of each rumination period was lower for sheep consuming diets 3 and 4 than for those on diets 1 and 2 but not significantly so ($P > 0·05$). Rumination time per 100 g neutral-detergent fibre (NDF) intake was significantly lower ($P < 0·01$) for diets containing ammoniated straw. Animals given ammoniated straw diets also regurgitated fewer boluses per unit NDF intake than did those on untreated straw diets. The results showed that increased intake and digestibility, which is usually associated with ammoniated straws, was due to sheep doing less work per unit of time to break down straw for digestion. In this way, potentially digestible tissues within a given amount of straw is more readily exposed. The foregoing suggests that ammonia treatment results in less rumination so that ruminants given ammonia-treated straw diets do less work ruminating.*

Keywords: *ammonia treatment, protein supplement, rumination, sheep, straw.*

Introduction

Fibre-residue load in the rumen of animals given low digestibility roughage is a major factor restricting voluntary food intake. The competitive processes of reduction in particle size and passage of small particles determine fermentation time and are modulated by the animal, allowing it to respond to different dietary conditions (Wilson and Kennedy, 1996). To meet maintenance and production requirements, ruminants given low quality roughage diets need to be supplemented with concentrates or be offered alkali-treated roughage. According to Harumoto and Kato (1979) and Fujihara and Nakao (1984), the process of rumination plays an important rôle in reducing the particle size of the food eaten, thereby also enhancing microbial degradation and rumen clearance. For animals given straw diets, the purposes served by comminution or chewing during eating and rumination is to reduce the size of large digesta particles so that they can be incorporated into a bolus and swallowed. This also helps to release soluble nutrients for fermentation through exposure of their internal structures for microbial invasion. Microbial digestion *per se* does not contribute to particle size reduction of rumen digesta (Dulphy *et al.*, 1980) but assists in weakening the internal structure of the plant cell-wall material in the rumen. Protein supplementation affects intake and rumen characteristics of straw while ammoniation also affects its physical (Zorrilla-Rios *et al.*, 1985) and chemical properties. Although there is much information on the effect of protein supplementation, there is little information on the effect of ammonia treatment of straw and its comparison with

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supplementary feeding of protein, regarding their effect on rumination behaviour of sheep.

The objectives of the study were to examine the effect of ammonia treatment and protein supplementation on rumination behaviour of sheep. It was postulated that such information could help explain further the differences in intake, digestibility and eating behaviour associated with treating or supplementing straw.

Material and methods

Ammonia treatment

Barley straw was chopped to 3 cm and ammoniated in polythene bags (130 × 70 cm) each holding 5 kg straw. Aqueous ammonia was added to the contents of the bag under vacuum to provide 35 g ammonia per kg straw dry matter (DM) allowing a 0·1 proportional loss of ammonia through volatilization while handling. The bags were kept tied for 8 weeks after which they were opened to disperse the excess ammonia before the start of the experiment. At the time of treatment minimal and maximal ambient temperatures were 9 and 15°C respectively.

Diets

The four diets formulated and used in the experiment were: untreated barley straw + 180 g molasses meal (diet 1), untreated barley straw + 100 g soya-bean meal + 66 g molasses meal (diet 2), ammonia-treated barley straw + 140 g molasses meal (diet 3) and ammonia-treated barley straw + 30 g soya-bean meal + 70 g molasses meal (diet 4). Diets were formulated to meet the maintenance requirements of sheep (Agricultural and Food Research Council (AFRC), 1993) and contained equal amounts of metabolizable energy and nitrogen (N). Table 1 shows the chemical composition of ammonia-treated, untreated straw and other food ingredients used in the formulation of the diets.

Experimental procedure

Four wethers were allocated to the four diets in a 4 × 4 Latin-square design experiment. Each experimental period consisted of an 11-day adjustment period to allow accustomization to harness and food followed by, from the 12th day, a 5-day period in which recordings were made. On the 12th day of this 16-day total period each wether was placed in a metabolism crate where it was maintained for the 5 days. The crates were placed in a well lit and well ventilated building and were constructed such that they allowed eating, drinking and other possible animal movements, except turning around, during the recording of jaw movement. Strain gauges were attached to the lower jaw of each animal. The gauges were connected to a pen recording chart (Rikadenki Kogyo Co. Ltd, Tokyo, Japan) through an amplifer, according to the procedure of Harumoto and Kato (1978). The recording chart was set at a speed of 1 cm/min to measure the number of rumination periods per day and the number of boluses per rumination period. Readings were taken continuously for 96 h after which the recording speed was changed to 6 cm/min for the remaining 24 h, in order to measure the number of chews per bolus and chewing rate. The information recorded allowed the calculation of the time spent eating, eating rate, ruminating time, idling time, duration of each rumination period, number of ruminations per day, number of boluses regurgitated per day, number of boluses per rumination, bolus time, number of chews per bolus and chewing rate.

Analyses

DM, crude fibre and ash for food ingredients were determined according to the Association of Official Analytical Chemists (AOAC, 1984). N was determined by the Kjeldahl method. Neutral- and acid-detergent fibre (NDF and ADF) were determined according to the method of Goering and Van Soest (1970).

Statistical analysis

Data obtained from the experiment were subjected to one-way analysis of variance using the general linear model (GLM) of the Statistical Analysis Systems Institute (SAS, 1985). Where differences between means were significant, comparisons were made using Duncan's multiple range test (Duncan, 1955).

Table 1 *Dry matter (DM) (g/kg) and chemical composition of DM (g/kg DM) of untreated barley straw, ammonia-treated barley straw, soya-bean meal and molasses meal*

Constituent	Untreated barley straw	Ammonia-treated barley straw	Soya-bean meal	Molasses meal†
Dry matter	913	898	836	783
Organic matter	926	925	931	876
Nitrogen	15	75	443	105
Crude fibre	384	412	49	16
Neutral-detergent fibre	746	704	126	236
Acid-detergent fibre	518	530	84	144
Cellulose	436	458	62	94
Hemicellulose	228	174	42	92
Lignin	82	72		50

† Molasses meal constituents (g/kg DM): molasses 450, sheanut cake 80, copra cake 300, rice bran 70, corn jam meal 40, calcium carbonate 60.

Results

Chemical composition of the experimental food ingredients are given in Table 1. The DM content of the food ingredients ranged from 836 g/kg in soya-bean meal to 783 g/kg DM in molasses meal. Ammoniation increased nitrogen concentration in the untreated straw from 15 to 75 g/kg DM. The rest of the chemical composition values were as indicated in Table 1. Data on DM, NDF and N intake, eating and rumination measurements are presented in Table 2. An increased DM intake was observed for diets 3 and 4 but this was not reflected in the time spent eating. N intake for the diets ranged from 5 to 10·5 g/day in diet 1 and diet 4 respectively. Animals given diet 4 spent a longer time eating followed by animals on diet 2 and diet 3 but the differences between diets 2 and 3 were not significant ($P > 0·05$). Eating rate was accordingly slower for animals on diets 2 and 4 than for those on diet 3. The number of rumination periods per day ranged between 16 to 18 for all treatments and there were no significant differences observed between treatments ($P > 0·05$). The duration of each rumination period was longer for diets 3 and 4 than for diets 1 and 2, but differences were not significant ($P > 0·05$). The total time spent ruminating per day was higher for wethers on diet 4 than for those on diet 1 ($P < 0·05$), but values for the other diets were not significantly different ($P > 0·05$). Rumination index (time spent ruminating per 100 g DM intake) was not different ($P > 0·05$) between treatment diets but time spent ruminating per 100 g NDF intake were clearly lower ($P < 0·05$) for ammonia-treated straw diets (diets 3 and 4) than for diets 1 and 2.

Table 3 shows the bolus and chewing characteristics of sheep on the experimental diets. The number of boluses regurgitated per day decreased in the order of diet 3 < diet 4 < diet 2 < diet 1, but the differences were not significant ($P > 0·05$). The number of boluses per rumination period followed a similar trend to the number of boluses per day but diet 4 had the highest ($P < 0·05$) value. Animals on diet 4 also had the highest ($P < 0·05$) values for bolus time and number of chews per bolus, which both measure the efficiency with which the bolus is chewed, while those on diet 1 had the lowest values. The values obtained for diets 2 and 3 did not differ significantly ($P > 0·05$). Chewing rate was faster for diet 4 than for the other diets ($P < 0·05$). Cyclic rate, which is the daily time spent ruminating per number of boluses regurgitated, was found to be lower for diets containing ammoniated straw but not significantly so ($P > 0·05$).

Table 2 *Dry matter (DM), neutral-detergent fibre (NDF) and nitrogen (N) intake, eating and rumination measurements in sheep given untreated and ammonia-treated barley straw as basal diet*

	Diet†				
	1	2	3	4	s.e.d.
DM intake (g/day)	528	628	692	694	35·00
NDF intake (g/day)	369	379	451	447	16·01
N intake (g/day)	5·40	9·12	9·18	10·49	1·47
Time spent eating (min/day)	278	346	337	393	17·02
Eating rate (g DM per min)	1·90	1·81	2·05	1·77	0·35
No. of rumination periods per day	17·40	17·40	18·25	16·05	2·35
Rumination period time (min)	26·72	26·85	30·20	27·08	3·16
Rumination time (min/day)	437·83	459·15	473·78	486·92	23·77
Fibre rumination index (min)‡	131·40	121·09	108·06	108·82	4·89
Rumination index (min)§	82·85	73·07	68·51	70·21	2·45

† In this table and Table 3 see text for details of diets.
‡ Rumination time per 100 g NDF intake.
§ Rumination time per 100 g DM intake.

Discussion

Changes in chemical composition of straw, due to ammoniation and those of other food ingredients were the same as those reported in a previous experiment (Mawuenyegah *et al.*, 1995). The time spent eating the diets used in this experiment agrees with the observation by Dulphy *et al.* (1980), who indicated that sheep normally eat for 200 to 400 min/

Table 3 *Bolus and chewing characteristics in sheep given ammonia-treated and untreated barley straw as basal diet*

	Diet				
	1	2	3	4	s.e.d.
No. of boluses per day	420·93	446·83	501·08	476·83	24·61
No. of boluses per rumination period	25·83	26·70	27·33[a]	28·60	2·28
Bolus time (s)	46·00	52·45	52·13	53·73	3·37
No. of chews per bolus	56·33	64·70	66·03	71·45	3·46
Chewing rate (per min)	73·65	74·55	75·75	80·25	2·46
Cyclic rate(s)†	62·40	61·70	56·70	59·30	3·38

† Daily time spent ruminating per number of boluses regurgitated.

day. Differences in eating time could however, be due to the slower eating rate recorded for diets 2 and 4 compared with the faster rate recorded for diet 3. In the present experiment, sheep given diet 3 reduced the time spent eating compared with the soya-bean meal-supplemented diets (i.e. diets 2 and 4). Fujihara and Nakao (1984) supplemented a hay diet with casein for sheep and observed a decrease in time spent eating. However, in the present experiment, animals on diet 4, which had the highest protein content, spent a longer time eating compared with the other animals. Time spent eating therefore, seemed to be affected by other factors including intake and nature of supplement. The number of rumination periods per day was similar in all the diets. Campling *et al.* (1962) and Fujihara (1980) found that the number of rumination periods per day in cows and in sheep were almost the same for both silage and hay, irrespective of intake. Harumoto and Kato (1978) also found that the number of rumination periods per day was a relatively stable parameter for sheep, irrespective of the level or type of food. In the present experiment rumination index (time spent ruminating per 100 g DM intake) could be considered a better indicator of rumination efficiency than total time spent eating, for ammonia-treated and untreated straw diets. Rumination index, being the true indicator of the amount of work done in comminuting a diet (Fujihara, 1980), was significantly lower for the ammonia-treated straw diets ($P < 0{\cdot}05$), possibly because of increased DM intake (De Boever *et al.*, 1990). The lower rumination index could also be due to the fragility and concomitant increase in digestibility due to ammoniation (P. O. Mawuenyegah *et al.*, unpublished results). In a previous report (Mawuenyegah *et al.*, 1995), it was observed that apart from intake, rumination index depends on the protein as well as the fibre content of the diet. The above results were further confirmed in the present experiment by the shorter time spent ruminating per 100 g NDF intake for animals eating ammoniated straw diets.

The higher number of boluses per rumination period for diet 3 could have been due to a higher intake (Luginbuhl *et al.*, 1989). Although similar responses have been reported for sheep (Harumoto and Kato, 1979), the lower number of boluses per 100 g NDF intake in the present experiment shows that animals on the ammonia-treated straw diets regurgitated fewer boluses per unit NDF than those on untreated straw diets. According to Zorrilla-Rios *et al.* (1985), a greater extent of comminution during mastication of the more fragile ammoniated straw could result in a larger rumen pool of small particles. Small particles, being cleared at a faster rate from the rumen, results in a reduced amount of undigested particles in the rumen, thereby causing fewer number of boluses to be regurgitated. The higher chewing rate and rumination time observed for the ammonia-treated straw diets in this experiment shows that the number of chews per min ruminating time increased while the number of boluses per min ruminating time decreased. This indicates a greater comminution for fewer boluses. According to Pond *et al.* (1984), the effect of mastication is the exposure of more potentially digestible tissues previously encompassed within indigestible 'barrier' tissues. In an earlier study (Mawuenyegah *et al.*, 1995), it was suggested that improved digestion and improved production of rumen fermentation products were due to increased consistency in chewing during eating and rumination. This may have increased the supply of substrate for ruminal fermentation. McLeod and Smith (1989) asserted that the ease with which the forage is eaten should be considered as the major factor influencing intake. Greater comminution of ammoniated straw provides more energy for microbial synthesis, compared with untreated supplemented straw. This could be another reason for the increased intake and digestion usually associated with ammoniated straw.

Cyclic rate is expressed as the total rumination time per number of boluses regurgitated during rumination (Gordon, 1955). In the present experiment, cyclic rate tended to be shorter for the ammonia-treated straw. According to Gordon (1955) and Fujihara (1980), a short cyclic rate implies a faster rate of reticulo-ruminal contraction. Whether this contributes to the faster rate of clearance of ammoniated straw is uncertain. According to Okine and Mathison (1991) however, the frequency of reticulo-ruminal contraction is not an important indicator of passage from the reticulo-rumen. Cyclic rate is therefore unlikely to be responsible for the increased intake of ammoniated straw but rather an indication of a reduction in the work done in comminuting straw. Considering the greater comminution of ammoniated straw observed in this experiment, this needs to be further investigated by studying the dynamics of ammoniated straw particles in the rumen. If the efficiency of rumination is defined as voluntary intake per unit of rumination time, and the quality of rumination is measured by the number of chews per bolus, then the foregoing discussion suggests that ammonia treatment results in less rumination. Thus ruminants do less work ruminating when given ammonia-treated, rather than untreated, straw diets. Considering the greater comminution of ammoniated straw, the relationship between ammoniation and particle size reduction needs to be investigated further.

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Evaluation of the efficiency of alternative selection schemes and breeding objectives in dairy sheep of Greece

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Abstract

The genetic and economic efficiency of alternative selection schemes and breeding objectives for the Karagouniki dairy sheep in Greece was investigated by model calculations. Criteria of efficiency were the annual genetic gain for the aggregate breeding value, the profit per ewe in the population and the annual selection responses for single traits. The introduction of a two-tier selection scheme, where the recorded ewes are separated into a nucleus and a pre-nucleus, was found superior in both genetic and economic terms for a breeding objective comprising the traits milk yield and number of lambs weaned per ewe per annum. Highest rates for annual genetic gain and selection responses were obtained when the nucleus size was proportionately 0·05 of the population (200 000 ewes), the size of the test matings 0·50 to 0·60 of the size of the pre-nucleus unit (0·10 of the population) and the number of daughters per test ram 40. Opening the nucleus to replacement ewes from the lower tier did not affect positively the annual genetic gain and the selection responses. Furthermore, a breeding objective comprising the traits fat yield and number of lambs weaned per ewe and per annum was found very efficient in both genetic and economic terms while selection on growth and carcass traits did not seem to be justified.

Keywords: *breeding programmes, dairying, Greece, selection programme, sheep.*

Introduction

In Greece, about 10·5 million sheep are kept which can be classified into various breeds and types. One of the most numerous indigenous breeds, being kept on the lowland areas of central Greece (Thessalia), is the Karagouniki breed. It is a medium-sized animal with adult ewe weight between 40 and 60 kg. Despite the extent to which the Karagouniki breed has been crossed with other indigenous and 'exotic' breeds there still remain about 200 000 purebred sheep in this region. The main objectives of current selection are the maintenance of hardiness and adaptability and the improvement of fecundity and milk production. However, there is no organized and consistent breeding policy being applied. About 20 000 ewes are milk recorded on an A-type recording system (monthly recording of the two daily milkings). The mean milk yield of the recorded ewes is 144 l per lactation after the weaning period (Rogdakis *et al.*, 1988) and the prolificacy 1·4 lambs per ewe per annum. The milk produced is almost entirely used for making traditional cheeses, the most well known being the Feta cheese. The lambs are weaned at about 42 days and slaughtered at a live weight of 9 to 14 kg. Only a small proportion is retained for fattening to heavier weights. Although consumers are considered to discriminate against fat deposition there are no official standards of quality assessment at any carcass weight. The main income of sheep farming comes from milk and meat. The wool produced is of medium to poor quality and therefore of no economic interest.

In the past, there has been some importation of 'exotic' dairy breeds in Greece, especially of the German Friesian milk sheep. The difficulties of maintaining this breed are its poor adaptability under Greek conditions and the unsatisfactory performance of its crosses with the Chios breed (Zervas *et al.*, 1975; Kalaisakis *et al.*, 1977) indicate the usefulness of developing modern, consistent purebreeding selection schemes for indigenous breeds. The development of selection schemes in pure breeds has the important advantage of

balancing the genetic improvement of traits with other essential qualities such as adaptation to the environment and the local production system. This paper deals with aspects of genetic and economic efficiency of various selection schemes and breeding objectives for the dairy sheep of Greece, taking the Karagouniki breed as an example.

Material and methods

Definition of the breeding objectives and selection criteria

The first component of the evaluation of the efficiency of alternative breeding schemes is the definition of the breeding objectives, i.e. the choice of traits to be genetically improved because they influence the producer's income. As market demands change over time, all breeding objectives, are, to some extent, speculative. For this reason, three possible breeding objectives were examined.

Objective 1. This objective comprises the traits milk yield and number of lambs weaned at 42 days per ewe per annum. This objective reflects the current production and marketing system.

Objective 2. This objective comprises the traits fat yield and number of lambs weaned at 42 days per ewe per annum.

Objective 3. This objective comprises the traits fat yield, number of lambs weaned at 32 days per ewe per annum, daily gain under field conditions and carcass fat depth. In objective 3 it is assumed that the breed may serve as a dual-purpose sheep for producing both, milk and meat. In that case, market demands may lead to an earlier weaning age of lambs, say at 32, in contrast to 42 days, allowing earlier exploitation of ewes' milk and fattening of lambs to heavier weights. In such an alternative, both fattening traits, such as daily gain from weaning to slaughter age (about 112 days) and carcass characteristics, such as carcass fat depth, are expected to play an economic rôle and therefore have to be incorporated in the breeding objective.

The economic values (v) of the various traits (Table 1) were calculated as marginal profits per unit of improvement for each trait in the aggregate breeding value, i.e. by subtracting the marginal costs from the marginal returns (Kominakis, 1991). The relative importance of the various, milk, reproduction, fattening and carcass traits within each breeding objective is given by its relative monetary genetic deviation, $\%s_A.v$. (Table 2). Milk production plays a predominant rôle even within a dual-purpose objective. In contrast, reproduction as well as fattening and carcass traits are of minor importance across the three breeding objectives.

Table 1 *Economic values (ECUs), genetic standard deviations (s_A), monetary genetic deviations ($s_A.v$) and unit of measurement of the traits in the breeding objectives*

Traits	Economic value (ECUs)	Genetic standard deviation (s_A)	$s_A.v$
Milk traits			
Milk yield (kg)	0·57	14·4	8·2
Fat yield (kg)	9·50	1·1	10·4
Reproduction traits			
No. of lambs weaned at 42 days	34·70	0·1	3·5
No. of lambs weaned at 32 days	22·70	0·1	2·3
Fattening and carcass traits			
Daily gain (field) (g)	0·066	15·5	1·0
Carcass fat depth (mm)	−6·00	0·5	−3·0

Selection criteria in objective 1 are milk yield and number of lambs born per ewe per annum, in objective 2, fat yield and number of lambs born per ewe per annum and in objective 3, fat yield, number of lambs born per ewe per annum, daily gain measured in a station and ultrasonic fat depth, measured by ultrasonic scanning. These traits were chosen because their recording is already applied, e.g. milk yield, or is feasible on-farm, e.g. number of lambs born and because of their high heritability and genetic correlations with the traits in the aggregate breeding value, e.g. ultrasonic and carcass fat depth.

Model calculations, population and genetic parameters

The model calculations were carried out using the computer program ZPLAN (Karras, 1984; Niebel *et al.*, 1989). Based on genetic, biological and economic input variables, the program calculates the annual genetic gain for the aggregate breeding value, the

Table 2 *Monetary genetic deviations (% $s_A.v$) of the various traits within the three breeding objectives*

Traits	Breeding objective		
	Objective 1	Objective 2	Objective 3
Milk	70	75	61
Reproduction	30	25	14
Fattening and carcass			25

Objective 1: milk yield + number of lambs weaned at 42 days per ewe per annum.
Objective 2: fat yield + number of lambs weaned at 42 days per ewe per annum.
Objective 3: fat yield + number of lambs weaned at 32 days per ewe per annum + daily gain (field) + carcass fat depth.

annual response for each selection and correlated trait defined and the profit per female animal in the population by subtracting breeding costs from returns. The calculations assume unchanged selection strategies and constant genetic, biological and technical parameters during the investment period. The gene flow method approach is used and only the effect of one round of selection on the performance of succeeding generations is accounted for. Maternal effects, reduction of genetic variance due to selection and inbreeding depression are neglected. Classical selection indices are calculated for breeding animals and order statistics are applied to get adjusted selection intensities for populations with finite sizes.

A total population of 200 000 ewes is considered and the size of the breeding unit, where milk recording is applied, is 0·10 of the population. Important parameters for the population structure are the proportion of the test matings which is assumed to be 0·35 of the breeding unit and the number of the daughters per test ram which is 40. The heritabilities used as well as the genetic and phenotypic correlations among the traits in the aggregate breeding values, the selection criteria and some correlated traits of interest (Table 3) were derived from literature (Purser, 1965; Ch'ang and Rae, 1972; Forrest and Bichard, 1974; Casu *et al.*, 1975; Mavrogenis *et al.*, 1980; Wolf *et al.*, 1981; Cameron and Smith, 1985; Atkins and Thompson, 1986a and b; Barillet and Boichard, 1987; Simm *et al.*, 1987; Parratt *et al.*, 1987; Barillet *et al.*, 1988; Simm and Dingwall, 1989). In some instances the information available was very limited and some assumptions had to be made. Where a phenotypic correlation was found but not a genetic correlation and *vice versa*, the genetic estimate was assumed to be 1·15 times greater than the phenotypic estimate.

Biological coefficients were 1·5 to 1·3 for litter size across the various tiers, age at birth of first offspring between 1·4 and 5·0 years, according to the point of time that selection of animals occurs, ram/ewe ratio 1/20 and 1/400 for natural mating and artificial insemination, respectively, two inseminations per successful pregnancy and annual survival rates of rams and ewes between 0·90 and 0·95.

For the profit estimation the following cost components were calculated. The required fixed breeding costs of a breeding unit with sizes 10 000 and 80 000 ewes are 28 300 and 75 500 European currency units (ECUs), respectively. Furthermore, costs for milk recording are 10·0 (milk yield) and 15·0 (fat yield) ECUs per ewe and year, respectively, for artificial insemination 2·0 per insemination, 410·0 ECUs for each waiting ram and year until progeny records are obtained and 125·0 ECUs for each tested ram and year for recording fattening performance in station. Other variable breeding costs are: recording of litter size 0·5 ECUs per ewe and year, planned

Table 3 *Heritabilities (on the diagonal), genetic correlations (below the diagonal) and phenotypic correlations (above the diagonal) of some milk, reproduction, fattening and carcass traits in sheep (average literature estimates)*

Traits	1	2	3	4	5	6	7	8	9	10	11
1. Milk yield (kg)	**0·25**	0·80	0·80	–0·20	–0·25	0	0	–0·20	–0·15	0·10	0·10
2. Fat yield (kg)	0·80	**0·30**	0·80	0·30	–0·05	0	0	–0·15	–0·15	0·10	0·10
3. Protein yield (kg)	0·80	0·80	**0·25**	0·05	0·10	0	0	–0·15	–0·15	0·10	0·10
4. Fat content (g/l)	–0·30	0·40	0·05	**0·50**	0·45	0	0	0	0	0	0
5. Protein content (g/l)	–0·35	0	0·05	0·50	**0·50**	0	0	0	0	0	0
6. No. of lambs born	0·05	0·05	0·05	0	0	**0·10**	0·10	0	0	0	0
7. No. of lambs weaned	0·10	0·05	0·05	0	0	0·70	**0·05**	0	0	0	0
8. Carcass fat depth (mm)	–0·30	–0·25	0·25	0	0	0	0	**0·30**	0·45	0·25	0·20
9. Ultrasonic fat depth (mm)	–0·25	–0·25	–0·10	0	0	0	0	0·65	**0·20**	0·20	0·15
10. Daily gain in station (g)	0·10	0·10	0	0	0	0	0	0·30	0·25	**0·30**	0·90
11. Daily gain in field (g)	0·10	0·10	0	0	0	0	0	0·20	0·15	0·75	**0·15**

matings 1·0 ECUs per ewe and year. The costs are assumed to be discounted at a rate of 0·03 and the returns at a higher rate of 0·05; the investment period is considered 25 years.

Selection schemes

The genetic and economic efficiency of three selection schemes was investigated for objective 1; these were devised by accounting for applicability and limited use of artificial insemination (AI) under current conditions of the Greek dairy sheep industry. The first selection scheme (Young Ram), assumes no progeny testing of rams, no use of AI, while selection of ewe lambs is based on pedigree information and of young rams on pedigree and paternal half-sib records. The second selection scheme (Old Ram) presumes progeny testing of rams for milk performance, selection of ewe lambs is based on pedigree information, of young rams on pedigree and paternal half-sib records and of the progeny tested rams on pedigree and progeny records. Furthermore, AI is used for accomplishing the test matings. The third selection scheme (Nucleus) is the same as the preceding but here the breeding unit is separated into two tiers: a nucleus (0·005 of the population) formed by 'elite' flocks, where ewes are selected on two successive lactation records and a pre-nucleus (0·095 of the population) where ewes are selected on pedigree and first lactation records. Males are selected as in the Old Ram scheme. The pre-nucleus serves as a basis for screening superior ewes for nucleus replacements, to provide the capacity for progeny testing and to operate as ram multiplier for commercial herds. In this selection scheme, AI is used for both planned and test matings. Furthermore, assuming standardized environmental conditions in the nucleus flocks, the heritabilities given for the selection traits can be higher, assumed to be 1·25 times. It is assumed that the breeding unit where milk recording is applied, has the same size across the three selection schemes, i.e. 0·10 of the population. In both the Old Ram and Nucleus schemes, the size of test matings is 0·35 of the breeding unit and the number of daughters per test ram 40. The nucleus is 0·40 open, i.e. 0·40 of the annual ewe replacements in the nucleus is coming from the pre-nucleus.

Variation of the population and biological parameters

The effect on annual genetic gain of altering four population parameters and one biological parameter was investigated. The four population parameters were: nucleus size in relation to the pre-nucleus size, proportion of the test matings in relation to the number of daughters per test ram and degree of opening the nucleus to replacement rates from the pre-nucleus. Nucleus size was varied in three levels: 0·005, 0·01 and 0·05 of the total population in relation to four pre-nucleus sizes: 0·10, 0·20, 0·30 and 0·40 of the population. Test matings were assumed to be 0·35 of the pre-nucleus and the number of daughters per test ram was 40. The degree of opening the nucleus was 0·40.

The proportion of the test matings was varied from 0·20 to 0·60 of the pre-nucleus and the number of daughters per test ram was varied from 20 to 80. The nucleus and pre-nucleus sizes were 0·005 and 0·095 of the population, respectively and the degree of opening the nucleus was 0·40.

The degree of opening the nucleus was varied from 0 to 100% replacement rates from the pre-nucleus. The nucleus and pre-nucleus sizes were 0·005 and 0·095 of the population, respectively, the test matings were 0·60 of the pre-nucleus and the number of daughters per test ram was 60.

The effect of the biological parameter investigated was the productive lifetime of the various selection groups. These animal groups were: sires of sires and sires of dams (variation of productive lifetime from 1 to 2 years), dams of sires (variation of productive lifetime from 1 to 3 years) and dams of dams (variation of productive lifetime from 3 to 4 years).

Results

Comparison of the economic and genetic efficiency of the three selection schemes

The economic and genetic efficiency of the three selection schemes is discussed in terms of annual genetic gain, profit per ewe in the population (in ECUs) and annual selection responses (Table 4).

Results reveal a clear superiority of the Old Ram scheme over the Young Ram in terms of profit 8·92 *v.* 5·17 ECUs) and of annual genetic gain (1·64 *v.* 0·86 ECUs). Proportionally higher rates of annual selection responses, between 0·10 and 0·32 in the case of reproduction traits and up to 0·92 for milk traits, were also obtained. This can be attributed to the progeny testing of the rams. However, the Old Ram scheme has higher variable breeding costs (0·24 *v.* 0·05 ECUs) arising from the costs of keeping the rams until their progeny records are obtained and the use of AI for accomplishing the test matings. Contrasting the Nucleus scheme to the Old Ram one, 0·65 higher genetic gain was attained (2·71 *v.* 1·64 ECUs). Furthermore, 0·60 higher rates of annual selection responses for milk traits and up to 1·60 to 2·00 for the reproduction traits were also obtained. This can be attributed, apart from progeny testing, to the planned matings and the higher selection accuracy of nucleus ewes. However, returns and profit were smaller compared to those achieved in the Old Ram

Table 4 *Economic and genetic efficiency of the three selection schemes under objective 1*

	Breeding schemes		
	Young Ram	Old Ram	Nucleus
Annual genetic gain (ECUs)	0·86	1·64	2·71
Returns (ECUs)	6·33	10·27	9·57
Costs (ECUs)	1·16	1·35	1·37
Profit (ECUs)	5·17	8·92	8·20
Annual selection response per trait			
(a) in the aggregate breeding value			
Milk yield (kg)	1·4	2·7	4·3
No. of lambs weaned	0·0021	0·0029	0·0075
(b) correlated			
Fat yield (kg)	0·084	0·169	0·267
Protein yield (kg)	0·061	0·124	0·195
Fat content (g/l)	−0·10	−0·20	−0·32
Protein content (g/l)	−0·07	−0·15	−0·24
No. of lambs born	0·0033	0·0036	0·0122

scheme (9·57 *v.* 10·27 ECUs) due to the limited size of nucleus and to the longer time lag required to disseminate the genetic gain from nucleus to the production unit as a result of the involvement of the pre-nucleus.

Variation of the size of the nucleus

A change in size of the nucleus from 0·005 to 0·05 of the population was associated with positive effect on annual genetic gain (Figure 1).

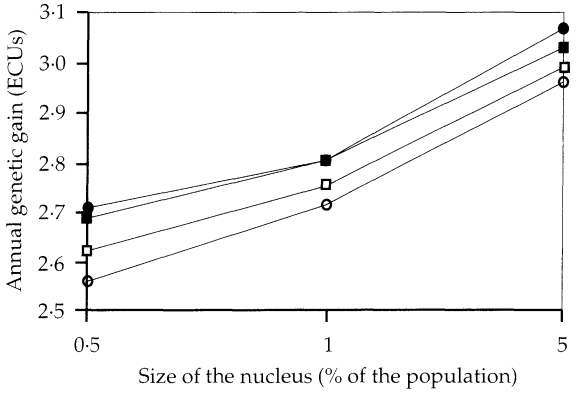

Figure 1 Annual genetic gain (ECUs) in relation to the size of nucleus. Test matings: 0·35 of the pre-nucleus. Number of daughters per test ram: 40. Degree of opening the nucleus: 0·40. Pre-nucleus: 0·10 (—●—), 0·20 (—■—), 0·30 (—❑—), 0·40 (—❍—) of the population.

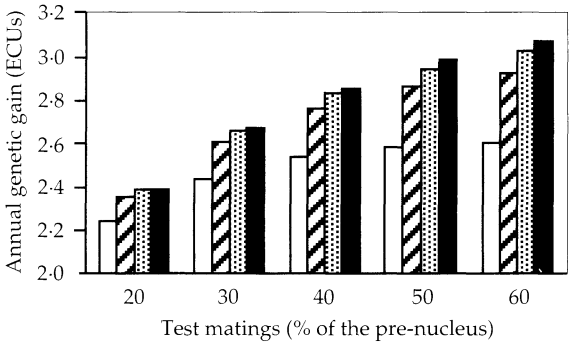

Figure 2 Annual genetic gain (ECUs) in relation to the proportion of the test matings and the number of daughters per test ram. Population size: 200 000. Nucleus size: 0·005 of the population. Pre-nucleus size: 0·095 of the population. Degree of opening the nucleus: 0·40. Number of daughters per test ram: 20 (□), 40 (▨), 60 (▩), 80 (■).

Variation of the proportion of the test matings and the number of daughters per test ram

The increase in the number of the test matings had a positive effect on the annual genetic gain (Figure 2). Increasing the number of daughters per test ram had also a positive effect on the annual genetic gain. However, the latter increase is not proportional, so that for small proportions of test matings (20 to 30% of the pre-nucleus), increasing the number of daughters beyond 40 does not affect the annual genetic gain.

Degree of opening the nucleus

Opening the nucleus by screening some of the female replacements from the pre-nucleus does not have a positive effect on the annual genetic gain (Figure 3).

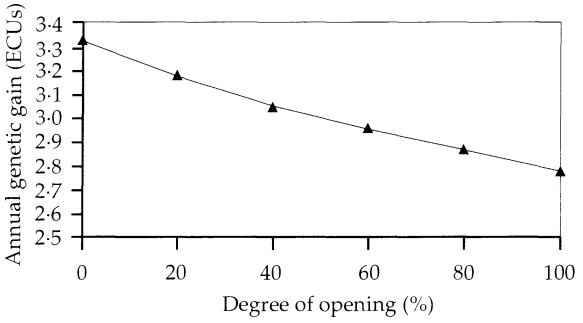

Figure 3 Annual genetic gain (ECUs) in relation to the degree of opening the nucleus. Population size: 200 000. Nucleus size: 0·005 of the populaton. Pre-nucleus size: 0·095 of the population. Test matings: 0·60 of the pre-nucleus. Number of daughters per test ram: 60.

Table 5 *Annual genetic gain (GG) in ECUs, in relation to the productive lifetime of various selection groups in the nucleus*

Productive lifetime (years) of sires to breed sires	Productive lifetime (years) of dams to breed		Productive lifetime (years) of sires to breed dams	
	sires	dams	1 GG (ECUs)	2 GG (ECUs)
1	1	3	3·33	3·13
		4	3·21	3·00
2		3	3·24	3·04
		4	3·13	2·93
1	2	3	3·33	3·12
		4	3·22	3·02
2		3	3·25	3·05
		4	3·14	2·94
1	3	3	3·29	3·09
		4	3·18	2·99
2		3	3·21	3·01
		4	3·10	2·92

Variation of the productive lifetime

Shorter productive lifetimes for the various selection groups resulted in increased annual genetic gains since it is linked to shorter mean generation intervals (Table 5).

Comparison of the relative efficiency of the three breeding objectives

The comparison of the relative efficiency of the three breeding objectives showed that the genetic and economic efficiency of objective 2 is about 0·97 that of objective 3 (Table 6). This figure implies that even in case where it would be necessary to define a breeding objective like objective 3, defining alternatively a breeding objective like objective 2 would result in about 3% losses in efficiencies. The high efficiency of objective 2 is also reflected in the high annual selection responses of single traits in the aggregate breeding value (Table 7). When comparing objective 2 with 1, losses of selection responses for milk yield (5·6 *v.* 4·9 kg) and for the number of lambs weaned (0·0102 *v.* 0·0071) are observed. This can be attributed to the smaller relative importance of reproduction traits in the second objective (see Table 2). In contrast, gains of selection response for fat yield (0·45 *v.* 0·35 kg) were found due to the favourable relative importance of milk traits (fat yield) in objective 2. In objective 3, responses for the reproduction traits were further reduced because of their smaller relative monetary genetic variation. In objective 3, relatively higher rates for daily gain, 0·74 *v.* 0·65 g (objective 2) and carcass fat depth –0·072 *v.* –0·065 mm (objective 1) were achieved as a result of selection on these traits.

Table 6 *Relative genetic and economic efficiency of the three breeding objectives*

Defined breeding objective	Alternative breeding objective					
	Objective 1		Objective 2		Objective 3	
	GG†	Profit	GG	Profit	GG	Profit
Objective 1	100	100	85·4	84·0	85·5	83·7
Objective 2	81·7	79·4	100	100	99·9	99·8
Objective 3	80·8	77·4	97·6	96·5	100	100

† Annual genetic gain (ECUs).

Table 7 *Annual selection response of traits in the aggregate breeding values for the three breeding objectives*†

	Objective 1	Objective 2	Objective 3
Milk yield (kg)	5·6	(4·9)	(5·0)
Fat yield (kg)	(0·35)	0·45	0·46
No. of lambs weaned	0·0102	0·0071	0·0051
Daily gain (g)	(0·61)	(0·65)	0·74
Carcass fat depth (mm)	(–0·065)	(–0·057)	–0·072

† Numbers in parentheses denote no emergence of the traits in the aggregate breeding value.

Discussion

Usually in establishing a breeding programme compromises have to be made between achieving a certain degree of selection accuracy and minimizing costs. The compromise is reflected in the extent to which milk recording and artificial insemination services can be operated and adapted for use in animal improvement (Hinks, 1978). In dairy sheep both activities are associated with either high costs or certain technical constraints, respectively. Costs of milk recording for ewes, per unit of milk quantity, are significantly higher compared with other dairy species (Flamant and Barillet, 1982). Until now, milk recording in the Karagouniki ewes has been carried out with the official method, A-type. Results have showed that even in the case where the selection programmes are charged with high variable breeding costs such as the recording of milk fat, genetic improvement in the Karagouniki breed could be coupled with respectable profit (20 to 30 ECUs) per ewe in the population. It should be pointed out, however, that these figures were attained under optimal population structure and selection strategies.

Another important constraint also to be considered is the use of the AI technique in combination with the synchronization of oestrus. Due to intensified research and technical development in recent years

these techniques have greatly advanced in countries of western Europe (e.g. France) where there is a high density of sheep. The inseminations are mainly carried out with chilled semen. This, however, allows the use of the semen only within a radius of 200 to 300 km around the AI centre. Although results are satisfactory, two inseminations are needed to achieve high rates of fertility. This makes AI in dairy sheep an expensive method and explains why it is not widespread. On the other hand, whilst the freezing process is simple and efficient there are some practical problems preventing the use of deep-frozen semen of progeny tested rams on a larger scale. This limits the efficiency of semen deep-freezing and makes it an expensive activity justified and reserved only for the best sires (Collas, 1979). AI in the Karagouniki breed is already being applied using fresh semen. In 1988, about 14 000 doses were used and thus about 7 000 ewes could be artificially inseminated per year. Since a certain amount of expertise is already achieved, a potential increase in the use of AI in breeding can be regarded as possible. This increase would, however, require high diversification of AI facilities and expenses.

As the first breeding scheme (Young Ram) showed, genetic improvement may be attained without AI. However, in order to achieve high rates of annual genetic gain, of profit and selection responses, the use of AI has to be introduced and diversified. Only AI enables the adoption of selection activities like progeny testing and planned matings in a most efficient way. This fact is clearly demonstrated by the high genetic and economic efficiency of the Old Ram and Nucleus schemes. Progeny testing of breeding rams can be carried out by natural mating, as practical experience in Italy and France has proved (Barillet *et al.*, 1986), but it is linked with some disadvantages. Only a small number of daughters per test ram, dispersed in a few herds able to provide testing facilities, can be attained. As a consequence, high selection accuracy of progeny tested rams cannot be achieved and possible genotype × environment interactions may lead to biased estimates for the breeding values of rams. Furthermore, in order to achieve high selection responses about 0·60 to 0·70 of the ewes in the recorded population should be mated with test rams (Barillet *et al.*, 1986).

Thus, a first step for developing a modern selection strategy for the Karagouniki breed could be to organize and diversify the test mating procedure by increasing the number of the test matings and recording up to 40 daughters per test ram.

A second step should be the establishment of a nucleus. The nucleus displays the advantage of the standardized environmental conditions which allow the increase in the accuracy of the estimation of the breeding values of breeding animals. The advantage of the open nucleus by screening female animals from a larger basis and thus achieving a higher selection intensity for the nucleus female replacements does not apply here. The reason for this can be found in the lower selection accuracy of the females outside the nucleus. The nucleus could therefore be kept closed to avoid costs arising from transferring larger number of animals from pre-nucleus herds. This is also found in other literature references regarding meat and wool sheep (Jackson and Turner, 1972; Hopkins, 1978). An increase in the size of the nucleus from 0·005 to 0·05 resulted in increased annual genetic gain. This result can be ascribed to the increased selection intensity of the elite ewes taking part in the planned matings and of the test rams to accomplish the test matings. The size of the nucleus should be large enough (about 0·05) of the population because of three reasons: (i) it would provide the whole system with rams of superior genetic qualities since only progeny tested rams would breed in the nucleus and young rams, offspring of the progeny tested rams, would mate in the production unit on a natural mating basis; as a consequence, the time-lag required to disseminate the genetic superiority of the nucleus to the production tier could be substantially reduced, (ii) it forms a wide selection basis for selecting the ewes taking part in the planned matings and (iii) it minimizes the rates of inbreeding. A larger nucleus may be also profitable but in such a case the limiting factor might be the associated higher costs.

The nucleus may be first introduced on institutional herds (supervised by the Ministry of Agriculture) and developed gradually over a certain period. This would also favour close supervision, continual monitoring and demonstration of techniques. As experience improves and profits accrue, the nucleus could be extended in size. Another possibility is the establishment of a nucleus in the form of a small enterprise. This enterprise could be funded by some of the breeders already participating in the milk recording scheme.

Results have also demonstrated that a breeding objective comprising fat yield and number of lambs weaned per ewe per annum represents a very efficient and realistic breeding objective for the Karagouniki breed. However, in order to achieve such a breeding objective, selection should be based on the traits fat yield and number of lambs born per ewe per annum. In such a case, however, recording of fat content has to be introduced widely to the whole recorded population. Alternatively, this could be achieved rationally by adopting simplified

recording methods, for instance, by reducing the number of samples (two to three) tested on fat content per ewe and lactation period. While the possibility of widespread use of fat recording seems rather unrealistic at least for the next 5 to 10 years, this proposal could serve as a guideline for distant future breeding activities. Selection on fat yield has the important advantages of no loss of protein and fat content and of an increase in fat and protein yields. These responses have an impact on the cheese production efficiency and therefore are of major importance for the regional creameries.

The incorporation of daily gain and carcass fat depth in the breeding objective did not prove to be much more profitable or genetically efficient overall. This result is in agreement with other literature references. In proposing a general improvement strategy for dual-purpose sheep Barillet *et al.* (1988) examined several selection schemes comprising selection on milk yield alone, on milk yield and prolificacy and finally on milk yield, prolificacy, growth rate and carcass traits. AI in the recorded population combined with natural mating in the commercial flocks was assumed. Results in this work showed that selection on fattening and carcass traits leads to increases of only about 0·08 for the discounted returns. It is, however, not clear whether the various proposed selection schemes shared the same or different breeding objectives. Furthermore the authors have concluded that in breeds with small adult weight (40 to 60 kg), such as the Manech and Sarda breeds, selection should be restricted to merely milk performance while big-sized breeds, such as the Lacaune and Manchega, with adult body weight 70 to 80 kg, combined selection on milk and fattening performance may be justified.

In the Karagouniki breed there are three types: the Kardista, Trikala and the Palama type. The latter one represents a heavier type than the other two. In cases where the production system changes to fattening lambs to heavier weights, selection on fattening performance and carcass composition could concentrate on this type. Genetic improvement is one of the most useful strategies for effective change in carcass composition, since it is permanent, cumulative and usually highly cost effective (Simm and Murphy, 1996). A number of selection experiments have been conducted involving divergent selection on measurements of ultrasonic fat depth, adjusted for live weight (Bennett *et al.*, 1988) or on indices based on live body weight and ultrasonic fat depth (Cameron and Bracken, 1992; Bishop, 1993). The indices were designed to alter body composition without a corresponding change in live weight. In all of these experiments rates of genetic change in excess of proportionately 0·01 to 0·04 per annum for ultrasonic backfat and muscle depths, increased carcass lean weight and proportion and decreased fat weight and proportion, have been achieved.

Another strategy, which allows rapid adaptation of the production system to changing market demands, is crossbreeding the ewes not needed for herd replacements in the hill and mountain regions, mostly small-sized animals, with rams of specialized meat breeds or with rams of heavier breeds or types. There is enough evidence that fattening performance of native meat ewes can be improved when mated with rams of exotic breeds. Crossing Suffolk rams with Ghezel (a fat-tailed Iranian sheep) ewes, resulted in improved birth weight, daily gain and final weight (Taleghani *et al.*, 1975). Tomov (1969) reported that crossing Dobricka ewes (a Bulgarian native breed) with Hampshire, Oxford, Suffolk and Clun Forest rams increased birth weight, weaning weight and post-weaning daily gain. Makarechian *et al.* (1977) in a study of crossing three fat-tailed Iranian ewes with Corriedale and Targhee rams found that crossbreds were superior to purebreds, 7% in pre-weaning average daily gain and up to 18·4% in the post-weaning daily gain. Such a strategy, however, may be linked with certain problems, the most important being the poor adaptability of exotic rams under the harsh environmental conditions in Greece.

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Estimating the metabolizable energy requirement for pregnancy in sheep

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Abstract

A model is developed in which the metabolizable energy (ME) requirement for growth and maintenance of the foetus, uterus and udder are described explicitly for sheep. The efficiency of the use of ME for the growth of the products of conception (k_p) is assumed to be the same as that for growth in non-pregnant animals. Using the results from the model to calculate the efficiency of the use of ME for conceptus growth (k_c) in the conventional manner yields values with a similar magnitude and similar variation with food quality as those estimated experimentally. It is concluded that separately accounting for the growth and maintenance of the foetus, uterus and udder is preferable in models but unnecessary in feeding standards.

Keywords: *efficiency, energy, mathematical models, pregnancy, sheep.*

Introduction

Agricultural Research Council (1980) suggested a value of 0·133 for the efficiency of use of metabolizable energy (ME) for growth of the conceptus (k_c). This or similar values have been used extensively for calculating the daily ME requirement of pregnant sheep (e.g. CSIRO, 1990; Agricultural and Food Research Council (AFRC), 1993) and in the simulation modelling sheep production (e.g. Vera *et al.*, 1977; Geisler and Jones, 1979; Finlayson *et al.*, 1995). The authors of the Australian Feeding Standards for Livestock (CSIRO, 1990) point out that this efficiency is surprisingly low when compared with the efficiency of use of ME for weight gain in non-pregnant animals. They suggest that this is because all the energy costs (the growth and maintenance of uterus, foetus and udder) are incorporated in the calculation of the efficiency of ME use for conceptus growth. This is not always the case; Rattray *et al.* (1974a) deducted the energy used for mammary growth when calculating the efficiency of energy use for conceptus growth. Nevertheless, it is usual to include the udder growth and cumulative additional maintenance costs in the calculation of k_c.

Calculating k_c in the above manner has implications for the way it can be used in estimating daily energy requirements or the daily consequences of a given level of energy intake by pregnant animals. To be theoretically justifiable, the following conditions must be met: (a) the energy deposition in the mammary tissue should be ignored; (b) the calculation of the maintenance energy requirement should be made on the basis of the live weight of the non-pregnant animal.

If these conditions are not met, there will be double accounting of the additional energy requirements during pregnancy.

Under some circumstances, it may be preferable explicitly to account for both the growth of mammary tissue and the maintenance requirement of the products of conception. Such accounting is possible if one can estimate the growth of the mammary tissue, the true efficiency of ME use for conceptus growth and the maintenance requirements of the conceptus and the additional mammary tissue. The following model is used to investigate how this may be achieved and the circumstances under which it might be worthwhile.

Modelling the ME demands for pregnancy in sheep

In the following text, the true efficiency of ME use for conceptus and udder growth is denoted by k_p and the efficiency of ME use for conceptus growth,

calculated in the conventional manner, is denoted by k_c. A key to the variables is in the **Appendix.**

General assumptions
For simplicity, the model is restricted to situations where (a) there is no remobilization of maternal tissue to support pregnancy and (b) the quality of the food remains constant throughout pregnancy.

The components of animal weight
The weight of the ewe ($M_{e,t}$; kg) is considered to be the fasted body weight minus that of the foetus ($M_{f,t}$; kg). $M_{e,t}$ is simulated as follows:

$$M_{e,t} = M_n + M_{pl,t} + M_{fl,t} + M_{u,t} + M_{g,t} \quad (1)$$

where t is the time since conception (days), M_n is the fasted body weight of the non-pregnant ewe and $M_{pl,t}$, $M_{fl,t}$, $M_{u,t}$ and $M_{g,t}$ are respectively the weights of the placenta, fluids, additional weight of the uterus and additional weight of the udder, all expressed in kg.

The simulation of the weights of the components of the conceptus uses the relationships derived by Robinson *et al.* (1977) and Geisler and Jones (1979). Foetal weight, $M_{f,t}$, is given by:

$$M_{f,t} = M_{f,T} \times \exp(2{\cdot}42 - 17{\cdot}6 \times \exp(-0{\cdot}0198 \times t) - 79 \times 10^{-5} \times N + 0{\cdot}0046 \times M_n)/5{\cdot}3 \quad (2)$$

where $M_{f,T}$ is the lamb birth weight and N is the number of foetuses carried. $M_{f,T}$ is given by:

$$M_{f,T} = \exp(0{\cdot}0046 \times M_n + 1{\cdot}34) \quad (3).$$

The weight of the placenta, $M_{pl,t}$, is given by:

$$M_{pl,t} = M_{f,T} \times \exp(-0{\cdot}387 - 245 \times \exp(-0{\cdot}1 \times t) - 0{\cdot}119 \times \mathrm{N})/5{\cdot}3 \quad (4).$$

The weight of the fluids, $M_{fl,t}$, is given by:

$$M_{fl,t} = M_{f,T} \times \exp(-11{\cdot}5 + 0{\cdot}326 \times t - 0{\cdot}0032 \times t^2 + 10{\cdot}2 \times 10^{-6} \times t^3)/5{\cdot}3 \quad (5).$$

The additional weight of the uterus, $M_{u,t}$, is given by:

$$M_{u,t} = M_{f,T} \times \exp(1{\cdot}56 - 3{\cdot}73 \times \exp(-0{\cdot}0038 \times t) + 0{\cdot}094 \times N + 0{\cdot}0099 \times M_n)/5{\cdot}3 - M_{u,n} \quad (6)$$

where $M_{u,n}$ is the weight of the uterus in the non-pregnant animal (kg).

The additional weight of the mammary tissue in pregnant sheep was derived from measurements by Mellor and Murray (1985). Using their measurements of udder dimension A, the relationship between the

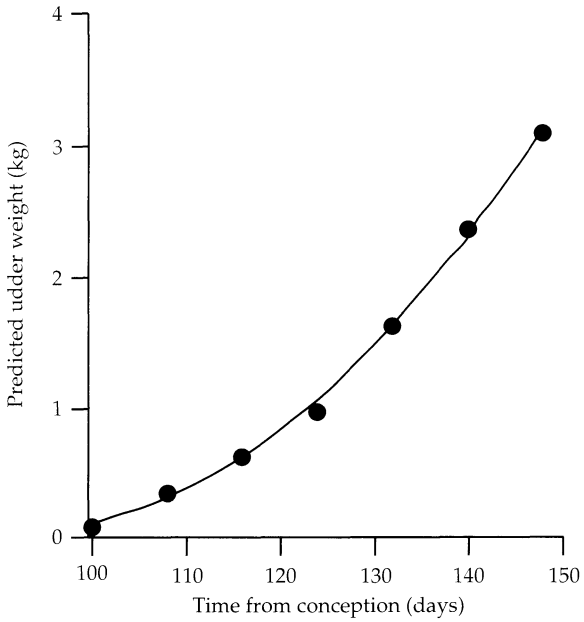

Figure 1 The predicted relationship between udder weight and time from conception. Data points calculated from Mellor and Murray (1985).

A dimension and the weight of mammary tissue was used to estimate the development of the udder with t (Figure 1). After scaling by the lamb birth weight in this study, the udder weight could be described by the following quadratic equation ($R^2 = 0{\cdot}99$):

if $t > 100$ then

$$M_{g,t} = M_{f,T} \times (1{\cdot}73 - 0{\cdot}037 \times t + 0{\cdot}0002 \times t^2) \quad (7)$$

otherwise $M_{g,t} = 0$.

Energy budget
The cumulative ME requirement for a pregnant sheep over the period of gestation (R_p; MJ) is given by:

$$R_p = \sum_{t=1}^{T} (E_{m,t}/k_m) + (E_c + E_g)/k_p \quad (8)$$

where T is the gestation period (days), $E_{m,t}$ is the daily maintenance energy requirement at time t (MJ), k_m the efficiency of ME use for maintenance and E_c and E_g the ME within the conceptus and the udder respectively at full term (MJ).

Maintenance energy
The maintenance energy demand is assumed to derive partly from the maintenance demand of the

foetus and partly from that of the ewe. It is assumed that the maintenance of the ewe and the foetus can be calculated using the formula used by (AFRC, 1993). This allows for the fasting metabolism plus an activity component:

$$E_{m,t} = 0{\cdot}23 \times (M_{e,t}^{0{\cdot}75} + M_{f,t}^{0{\cdot}75}) + 0{\cdot}0054 \times (M_{e,t} + M_{f,t}) \quad (9).$$

Energy within the products of conception
The energy within the conceptus at full term was simulated by the relationship found by Langlands and Sutherland (1968). After conversion of units and scaling by birth weight, this yields the, relationship:

$$E_c = 4{\cdot}8 \times M_{f,T} \quad (10).$$

The energy within the udder at full term (E_g) was simulated by multiplying the additional weight of the udder at term (from equation 7) by the energy concentration at term (11 MJ/kg) found by Rattray *et al.* (1974b).

Efficiency of energy use for maintenance and growth
The efficiency of energy use for maintenance is taken from AFRC (1993) and is given by:

$$k_m = 0{\cdot}35 \times q_m + 0{\cdot}503 \quad (11)$$

where q_m is the metabolizability of the food.

The efficiency of energy use for growth of the products of conception (k_p) was simulated using the equation quoted by AFRC (1993) for the efficiency of energy use for growth in non-pregnant animals (k_f):

$$k_p = 0{\cdot}78 \times q_m + 0{\cdot}006 \quad (12).$$

No adjustment was made for the effect of level of feeding.

k_c was calculated by dividing the energy in the conceptus at full term (E_c) by the additional energy requirement of pregnancy:

$$k_c = E_c/(R_p - E_{m,n}) \quad (13)$$

where $E_{m,n}$ (MJ) is the cumulative maintenance energy requirement of a non-pregnant animal for a period equivalent to the gestation time, calculated with the formula used in AFRC (1993).

Simulations
The model was used to simulate the energy demand of a ewe with a non-pregnant weight of 37·6 kg, corresponding to the mean of the animals used in the experiment of Rattray *et al.* (1974a).

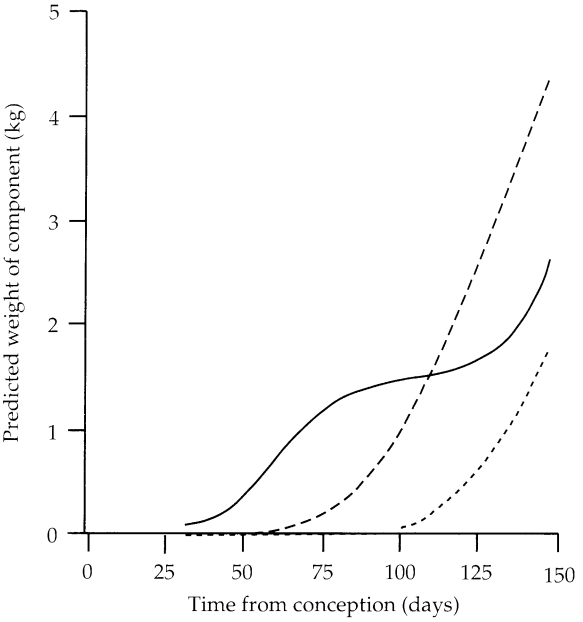

Figure 2 The predicted weight of udder (- - - -), foetus (— — —) and the remaining components of the conceptus (——) over the period of gestation.

Results

The predicted growth of the foetus, udder and remaining components of the conceptus are shown in Figure 2. The efficiencies of energy use for conceptus growth (k_c), calculated using equation 13 from the results of the simulations, are given in Table 1. The corresponding values of k_p, calculated using equation 12, are shown for comparison. The variation with diet quality of the predicted energy demand, summed over gestation, is shown in Figure 3.

Table 1 *Predicted variation of* k_p *and* k_c *with the metabolizability of food* (q_m)†

q_m	k_p	k_c
0·4	0·32	0·11
0·5	0·40	0·13
0·6	0·47	0·15
0·7	0·55	0·17
0·8	0·63	0·18

† k_p = efficiency of energy use for the growth of the conceptus (equation 12). k_c = energy deposited in conceptus/additional energy required for pregnancy (equation 13).

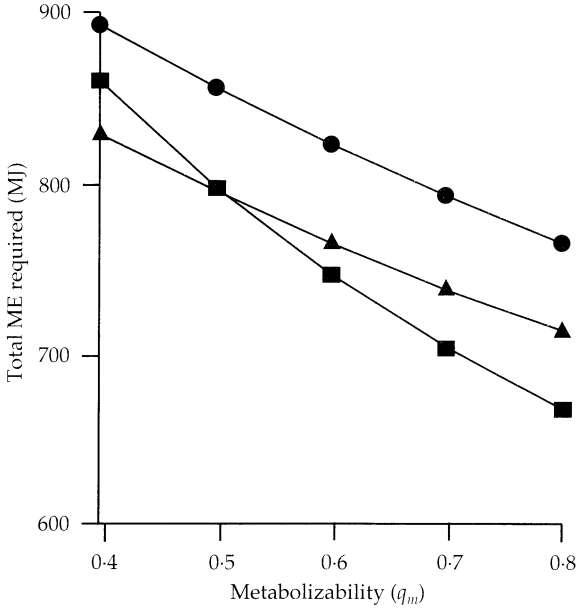

Figure 3 The relationship between the total metabolizable energy (ME) required during gestation and the food metabolizability, predicted by the model (■), AFRC-A (●) and AFRC-B (▲).

For comparison, the total energy demand over gestation was also calculated using the method recommended by AFRC (1993). AFRC (1993) does not specify whether the pregnant or non-pregnant live weight should be used in calculating the maintenance energy demand so here this was calculated on the basis of either (1) the current fasted body weight (AFRC-A) or (2) the fasted body weight of the non-pregnant ewe (AFRC-B). In both AFRC-A and -B. the total energy requirements over gestation are calculated as:

$$R_p = \sum_{t=1}^{T} (E_{m,t}/k_m) + E_c/k_c \qquad (14)$$

where $k_c = 0{\cdot}13$. For AFRC-A, all other variables are as defined in the main model. AFRC-B is the same as AFRC-A except $E_{m,t} = E_{m,n}$ (equation 14). The total energy requirements over gestation as calculated by these two methods are shown in Figure 3.

Discussion

The predicted efficiencies of energy use for conceptus growth (k_c) are much lower than the actual efficiency of energy use for growth that was used in the model (Table 1). This is because the calculation of k_c includes the maintenance costs of the products of conception and the cost of udder growth. The predicted values are within the range found by Robinson *et al.* (1980) and show a similar dependence on the metabolizability of food as described in that study. This suggests that in systems that separately account for the weight gain and maintenance of pregnancy related growth, it may be assumed that the efficiency of use of ME for the weight gain may be calculated with the relationship used for non-pregnant animals (e.g. k_f in AFRC, 1993).

Using k_c and calculating maintenance energy on the basis of actual rather than non-pregnant fasted body weight (AFRC-A) generates estimates for the total energy requirement during gestation that are higher than those predicted by AFRC-B and by the model (Figure 3). The effect of double accounting for the maintenance costs of the weight gain associated with pregnancy is relatively small because most of the gain occurs towards the end of gestation (Figure 2). The differences between the estimates obtained with the different methods are also small in relation to the between-animal variation in intake and energy utilization. The similarity between the predictions of AFRC-B and the model suggests that the effect of food quality on the additional energy requirement during pregnancy may be ignored without greatly affecting the estimate of the total energy requirement.

The results of the simulations suggest there is no pressing need separately to account for the growth and maintenance of the udder and the components of the conceptus when calculating the energy requirements in connection with feeding recommendations. There is a need for greater clarity in defining the weight term used in the calculation of the maintenance energy requirements during pregnancy. The present study might also alert readers to the differences between the methods of calculating k_c and k_f if the former were termed the cumulative efficiency. Models, in contrast to feeding recommendations, are often intended to encapsulate our current knowledge, in addition to yielding predictions. In this case, it is preferable to include an explicit description of the energy costs of the growth and subsequent maintenance of the weight gain associated with pregnancy.

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Appendix

Key to variables

E_c ME within the conceptus at full term (MJ)
E_g ME within the udder at full term (MJ)
$E_{m,t}$ maintenance energy requirement at time t (MJ/day)
$E_{m,n}$ maintenance energy requirement of a non-pregnant animal for a period equivalent to the gestation time (MJ)
k_c cumulative efficiency of ME use for conceptus growth (includes maintenance)
k_m efficiency of ME use for maintenance
k_p efficiency of ME use for conceptus growth
M_n live weight of the non-pregnant ewe (kg)
$M_{e,t}$ live weight of the ewe minus the weight of the foetus (kg)
$M_{f,T}$ lamb birth weight (kg)
$M_{f,t}$ weight of the foetus (kg)
$M_{fl,t}$ weight of fluid in the uterus (kg)
$M_{g,t}$ additional weight of the udder during pregnancy (kg)
$M_{pl,t}$ weight of the placenta (kg)
$M_{u,t}$ additional weight of the uterus during pregancy (kg)
$M_{u,n}$ weight of the uterus in the non-pregnant animal (kg)
N number of foetuses carried
q_m metabolizability of the food
R_p cumulative ME requirement for a pregnant sheep over the period of gestation (MJ)
t time since conception (days)
T gestation period (days).

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Modelling responses to selection for resistance to gastro-intestinal parasites in sheep

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Abstract

This paper describes a general framework which enables responses to selection for resistance to gastro-intestinal parasites in sheep to be stochastically modelled. The model incorporates between-animal variation for pasture intake, the proportion of larvae ingested from the pasture which survive to become adults, the fecundity of the mature worm, along with density-dependent control of this trait and the mortality rate of the worms. The between-animal variation for each component is partitioned into genetic, permanent and temporary environmental components which vary with age. Infection rates are estimated from existing pasture larval contamination and new contamination from infected animals. Using this framework, selection for reduced mean faecal egg count was practised, in silico, *for a period of 10 years. Several general patterns emerged. First, a curvilinear response to selection was observed, with responses initially being large then declining over time. Mean faecal egg count declined from approximately 500 to 140 eggs per g in 10 years and worm burdens and pasture larval contamination showed similar patterns of response. The initial responses to selection were approximately 1·7 times that predicted by quantitative genetic theory because the epidemiology of the disease changed as the animals' genetic resistance improved. A method of partitioning selection responses into components due to the altered genotypes of the animals and components due to altered disease epidemiology is outlined. Secondly, the faecal egg count distribution became more aggregated, or skewed, as selection progressed. Thirdly, correlating pasture contamination levels across years (carry-over effects) resulted in even greater apparent responses to selection. Finally, regular anthelmintic treatment reduced mean faecal egg counts but did not alter the patterns of response to selection, indicating that selective breeding should be feasible under a variety of anthelmintic regimes.*

Keywords: *disease resistance, epidemiology, parasites (gastrointestinal), selection, sheep.*

Introduction

Gastro-intestinal parasitism presents a major constraint on livestock production, resulting in reduced animal performance and welfare, along with increased costs associated with both anthelmintic and management control measures. Resistance to gastro-intestinal parasitism in sheep has been shown to be heritable (e.g. Baker *et al.*, 1991; Woolaston *et al.*, 1991; Bishop *et al.*, 1996) with heritabilities for faecal egg count (FEC), a convenient measure of resistance to gastro-intestinal parasites, being of the order of 0·2 to 0·3. This suggests that selection for increased resistance may be used as a long-term control measure, in addition to existing management strategies.

However, predicting responses to selection for decreased FEC, in grazing ruminants, is not straightforward. Measured FEC is the result of a complex series of interactions between the parasite and the host, it commonly shows a negative binomial rather than a normal distribution and FEC levels also indicate the likely reinfection probabilities of other animals grazing the same pasture. Thus, in the situation of continual reinfection, interactions exist between animals in terms of their expression of resistance and this contravenes the normal (unstated) assumption made when calculating likely rates of genetic response that animals express their traits independently of each other. A possible consequence of these interactions between animals is that selection

which alters mean FEC over several years may also alter the epidemiology of the disease, as suggested by Barger (1989).

This paper addresses the issues of responses to selection for disease resistance and possible effects on disease epidemiology. Using gastro-intestinal parasitism in sheep as a case study, this paper aims to define a framework which may be used to model the effects of selection for disease resistance. Having defined the framework, the model will be investigated by means of stochastic simulation and likely patterns of selection responses will be calculated.

Material and methods

Epidemiological model

A model will be defined in general terms, then tailored to the specific example of gastro-intestinal parasitism in sheep. Unlike most previous attempts to model nematode populations, the model will concentrate on the interactions between the animal host and the pathogen, keeping epidemiological aspects of the model deliberately simple. Whilst it is recognized that external factors such as weather conditions are of overriding importance in defining potentially infective parasite populations, they are outwith the scope of this model.

(i) Basic model and equilibrium assumptions. Consider a simple infection where there is a constant infection rate of the host (I), a constant mortality rate of the adult parasite (m) and the number of adult parasites at time t is $M(t)$ (Anderson and May, 1992). Therefore:

$$dM_{(t)}/dt = I - mM_{(t)}$$

with solution:

$$M_{(t)} = M^*(1-e^{-mt}) \qquad (1).$$

Under this model the infection rate of a population of hosts will rise monotonically with time until it reaches an equilibrium value, $M^* = I/m$.

This concept may be extended to consider actual interactions between the parasite and the host. In the simplest form for gastro-intestinal parasites, three major factors may be defined: (i) *establishment* (E), i.e. the proportion of ingested larvae which survive to become adult parasites; (ii) *fecundity* (n), the number of eggs laid per adult worm per day (assuming a constant sex ratio); and (iii) *mortality* (m), the daily death rate of the adult worm, as defined above. With great simplification, and assuming constant weather conditions, the free-living stage of the parasite may be summarized by the following parameters: the probability that the egg hatches and develops to form a third stage lavae and is ingested at time t (y), and the mortality rate of the free living stage of the parasite (z).

An actual epidemic of the disease is assumed to be triggered by an initial ingestion rate of a high concentration of infective larvae (L_o). In practice, this may be caused by pasture contamination with infected larvae by periparturient ewes or by 'favourable' weather conditions resulting in dormant larvae becoming active. Therefore, the following model may be defined to take into account both the initial infection and, after time, continual reinfection:

$$M_{(t+1)} = M_{(t)}(1 - m) + L_{(t-j)}E \qquad (2)$$

where $L_{(t-j)}$ is the ingestion rate of larvae j time units ago, where j is the time assumed for larvae, which become established, to reach adulthood, and summing over time

$$L_{(t)} = L_o(1 - z)^t + \sum_{i=0}^{t} M_{(i)}\, n\, y(1 - z)^j \qquad (3).$$

Under this model, the parasitic population will reach an equilibrium which can be defined as follows:

$$M^*_{(+1)} = M^*_{(\,)}(1 - m) + \sum_{i=0}^{-j} M^*_{(-j-i)}\, n\, y(1 - z)^i E \qquad (4).$$

This equation holds true whilst:

$$\begin{aligned} m &= nEy/z \\ &= nEp \end{aligned} \qquad (5)$$

where p is the overall probability of an egg hatching and developing to third larvae stage and being ingested before it dies. If this relationship breaks down, then the parasite population (M) quickly approaches either zero (extinction) or infinity (a runaway epidemic).

Although there may be a threshold above which the worm burden is fatal to the host, it is assumed that this threshold is not exceeded in unselected populations of lambs.

(ii) Density dependent constraints. Consider the situation of an outside perturbation of the worm population, e.g. biocide from anthelmintic treatment. The above model will once again establish an equilibrium worm burden, but a lower level than previously. This is at odds with observed field data, where a similar equilibrium appears to be re-established. Moreover, the model is also very

sensitive to breakdowns in the assumptions, with irreversible epidemics, or entire loss of the parasitic population, occurring. Population regulation of the parasite may be controlled to a large extent by density dependent constraints, which may act at any of the *establishment*, *fecundity* or *mortality* levels, whereby the levels of any one of these stages is an inverse function of the worm population present.

Density dependent constraints may be simply modelled for *fecundity*, i.e. for worm densities greater than average, *per capita* fecundity decreases and *vice versa* for below average worm densities. This both stabilizes the recursive equations given above, as well as ensuring that when perturbations hit the worm population the population returns towards the pre-perturbation level. Setting a constraint that the total egg production per animal rises with increasing worm burden, then the density dependent effects acting on fecundity (n) may be modelled as being proportional to worm burden (M):

$$n \propto M_{(t)}^{\,b} \qquad (6)$$

where the exponent b defines the strength of the density dependent effects, such that $0 \quad b > -1$.

(iii) Between-animal variation. The factors above describe the dynamics of the population mean. The model now has to be extended to an individual animal basis. Between-animal variation exists for worm burden and FEC, with FEC most commonly showing a negative binomial distribution. It is 'overdispersed', with a small number of animals having high FECs and thus being responsible for a large proportion of the pasture infection. Between-animal variation can conceivably exist for *establishment*, *fecundity* and *mortality*, with each being a manifestation of the animal's immune response to the infection. Between-animal variation for *fecundity*, as a trait of the animal, may be thought of largely as between-animal variation in adult worm size, since the correlation of worm length and eggs per worm is very high (Stear *et al.*, 1995b).

The forms of the distributions of the between-animal variation for *establishment*, *fecundity* and *mortality* are unknown, however they should be defined such that measured FEC has an empirical distribution similar to the negative binomial. Preliminary assumptions, made in the absence of information to the contrary, are that *establishment* and *mortality* are normally distributed (mean values are E and m, respectively), and that *fecundity*, being a biological count measurement, has a Poisson distribution (mean = n).

Further variation between animals exists for the number of ingested larvae per day and FEC measurement error. The number of larvae ingested per day is comprised of two components — first, pasture intake and secondly, sampling of larvae off the pasture. Pasture intake is assumed to be a normally distributed trait, being zero in very young lambs and rising to a plateau in lambs 4 months and older. For an animal with a given food intake, the number of larvae ingested per day due to sampling effects is a biological count, and hence may be assumed to be a Poisson variable (mean at time t = $L_{(t)}$, where $L_{(t)}$ is proportional to the animals food intake at time t). Measurement errors in FEC may be assumed to be proportional to the true (unknown) FEC, i.e. the measurement scaling error is normally distributed (mean = 1·0).

(iv) Development of immunity. Exposure to the parasite population will result in lambs developing immunity, i.e. they will improve their ability to suppress *establishment* and *fecundity* and increase *mortality*. Two components to this may be considered. First, the mean level of immunity for the population as a whole will improve with time. Secondly, genetic differences between animals in *establishment*, *fecundity* and *mortality* will become apparent (see below), representing differences between animals in their immunocompetence. The increase in mean immunity is assumed to follow a pattern similar to that presented by Roberts and Grenfell (1991), rising from zero in young lambs to essentially a plateau in lambs 4 months and older. Thus, under these assumptions the increase in pasture intake with age in young lambs effectively balances the increase in immune ability.

(v) Partitioning animal variation. The animal controlled components, *establishment*, *fecundity*, *mortality* and pasture intake, may be assumed to be both genetically and environmentally controlled in lambs, with both the mean levels and the degree of genetic variation between animals reflecting the immune status of the population (see above). Heritabilities may thus be defined for these traits such that the heritability for FEC is similar to that observed in field situations. *Establishment*, *fecundity*, *mortality* and pasture intake are assumed to be genetically uncorrelated. Further partitioning of between animal variation into permanent environmental components, associated with either the whole lifetime or a particular season, and temporary environmental effects, mimicking time trends and chance effects for individual animals is also necessary. Modelling these permanent and temporary environmental effects serves two purposes: it allows realistically low repeatabilities between FEC measured within the same season (e.g. r 0·5) and it also models the observed phenomenon of the correlation between FEC

measurements decreasing as the time interval between measurements increases (Stear *et al.*, 1995c).

(vi) Defining initial infection rates. The model outlined above describes the course of an infection in a static host population within one season. When considering the effects of selection, and hence changes in infection rates across seasons, an issue arises as to the definition of the initial infection rate at the start of each season. An arbitrary function, f(*LC*,*w*) may be used to define the mean pasture larval count at the start of a season as a function of that present at the end of the previous season, accounting for the genotypes and immune status of animals grazing the pasture in the interim. These will be termed carry-over effects. For example, if $w = 0$ then the initial infection rate each season is constant, whereas if $w = 1$, the initial infection rate is directly proportional to the previous season's larval count.

Model investigation

The use of the model outlined above for the prediction of responses to selection for reduced FEC in lambs was investigated using stochastic simulation. The parameters of the model were set so that heritabilities and mean values for FEC mimicked results obtained for unselected Scottish Blackface lambs naturally infected with *Ostertagia circumcincta* (Bishop *et al.*, 1996).

(i) Mean values. L_0, m, n, E and p were defined such that mean FEC and adult worms burdens were close to 500 eggs per g and 2500 worms, respectively, prior to selection. Setting $E = 0{\cdot}6$, $m = 0{\cdot}06$, $n = 100$ (i.e. 500 eggs per g × 500 g faeces per day per 2500 worms) requires $p = 0{\cdot}001$ for equilibrium conditions to hold. Setting $L_0 = 1000$ achieved the desired mean infections. A constant L_0 was assumed for every year during the selection procedure. This represents the case, for example, where periparturient ewes graze the pasture prior to the lambs and these ewes are either unselected (in the initial years of selection) or unaffected by the selection (in later years). Additional scenarios were also investigated where L_0 was directly proportional to mean pasture contamination from the previous year, and where L_0 was mid way between these two extremes.

(ii) Density dependent effects. b was set to either 0, –0·25 (weak effects) and –0·5 (strong effects), for different simulation runs, following results of S. C. Bishop and M. J. Stear (unpublished data) and values presented for humans by Anderson and May (1992).

(iii) Component trait variation. A coefficient of variation of 0·3 was defined for *establishment*, *mortality* and food intake. Setting the *fecundity* mean to 100 automatically defines the coefficient of variation to be 0·1, as it is a Poisson variable, somewhat lower than for *establishment* and *mortality*. Experimental data (S. C. Bishop and M. J. Stear, unpublished data) suggest that this underestimates the variability in this trait. Moreover, assuming mean *fecundity* to be 100 resulted in unrealistic, i.e. too large, values for the negative binomial aggregation parameter (k), when this distribution was fitted to FEC. A solution was found to this problem by redefining n to be 10, rescaling the conversion from total daily egg output to eggs per g, so that mean FEC remains close to 500 eggs per g, and altering p to 0·01 so that an equilibrium was still maintained.

(iv) Animal effects. h^2 values were set to 0·2 for *establishment*, *fecundity* and *mortality*. Results of Bishop *et al.* (1996) were mimicked such that genetic control of these three traits was only apparent after 3 months of age, i.e. prior to 3 months all h^2 values were zero. Prior to 3 months the permanent environment effect (conceptually defined as a maternal c^2 effect) was set to 0·3 for all traits, and after 3 months it was reduced to 0·15. Food intake was assumed to have a constant heritability of 0·2 and a permanent environment effect of 0·15.

(v) Population structure. A simple population structure was used, for ease of calculations. A flock of 500 ewes was simulated, mated to 25 rams, with each ewe producing twins. Males were selected on FEC (see below) and used for only one breeding season at 7 months of age. Females were selected at random. Ewes were for randomized mating each year, mated first at 7 months of age and culled at random such that they had a maximum flock life of three parities.

(vi) Simulation procedure. Each year the model was run for lambs from 30 to 180 days of age. Individual genetic, phenotypic and environmental values were simulated for each trait for every lamb, as described above, with mean L values estimated from equation (3) after averaging pasture contamination effects across the whole flock. Mean FEC, averaged from samples taken on days 120, 150 and 180 was used as the selection criterion, this being a period during which heritabilties are significantly different from zero (Bishop *et al.*, 1996) and thus there are differences between animals in immunocompetence. Using these values the best 25 ram lambs every year were selected. Ten years of selection were practised and 20 replicates were run for each scenario, this being sufficient to show general patterns.

(vii) Comparing selection responses to expectation. Comparison of observed and expected responses to selection may be done by two methods. First, from

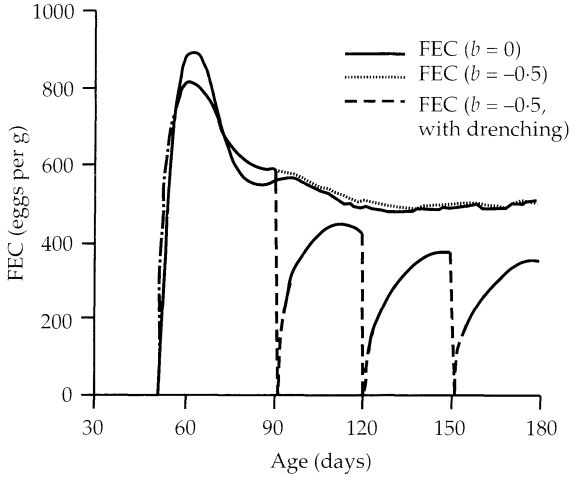

Figure 1 Faecal egg count (FEC) profiles in unselected sheep for different density-dependent effects and with anthelmintic treatment at 90, 120 and 150 days.

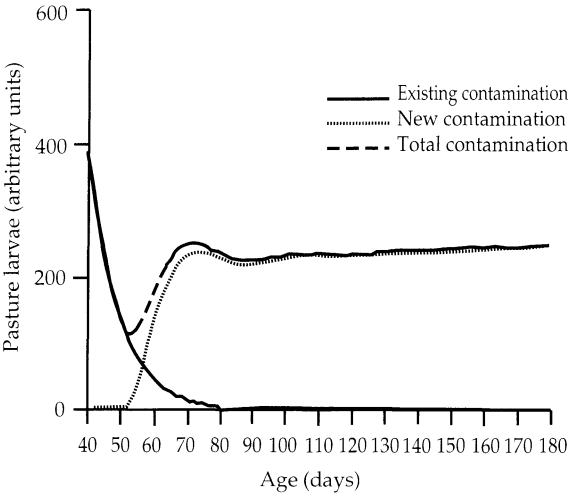

Figure 2 Pattern of pasture larval contamination.

quantitative genetic theory, i.e. $R = h^2S$, where S is the achieved selection differential and the h^2 value describes the base population. This simple approach is effective for the first generation, however it becomes more complicated in subsequent generations as the genetic and phenotypic variances change. Secondly, expected responses to selection may be inferred from the mean values for *establishment*, *fecundity* and *mortality* available from the model, each generation. Suppose unselected and selected animals are each given a dosage of a known number of infective larvae, then these mean values

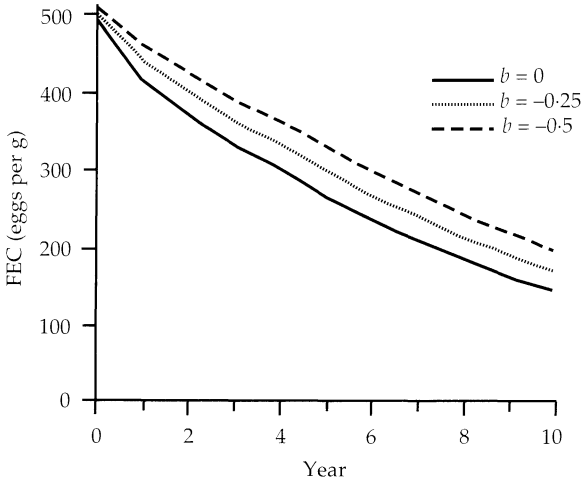

Figure 3 Responses to selection for reduced faecal egg count (FEC), assuming no carry-over effects between years.

can be used to calculate the approximate daily mean FEC values for the two groups. Comparison of the expected mean values relative to those observed from the model will determine how much of the response is genetic and how much is environmental (epidemiological) in origin.

Results

The behaviour of the model across a season for unselected animals is shown for undrenched animals (b = 0 and –0·5) and for lambs regularly drenched with a short-acting anthelmintic targetted at adult worms (b = 0·5) in Figure 1. Altering the density dependent b value has little effect on population means. However introducing a regular drench does result in an overall lowering of FEC values. Associated pasture larval contamination is shown in Figure 2, for untreated lambs.

Responses to selection for decreased mean FEC over a period of 10 years are shown in Figure 3 for density dependent exponents of b = 0, –0·25 and –0·50, mean worm fecundity of n = 10 and no carry-over effects from one year to the next in pasture larval contamination (w = 0). No anthelmintic treatment is given in this scenario. Selection responses show a general curvilinear pattern especially in the early years of selection, initially being large then decreasing and becoming more linear with time. Increasing the density dependent effect on worm fecundity decreased the responses to selection.

Using the GENSTAT package (Lawes Agricultural Trust, 1983), parameters from the negative binomial distribution were fitted to FEC values taken prior to

Table 1 *Negative binomial parameters for FEC distributions (replicate 1 data)*

	Measurement age (months)	Mean	k	Variance	Probability†
Before selection	4	489	3·89	71838	<0·05
	5	497	3·54	79628	<0·05
	6	500	3·46	81534	>0·50
	mean	497	7·00	38391	<0·05
After 10 years of selection	4	244	1·16	35168	<0·01
	5	121	1·23	7935	<0·01
	6	71	1·32	3450	<0·01
	mean	145	2·97	6698	>0·05

† Probability of fitting negative binomial distribution.

selection in the first replicate and to FEC values after 10 years of selection in the final replicate (Table 1). The fit of the negative binomial distribution was generally poor, only giving an adequate empirical description of FEC for two of the eight examples. However, the k values which describe the aggregation of the distribution were similar to those commonly observed in field data (e.g. Smith and Guerrero, 1993; Stear *et al.*, 1995a). In the cases where the negative binomial distribution was a very poor fit ($P < 0{\cdot}01$) it was because there were more zero FEC values observed than would be predicted from the fitted parameter values. Two important results are apparent in Table 1. First the distribution of FEC becomes more aggregated with selection, as mean FEC decreases. This is illustrated in Figure 4, where

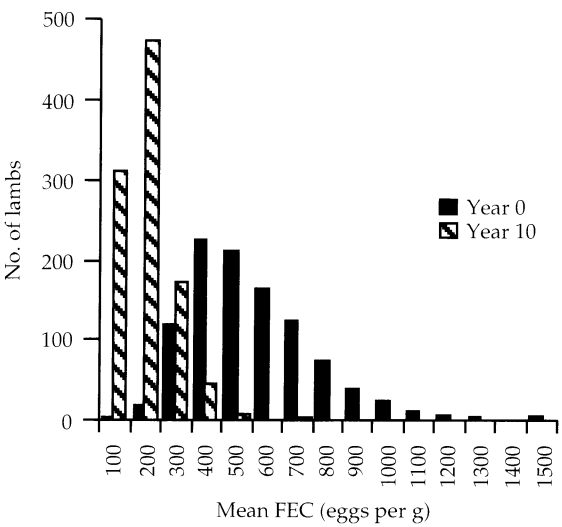

Figure 4 Distribution of mean faecal egg count (FEC) in 6-month-old lambs, before and after 10 years of selection for reduced FEC.

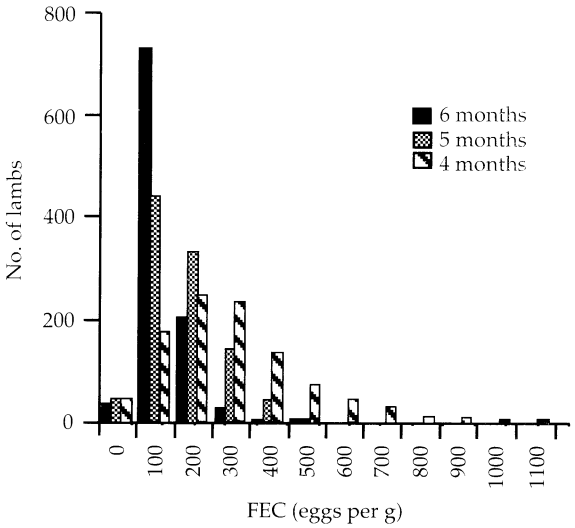

Figure 5 Distribution of faecal egg count (FEC) in lambs at different ages, after 10 years of selection.

the distribution of mean FEC before and after selection is plotted. Secondly, after effective selection has been practised and animals with superior genotypes exist in the flock, both the mean and variance of FEC decrease across the season, as illustrated in Figure 5. This results from both an increased immunocompetence of the selected animals and a consequent decreased reinfection rate.

After taking log transformations, the heritability of mean FEC in unselected animals in replicate 1 was 0·41, and the mean heritability for a single FEC was 0·19. These values dropped to 0·14 and 0·07, respectively, after 10 years of selection. The mean correlation between the three FEC measurements on which selection decisions were made was 0·25, in unselected animals.

Correlated responses to selection were also observed for worm burden and pasture larval contamination. Again, curvilinear responses to selection were apparent for both traits, and increasing the density dependent effects reduced the responses to selection. The relative reduction in worm burden was approximately half of that for mean FEC but the relative reduction in pasture larval contamination was almost as great as that for FEC.

Unlike the curvilinear responses in FEC, the responses to selection in the component traits of *establishment*, *fecundity* and *mortality* were essentially linear. Clearly, absolute responses are dependent on the assumptions made about the variability and

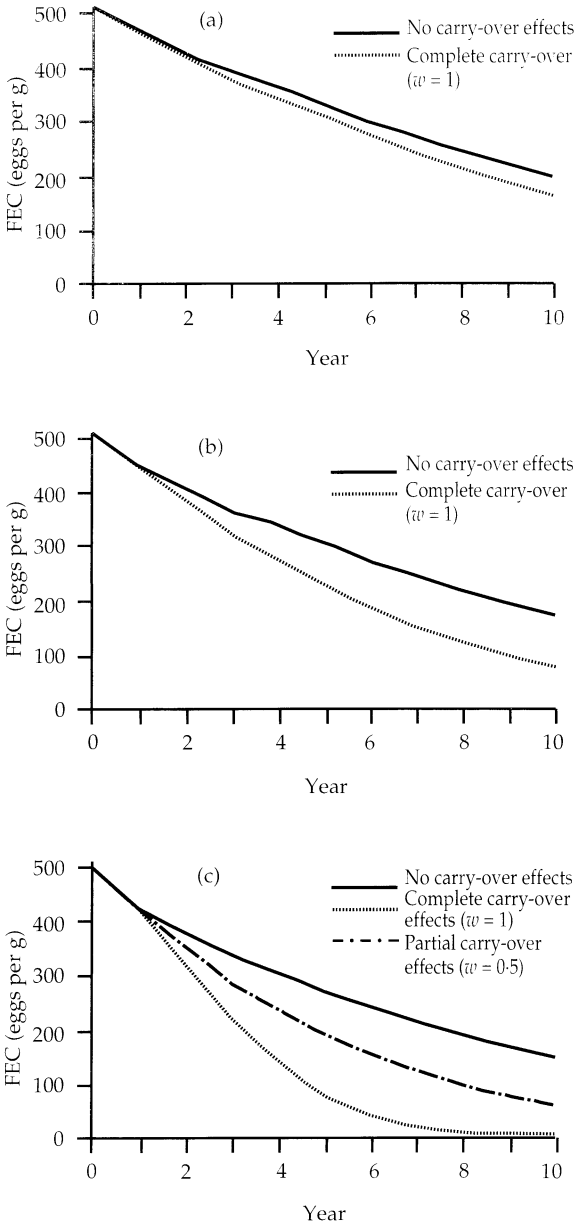

Figure 6 Carryover effects on selection response (a) for b = 0·5; (b) for b = –0·25; (c) for b = 0.

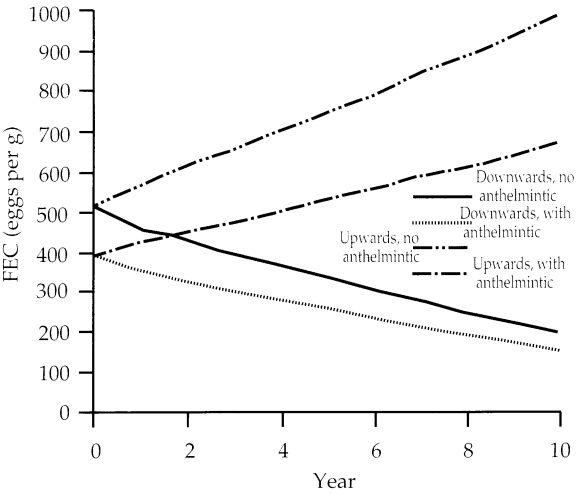

Figure 7 Comparison of divergent selection on faecal egg count (FEC) with and without anthelmintic treatment.

heritability for each component, however all three components will respond to selection on FEC provided that they show genetic variation during the time period the FEC measurements are being made. A more general observation was that imposing density-dependent effects on *fecundity* had a large effect on the other two components, significantly reducing the selection responses in *establishment* and *mortality*, yet it slightly increased the response in *fecundity*. This is explicable by the observation that such density-dependent effects will have the general effect of negating variation in other component traits. Consider *establishment*, the greater the value of this trait the greater the number of worms present in the host and hence the greater the impact of the density-dependent effect. A similar line of reasoning explains the sensitivity of mortality density-dependent effects. Food intake did not alter with selection for b = 0. However, the presence of density-dependent effects resulted in a small increase in food intake, as larval intake and total faecal output are proportional to intake but total egg output is modulated by the density-dependent effects.

The influences of carry-over effects between years in pasture larval contamination are presented in Figures 6a, 6b and 6c. With strong density-dependent effects (Figure 6a) the influence of carry-over effects on selection responses is small. It is only in the presence of weak or no density-dependent effects (Figures 6b and 6c) that the definition of carry-over effects becomes important. Clearly, the situation of L_0 being directly proportional to the previous season's pasture larval contamination gives unrealistic selection responses in the absence of density-dependent effects, however it is also unlikely that such integrity of pasture management could be maintained for several years on a farm containing a mixture of selected and unselected sheep, including periparturient ewes.

Consider selection during a rigorous anthelmintic treatment regime where the lambs are drenched immediately following the faecal sampling on days 90, 120 and 150 with a short-acting anthelmintic killing only adult worms resident in the sheep. The pattern of FEC for unselected animals under this scenario is shown in Figure 1 (for $b = -0{\cdot}5$). Responses to selection for lambs under this treatment regime are shown in Figure 7, compared with those obtained for the same b value but with no drenching. The responses obviously differ, with lower mean FEC at all stages for the drenched flock of animals. However, the ratio of mean FEC in the drenched flock to that in the undrenched flock stays almost constant at 0·76 (±0·01), each year. Therefore, the nature of the response to selection for decreased FEC is unaffected by whether the animals are selected in a drenched or an undrenched environment. Moreover, the mean values for *establishment*, *fecundity* and *mortality* did not differ significantly between the two scenarios.

Also shown in Figure 7 are responses for selection for increased FEC, with and without anthelmintic treatment. These selection responses are linear, in contrast to the curvilinear responses for downwards selection. Only under the assumptions of no anthelmintic treatment and no density-dependent effects is a runaway epidemic predicted by the model.

The issue of whether the responses achieved in decreasing mean FEC were greater than predicted by quantitative genetic theory was addressed, firstly, using the equation $R = h^2S$, where S is the achieved selection differential. For generation 1 (replicate 1), the observed response was 1·72 times the expected response, using the heritability for untransformed mean FEC, and 1·67 times the expected response if the heritability of mean log-transformed FEC was used. Therefore, observed responses are considerably greater than expected, at least in early generations, indicating that selection has indeed altered the epidemiology of the disease.

The mean values for *establishment*, *fecundity* and *mortality* were also used to infer the relative contributions of genetics and epidemiology to the observed responses to selection in early and later generations. Consider results for $b = 0{\cdot}0$, with no carry-over effects between years in larval contamination. Conceptually dosing unselected and selected animals with 1000 infective larvae results in approximately 119 eggs per g on day 21 for unselected animals and 55 eggs per g for selected animals. This is a genetic reduction to 0·46 of the original value, whereas the observed reduction was from 493 to 141, i.e. to 0·29. Therefore, after 10 years of selection, proportionately 0·76 of the reduction was genetic and 0·24 was due to the change in the environment. After 1 year of selection the relative contributions were 0·51 and 0·49, implying that the epidemiological effect was greater in the early years of selection, for the assumptions made in this example. Relating the initial larval challenge at the start of each season to pasture larval contamination from the previous season will increase the contribution of the epidemiological influences to the apparent selection responses.

Discussion

This paper has described a very general framework which enables individual animal variation in resistance to gastro-intestinal parasites to be modelled. This model has many potential uses, of which investigating responses to selection under different scenarios, as investigated here, is but one. No attempt has been made to make the model sophisticated from either a parasitological or host immunity viewpoint, as this has been successfully achieved by other authors (e.g. Gettinby *et al.*, 1989; Barnes and Dobson, 1990; J. A. Beecham *et al.*, unpublished data). Many of the published models describing parasite infections have been weather-based (Barnes and Dobson, 1990; Gettinby and Byrom, 1991) and have concentrated on how external factors affect parasite populations and hence likely infection rates. Some have also investigated selection for anthelmintic resistance in the parasite (Gettinby *et al.*, 1989; Barnes and Dobson, 1990). However, little attempt has hitherto been made to model specific interactions between the parasite and individual hosts within a population, as is done in this paper.

The performance of the model described in this paper is of course limited by the generality of the assumptions made. Between-animal variation in faecal egg count has been well described for a variety of host populations infected by a variety of parasites. Less well described, at the population level, are the component traits of *establishment*, *fecundity* and *mortality* as well as other important regulating factors such as density-dependent effects. Although some information is available to describe mean values of the component traits, little information is available on their variability between animals (including distributional properties), heritabilities, maternal effects (and their duration) and other repeatable sources of variation.

The results described in this paper will of course change as the assumptions regarding the component traits change with more experimental information. However, some properties which may be general have emerged.

First, a curvilinear response to selection is observed for FEC and to a lesser extent for true worm burden and pasture larval contamination. Responses to selection are initially large, then decline and become more linear. However, as described above, they are greater than predicted by quantative genetic theory. Density-dependent constraints appear to act to reduce selection responses, as they reduce between-animal variation for the traits under consideration. Reduced pasture contamination will benefit both the selected animals and also any unselected animals grazing the same pasture.

Secondly, the distribution of FEC becomes more aggregated over time. This is equivalent to the distribution becoming more skewed, resulting in selection differentials decreasing. However, as the distribution becomes more aggregated proportionately fewer animals are contributing a disproportionate number of eggs onto the pasture, hence culling of 'wormy' animals may become relatively more effective than in unselected animals. This possibility must be balanced by the relatively low repeatability of FEC within a season and requires further investigation. For selected animals, FEC also decreases within a season, this being a function of both an increased immunocompetance and lower resulting reinfection rates.

Thirdly, carry-over effects of pasture larval contamination from one year to the next have large effects on apparent selection responses in the absence of density-dependent effects but were largely cancelled out when strong density-dependent effects were present. These carry-over effects are perhaps the most difficult aspect of the system to model adequately and represent an area where this model could usefully interface with models describing animals management and the free-living stages of the parasite.

Finally, selection under regimes of no anthelmintic treatment compared with regular treatment results in different mean FEC values but similar patterns of response. Moreover, responses in the component traits (*establishment*, *fecundity* and *mortality*) are unaffected by the dosing regime.

Experimental verification of the results predicted by the model described in this paper are difficult to come by. Published selection experiments for reduced faecal egg count in sheep appear either to have offered sheep a fixed experimental dosage of larvae (e.g. Windon, 1990; Woolaston and Piper, 1996), or in the case of natural infections they have generally run divergently selected sheep together (Baker *et al.*, 1991). Whilst these are robust experimental protocols, they do not mimic selection under commercial conditions where the entire flock would be selected in the same direction and epidemiological factors would be important. It is this commercial situation that the model in this paper attempts to mimic. For these results to be predictive, however, the flock must remain closed or purchased sires and/or semen must come from equally resistant sources. A flock which allows importation of susceptible animals after several years of selection will negate some of the benefits of the several years worth of selection. Clearly, it is necessary to define circumstances under which epidemiological benefits are likely to be realized.

Although there appear to be no published experimental verifications of the epidemiological effects of selection, two simulation studies (Barger, 1989; Windon, 1990) have addressed this problem, comparing 'susceptible' and 'resistant' sheep using the model of Barnes and Dobson (1990). Both studies found that 'resistant' sheep had lower worm burdens which led to a markedly reduced pasture larval contamination, in broad agreement with the results in this paper. Moreover, the 'resistant' sheep required less anthelmintic intervention.

The results presented in this paper have general implications which are much wider than the simple situation of selecting sheep for resistance to worms, i.e. what are the epidemiological consequences of selecting domestic animals for resistance to infectious diseases? If the finding of an interaction between selection responses and the epidemiology of the disease in question is generally true, then the benefits of selecting for disease resistance relative to selecting for production traits will have been underestimated by animal breeders. This could have important consequences for future selection objectives. Clearly, there is a need to develop theory which combines genetics with epidemiology and enables us to predict such responses to selection. The arguments in this paper have been couched in terms of traditional selection techniques, however they will apply equally well to possible marker assisted selection. Therefore, results presented here further highlight the importance of mapping and utilizing disease resistance alleles in domestic animal populations.

Further work is required to verify and develop the results suggested in this paper. Numerous refinements can be made to parasitological and epidemiological aspects of the model or, alternatively, this model could be interfaced with other models which provide a more detailed description of animals' immune mechanisms, the parasite life cycle and environmental factors affecting it (e.g. those of Barnes and Dobson, 1990, and

Beecham *et al.*, 1994). Other problems of interest which should be addressed include (i) defining an optimal balance between the selection of resistant animals and the culling of 'wormy' animals, (ii) the incorporation of production traits into the model and (iii) consideration of the effect of selection on the required frequency of drenching which will influence the rate development of anthelmintic resistance amongst the parasite. Finally, as mentioned above, there is a need to develop a general theoretical framework linking quantitative genetics and epidemiology.

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Relationship of body condition score and live weight with body composition in mature Churra ewes

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Abstract

Thirty-five mature Churra ewes, ranging in live weight from 30·3 to 52·6 kg and in body condition score from 1·25 to 4·00 were used to study the relationship between body condition score (BCS), live weight (LW) and body composition and fat distribution in ewes of this breed, which is one of the major sheep breeds of northern Spain. The procedure at slaughter and at subsequent dissection was designed to partition each body into two components, carcass and 'non-carcass'. Right side carcasses and 'non-carcass' components were used to analyse the chemical composition. From the left side of the lumbar region a joint was cut and dissected into muscle, bone, subcutaneous and intermuscular fat. According to the results obtained, omental fat represented the highest proportion of total internal fat regardless of the level of fatness. Distribution of internal fat was similar to that observed in other milk production breeds. Regressions on LW explained more of the variation than those on BCS for individual internal fat depots and chemically determined 'non-carcass' fat. The prediction of total body fat afforded by LW was better than that provided by BCS. The subcutaneous and intermuscular fat depots in the lumbar joint were well correlated with BCS, carcass fat and total fat in the body, validating the use of this region for assessing BCS in Churra ewes. Nevertheless, the correlation coefficient with the omental depot was not statistically significant. The results of this study suggest that BCS was not as accurate for estimating body composition and fat depots in mature Churra ewes as has been shown previously in other breeds. The single most effective prediction index was LW. However, the utilization of both BCS and LW together provided more accurate estimations.

Keywords: *body composition, body condition score, ewes, live weight.*

Introduction

In most sheep production systems under arid or semi-arid conditions, the ability of the animal to retain and mobilize body reserves is of considerable importance in determining the sheep's productivity or even its survival (Russel *et al.*, 1971). In such extensive conditions, a method for estimating body composition in a simple way would prove extremely useful.

Body condition was defined by Murray (1919) and a system for describing it in sheep, based on a five-point scale assessed by palpation of the lumbar region, was devised by Jefferies (1961). The lumbar region was proposed by these authors because the loin is the last part of the growing animal to develop; it is the last to put on fat and the first to lose it. Russel *et al.* (1969), using an adaptation of the Jefferies technique, showed that body condition score (BCS) was related closely to the proportion of chemical fat in the body.

The system is considered a good predictor of the level of fatness and has proved very useful in quantifying relationships between body condition and certain production parameters. Since it is a simple system, it has been used in many aspects of sheep husbandry and management.

On the other hand, it is accepted that individual breeds have a distinctly different distribution of fat within the body (Taylor *et al.*, 1989) and relationships derived from studies in one breed cannot be applied with confidence to others.

This experiment was carried out with the aim of studying the relationship between body condition score, live weight and body composition and fat

distribution in mature ewes of the Churra breed. This breed is one of the major sheep breeds of northern Spain and its most important function is milk production. As an autochtonous breed, and because of the environment in which it is kept in which food availability undergoes significant seasonal changes, it is well adapted to the utilization of natural resources through mobilization of its body reserves.

Material and methods

The study was conducted using 35 non-pregnant, non-lactating mature Churra ewes (averaging 5 to 7 years of age), ranging in live weight (LW) from 30·3 to 52·6 kg and in body condition score (BCS) from 1·25 to 4·00. Body condition score was assessed to nearest 0·25 score using the Russel *et al.* (1969) technique which employs a 0 to 5 score. The animals belonged to the flock of the research farm of the Spanish Council for Scientific Research (CSIC) in León (Spain). Before slaughter, the ewes were shorn and weighed without having been fed overnight and the BCS assessed independently by two experienced people.

The sheep were slaughtered by exsanguination from the jugular vein, after being injected intravenously with 1 ml Xilocain (Rompum®). Blood collected was not analysed but was assumed to contain proportionately 0·80 water, 0·183 protein and negligible amounts of fat (Agricultural Reseach Council, 1980). The procedure at slaughter and at subsequent dissection was designed to partition each body into two components, carcass and 'non-carcass'.

The gastrointestinal tract was emptied and its contents weighed in order to obtain the fleece-free empty body (FFEB) weight. The fatty tissues surrounding the alimentary tract (omental and mesenteric fat) were removed, along with any associated connective tissue, and weighed. Kidney and pelvic fat was also weighed after removal of the kidneys. Finally, all the internal fat depots and the gastrointestinal tract were added to the 'non-carcass' fraction.

Carcasses were divided down the backbone: both sides of each carcass were weighed while warm and again 1 day later when cold. Right sides were used to study the chemical composition. From the left side of the lumbar region (which was palpated to assess body condition score) a joint was cut from between the 2nd and 3rd to the 6th and 7th lumbar vertebrae and dissected into muscle, bone, subcutaneous and intermuscular fat. Weight losses occurring during dissection were considered to be proportional to each component.

Right side carcasses and 'non-carcass' components were stored in separate polyethylene bags at –20°C until preparation for analysis. After thawing, they were cut into pieces and minced successively, using a blender fitted with end plates containing holes of 7 mm and 4 mm diameter. The mince was then mixed thoroughly and subsamples freeze dried to determine water content.

All samples were analysed for crude protein and ash by the Association of Official Analytical Chemists (1984) procedures. Fat content of carcass and 'non-carcass' was estimated by difference. The chemical composition was calculated assuming that all weight losses occurring during freezing and storage were 100% water.

Statistical analysis was conducted using GENSTAT 5 (Lawes Agricultural Trust, 1993). Regression analysis was used to evaluate relationships between BCS, LW and a combination of both to several body components. Correlation analysis was used to relate subcutaneous fat and subcutaneous plus intermuscular fat in the dissected lumbar joint with the weight of individual fat depots of ewes.

Results

Table 1 shows means and standard errors of the characteristics measured. The full range of BCS (from 0 to 5) was not represented at slaughter; however, the range of BCS from 1·25 to 4·00 as well as the range of LW are representative of those generally found in ewes of the Churra breed.

BCS and LW were correlated, with a correlation coefficient of 0·683 ($P < 0·001$). The regression equation for estimating LW from BCS indicated that per unit change in BCS, a corresponding change of 5·57 kg of LW could be expected:

$$\text{LW} = 30·12 \text{ (s.e. } 2·467) + 5·57 \text{ (s.e. } 1·004) \text{ BCS}; \quad \text{s.e.} = 4·27$$

The omental fat depot represented the highest proportion (mean 0·438, s.e. 0·0186) of the total internal fat (kidney and pelvic depot plus omental depot plus mesenteric depot). This was the case for the whole range of BCS and LW values studied. The mesenteric fat depot represented a higher proportion of internal fat at low BCS. In contrast, kidney and pelvic fat depot represented a higher proportion of the total internal fat at high levels of total fatness, as indicated in equations given in Table 2.

Chemical non-carcass fat represented a high proportion of the FFEB fat in ewes of the Churra breed analysed in this experiment (mean 0·416, s.e. 0·0104). Carcass fat was proportionately only about 0·58 of the total fat weight and 0·24 of the carcass weight (mean 0·241; s.e. 0·0127).

Table 1 *Means and standard errors of characteristics measured (no. = 35) (all values denote means for individual sheep)*

	Mean	s.e.
Live weight (kg)	43·22	0·990
Body condition score	2·35	0·123
Kidney and pelvic fat:		
weight (g)	528·8	54·02
proportion in total internal fat	0·227	0·0122
Omental fat:		
weight (g)	1041·0	124·12
proportion in total internal fat	0·439	0·0188
Mesenteric fat:		
weight (g)	686·3	50·36
proportion in total internal fat	0·334	0·0168
Total internal fat (g)	2256·2	206·16
Non-carcass fat:		
weight (g)	3231·9	251·32
proportion in the FFEB fat	0·416	0·0104
Carcass fat:		
weight (g)	4548·4	347·14
proportion in the carcass weight	0·241	0·0126
proportion in the FFEB fat	0·582	0·0104
Fleece-free empty body (FFEB):		
weight (g)	33312·8	976·78
dry matter (g)	14912·2	701·70
fat (g)	7772·3	567·37
protein (g)	5127·6	130·68
ash (g)	2002·3	56·92
Lumbar joint (proportion of):		
subcutaneous fat	0·118	0·0127
intermuscular fat	0·090	0·0057
muscle	0·516	0·0114
bone	0·275	0·0128

Estimates of parameters for prediction equations for estimating weight of fat depots and FFEB composition of ewes of the Churra breed from BCS and from LW and correlation coefficients are presented in Table 2. As shown in that table, similar R^2 values were observed when either BCS or LW were used to predict kidney and pelvic fat depot weight. Variation in weights of omental fat depot, mesenteric fat depot and total internal fat was more accurately predicted by equations involving LW than with BCS. Including both BCS and LW in the regression model only significantly improved the model for estimating the weight of kidney and pelvic fat depot.

LW accounted for more of the variation in non-carcass fat, with essentially no improvement in the regression model when both BCS and LW were included. BCS accounted for more variation in carcass fat weight (R^2 = 0·70) than did LW (R^2 = 0·64). Inclusion of both fitted terms together (BCS and LW) improved the accuracy of its estimation (R^2 = 0·79).

Chemical composition of the FFEB was better predicted using LW than using BCS. Variation in LW accounted for more of the variation in dry matter, fat, protein and ash contents (0·77, 0·72, 0·65 and 0·26, respectively) than did BCS (0·58, 0·64, 0·27 and 0·004, respectively for dry matter, fat, protein and ash. Table 2).

Table 2 *Estimates of parameters for prediction equations for estimating weight (g) of fat depots and fleece-free empty body composition of ewes of Churra breed from body condition score (BCS, condition score units) and from live weight (LW, g) (S_a and S_b are standard errors of estimated parameters; s.e. is standard error of observations). All* R^2 *values are significant (P < 0·001) except those indicated*

	BCS						LW						LW + BCS
	a	S_a	*b*	S_b	s.e.	R^2	*a*	S_a	*b*	S_b	s.e.	R^2	R^2
Kidney and pelvic fat	−233	126	324	51·1	218	0·54	−1244	273	0·041	0·0063	214	0·55	0·64
Omental fat	51	390	421	159	677	0·15[a]	−2355	741	0·078	0·0170	581	0·38	0·36
Mesenteric fat	112	140	244	56·8	242	0·34	−1122	219	0·042	0·0050	172	0·67	0·66
Total internal fat	−70	575	990	234	997	0·33	−4721	997	0·161	0·0229	781	0·59	0·58
'Non-carcass' fat	166	661	1307	269	1145	0·41	−5337	1179	0·199	0·0271	922	0·62	0·62
Carcass fat	−995	654	2359	266	1134	0·70	−7667	1573	0·283	0·0361	1233	0·64	0·79
Fleece-free empty body													
Dry matter	4789	1151	4316	631	2687	0·58	−11769	2528	0·618	0·0581	1978	0·77	0·82
Fat	−823	1156	3665	470	2003	0·64	−13018	2288	0·482	0·0526	1790	0·72	0·80
Protein	3800	381	566	155	660	0·27	554	585	0·106	0·0134	457	0·65	0·64
Ash	1805	194	84	78·9	336	0·004[b]	694	370	0·030	0·0085	289	0·26	0·31

[a] $P < 0·01$.
[b] $P > 0·05$.

Table 3 *Estimates of parameters for prediction equations for estimating weight of fat depots and fleece-free empty body (FFEB) composition of ewes of Churra breed, using both body condition score (BCS) and live weight (LW) and/or fitting logarithmic variates(S_a, S_b and S_c are standard errors of estimated parameters; s.e. is standard error of observations)*

	a	S_a	b	S_b	c	S_c	s.e.	R^2
Equation: component log$Y$(g) = a + b log LW (g)								
Omental fat	−15·17	2·490	3·98	0·662			0·543	0·51
Mesenteric fat	−12·73	1·420	3·25	0·77			0·309	0·68
FFEB protein	−1·44	1·190	0·93	0·112			0·091	0·68
Equation: component log$Y$(g) = a + b log LW (g) + c log BCS								
Kidney and pelvic fat	−11·44	2·760	2·59	0·782	1·02	0·330	0·450	0·67
FFEB ash	−2·78	2·570	0·98	0·247	−0·16	0·104	0·142	0·34
'Non-carcass' fat	−19·99	5·59	0·43	0·226	2·58	0·536	0·308	0·71
Equation: component Y(g) = a + b LW (g) + c BCS								
Carcass fat	−5553	1281	0·15	0·038	1515	309	945	0·79
FFEB fat	−10343	2017	0·32	0·061	1907	485	1486	0·80
FFEB dry matter	−9386	2416	0·47	0·073	1699	581	1779	0·82

Very little variation in ash content was predicted with either BCS or LW. Even the relationship between BCS and ash content in the FFEB was not statistically significant ($P > 0·05$). Likewise, proportionately only 0·27 of the variation in protein content was accounted for by variation in BCS.

Including both BCS and LW in the regression equation improved the estimation of dry matter and total fat content. However, despite its statistical significance, little improvement was noted in estimating ash content.

Linear equations were used to compare the relationship of LW and BCS with body composition. However, for the purpose of predicting body composition in Churra ewes, equations giving the best fit to the data, using both BCS and LW (in some cases with logarithmic transformations) are shown in Table 3.

Table 4 *Correlation coefficients for proportion of dissected subcutaneous fat in lumbar joint, proportion of dissected subcutaneous + intermuscular fat in the lumbar joint and body condition score (BCS) and fat depots in mature ewes of Churra breed (all values are significant (P < 0·05) except those indicated with †)*

	Subcutaneous	Subcutaneous + intermuscular
BCS	0·774	0·764
Kidney and pelvic fat	0·693	0·679
Omental fat	0·313†	0·371†
Mesenteric fat	0·586	0·661
Total internal fat	0·508	0·557
'Non-carcass' fat	0·625	0·669
Carcass fat	0·789	0·772
Fleece-free empty body fat	0·766	0·774

As shown in Table 4, the proportions of dissected subcutaneous fat and subcutaneous + intermuscular fat in the lumbar joint were well correlated with carcass fat (r = 0·789 and r = 0·772, respectively) and total fat in the body (r = 0·766 and r = 0·774, respectively). Correlation coefficients found between those two depots and individual internal fat depots were lower, especially with the omental depot (r = 0·313 and r = 0·371, respectively for subcutaneous depot and subcutaneous + intermuscular depot). BCS and proportion of subcutaneous fat in the lumbar joint showed a coefficient of 0·774. The correlation coefficient between BCS and the proportion of total fat (subcutaneous plus intermuscular) in the lumbar joint dissected was similar (r = 0·764).

Discussion

BCS and LW were correlated with a coefficient of 0·683 ($P < 0·001$) and logarithmic transformation of the variates did not improve the precision of the equation. The correlation coefficient was lower than expected from previous studies in other breeds (Russel *et al.*, 1969; Purroy *et al.*, 1987; Teixeira *et al.*, 1989; Sanson *et al.*, 1993). However, it was similar to that reported by Oregui (1992) in the Latxa breed. The high variability in adult size and weight observed in sheep of these breeds compared with other breeds might account for this. Thus, the inclusion of both BCS and LW in the prediction model increased, in most equations, the amount of variation accounted for compared with either BCS or LW alone.

The change in LW per unit change in BCS, according to the regression equation, corresponded proportionately to approximately 0·13 of mean live

weight in Churra ewes. This value is lower than most of the values found in the literature, except again that indicated by Oregui and Garro (1989) in Latxa ewes and the average of three Greek dairy breeds reported by Zygoyiannis *et al.* (1997) despite the inevitable differences between studies due to the method used for estimating the mature LW, the BCS assessor, etc. Important differences in changes in LW for one unit change in BCS associated with genotype have been reported widely.

The general distribution of mesenteric and kidney and pelvic fat depots was in broad agreement with those indicated by other authors (Russel *et al.*, 1971; Teixeira *et al.*, 1989; Oregui, 1992). However, while others have found that the omental fat depot was markedly increased in animals having BCS higher than three, in Churra ewes, according to the results obtained in this experiment, the omental fat represented the highest proportion of total internal fat regardless of the level of fatness. This may be a characteristic of the breed related to milk production and mobilization of internal fat depots.

Comparisons between breeds must be interpreted carefully since the amounts of fat and their proportions can be very different if they have been determined chemically or by dissection. The amount of total internal fat (dissected) or non-carcass fat (determined chemically) as well as its proportion in the empty body found in Churra sheep was similar to that observed in other milk production breeds and higher than shown by breeds noted for meat production (Russel *et al.*, 1971; Butterfield, 1988). This kind of dichotomy of fat partitioning has been found previously in other sheep breeds (Russel *et al.*, 1972; Butler-Hogg, 1984; Taylor *et al.*, 1989). In general, ewes bred for milk production tend to deposit more fat in internal depots and those bred for meat production deposit more in the carcass depot. Thus, the amount of carcass fat, as a proportion of the carcass weight and as a proportion of the total fat, was also lower than most of the values observed in other breeds and cited in the literature (Wood *et al.*, 1980; Butler-Hogg, 1984; Butterfield, 1988; Taylor *et al.*, 1989).

For BCS over 3, increments in fat depots in Churra ewes were quite low in comparison with higher increments at the highest level of fatness found in other breeds. This may be a characteristic of the Churra breed but it must be noted that there were few animals with scores higher than three which could have affected the reliability of the relationship at the high extreme (see Table 3).

Regressions on LW explained more of the variation than those on BCS for individual internal fat depots and chemically determined non-carcass fat (see Table 2). Only for the kidney and pelvic fat depot, did the inclusion of BCS together with LW increase the accuracy of the estimation, possibly because this depot is closely associated with the carcass fat which shows a better relationship with BCS.

The prediction of total body fat afforded by LW was better than that provided by BCS. Similar results have been obtained in other breeds, in which LW was a more accurate predictor of body reserves than BCS (Castrillo *et al.*, 1988; Treacher and Filo, 1995). In the ewes of the Churra breed, there are at least two possible explanations for this. On the one hand, the high proportion of non-carcass fat in the total fat and its lower correlation with the BCS than that between BCS and carcass fat, can decrease the accuracy of the BCS as index of prediction. On the other hand, the best results with BCS have been found in studies including a wide range of BCS values and in animals of a narrow range of sizes. In this experiment, those ranges were narrower and wider, respectively, because they were representative of those generally found in Churra flocks.

LW was by far the best predictor of body protein as has been reported previously (Wright and Russel, 1984). The relationship with the BCS was much poorer since, typically, protein reserves are not depleted to a great extent until animals are very emaciated.

The subcutaneous fat and subcutaneous plus intermuscular fat in the lumbar joint were highly correlated with BCS. They also correlated well with carcass fat and total fat in the body, validating the use of this region for assessing BCS in Churra ewes. Nevertheless, the correlation coefficients were not as high as found in previous studies in other breeds (Delfa *et al.*, 1989). Furthermore, the correlation coefficient found between these fat depots of the lumbar joint and the omental depot was not statistically significant. This lack of correlation may explain the poor relationship found between BCS and internal fat (or non-carcass fat) in ewes of the Churra breed, in which the omental depot plays an important rôle.

The results of this study suggest that BCS was not as accurate for estimating FFEB composition and fat depots in mature ewes of the Churra breed, as has been shown previously in other breeds. This is probably due to the pattern of fat partitioning found in this breed, with a high proportion of the total fat deposited in internal depots. The single most effective prediction index was LW. However, the utilization of both BCS and LW together provided more accurate estimations.

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Interactions among tannins, supplementation and polyethylene glycol in goats given oak leaves: effects on digestion and food intake

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Abstract

Effects were studied on food intake and diet apparent digestibility of giving to goats once daily a basal diet rich in tannin from inclusion of leaves of Quercus calliprinos *either supplemented with a high carbohydrate or a high protein food. Also interactions with polyethylene glycol (PEG) were investigated. The results of the present work confirmed our presumption that the content of tannin in some Mediterranean browse is so high that it may negatively affect the utilization of protein in supplementary foods. Neutralizing the tannins with once-daily provision of PEG proved to be an effective means of preventing the negative effect. Providing 10 g/day PEG to goats given* Quercus calliprinos *leaves* ad libitum *and supplemented with 300 g/day concentrates containing 160 g crude protein per kg DM increased digestible crude protein intake by 50 g/day. When the concentrate food was given to goats, leaf dry-matter intake decreased significantly (from 664 to 565 g/day) and the goats lost weight rapidly. Therefore, supplementing tannin-rich leaves with concentrate food is recommended only if done in combination with PEG. High protein supplementation increased leaf (from 664 to 844 g/day) and digestible protein intakes (from 4·8 to 92·3 g/day) but a considerable portion of the protein supplementation was wasted due to interaction with tannins. PEG may allow economies in the use of such high-cost foods due to the greater efficiency of protein utilization (digestible crude protein intake increased from 92·3 to 122 g/day) of the supplementary food and to increased intake and protein utilization of the basal leaf diet (from 844 to 1023 g/day).*

Keywords: *digestion, food intake, goats, supplements, tannins.*

Introduction

Mediterranean woodlands have been exploited for millennia for raising range-fed goats in areas where few agricultural alternatives are available (Meuret *et al.*, 1990). Browse constitutes the majority of natural vegetation in the diet selected by goats in various regions of the Mediterranean basin (Kababya, 1994; Meuret *et al.*, 1990). However, the use of browse sources by herbivores is restricted in many cases by defensive or deterrent mechanisms related to the high content of tannins in woody plants (Provenza, 1995). The inverse relationship between high tannin level in the forage and voluntary intake, digestibility and nitrogen retention in ruminants has been well established (Robbins *et al.*, 1987; Silanikove *et al.*, 1994 and 1996). It was concluded that the protein-binding capacity of tannin-containing leaves may exceed considerably the protein content in the leaves (Silanikove *et al.*, 1996). Thus, tannins ingested with the browse may also affect the protein utilization of supplementary food.

Once-daily provision of polyethylene glycol (PEG) with a molecular weight of 4000 (a tannin-binding agent) increased markedly the intake and digestion of tannin-containing leaves offered to sheep and goats (Silanikove *et al.*, 1994 and 1996). The aims of the present experiment were to: (i) study the effect of feeding a basal tannin-rich leaf diet supplemented with a high-carbohydrate or high-protein food on food intake and apparent digestibility, and (ii) examine whether PEG, given to goats once daily, increases the goats' intake of the leaves and the protein utilization of the supplementary and basal foods.

Material and methods

Animals

The experiments were carried out with six 2- to 3-year-old non-lactating and non-pregnant Mamber goats (a breed indigenous to the Mediterranean woodland) weighing 35 (s.d. 5) kg. The animals were

stall-fed individually in a yard protected from rain and wind and equipped with troughs which enabled quantitative measurement of food intake.

Foods

Quercus calliprinos (common oak, or simply oak) leaves attached to small edible branches (diameter of 2 to 3 mm) were harvested once a week early in the morning and stored at –20°C. The leaves contained per kg approximately 500 g dry matter (DM) and on a DM basis per kg, 955 g organic matter (OM), 730 g crude protein (CP), 509 g neutral-detergent fibre (NDF), 444 g acid-detergent fibre, and 160 g acid-detergent lignin (Silanikove *et al.*, 1996). The oak leaves may be defined as high-tannin and had 40 g soluble phenolics and 95 g condensed tannins per kg. The daily allotment of the leaves was removed from the freezer, allowed to thaw and offered once daily *ad libitum* at 08.00 h. Two types of supplementary food were used: (i) high-carbohydrate commercial concentrate pellets (cereal concentrate, CC) composed mainly of cereal grains with DM content of 860 g/kg and which contained on a DM basis per kg, 160 g CP, 194 g NDF, and 7·0 MJ net energy, and (ii) high-protein soya-bean meal (soya concentrate, SC) with a DM content of 88 g/kg and, on a DM basis per kg, 500 g CP, 70 g NDF and 7·4 MJ net energy. The supplementary foods were given at 08.00 h and were consumed within 15 to 30 min. PEG (molecular weight of 4000, Degussa, Germany, pharmacological grade) at 10 g/day was given in the morning, before the leaves and supplemented foods. PEG was given mixed with a small amount (3 to 5 g) of concentrates (160 g CP per kg in mash form) and was consumed immediately. Water was available at all times.

Treatments

Digestibility trials were carried out using oak leaves as the basal diet with addition of 300 g/day of either cereal or soya concentrate foods, with or without 10 g/day PEG. The six dietary treatments using six goats were: (i) oak, (ii) oak + PEG, (iii) oak + CC, (iv) oak + CC + PEG, (v) oak + SC, (vi) oak + SC + PEG.

Digestibility trials

The animals were adapted to treatments for at least 2 weeks as described previously (Silanikove *et al.*, 1996). Daily food intake on each treatment was measured for 2 weeks, and a digestibility trial was carried out during the 2nd week. Daily subsamples from the fresh food offered and from total food refusals were stored at –20°C for further analyses. Faecal grab samples were taken every 6 h over a period of 4 days and stored at –20°C. Apparent digestibility of OM, protein (N × 6·25) and cell walls (NDF) was determined by measuring their concentration and the concentration of external marker in the food and faecal samples. Wheat hay cell-wall-chromium mordant was used as an external marker (Silanikove *et al.*, 1996). Every day, 10 g of the marker was given at 08.00 h, for a week before the digestibility trial and during the week of digestibility measurements, along with PEG supplementation. The animals were weighed routinely on electronic scales every 2 days at 10.00 h.

At the end of each experimental period, rumen samples were taken by stomach tube before the presentation of food (07.30 h) and three more times at intervals of 2 h after the presentation of food.

Chemical and statistical analyses

The apparent digestibility of OM, CP, and cell walls, the concentration of volatile fatty acids (VFA) (acetic, propionic, butyric and valeric acids) and ammonia, the pH of rumen fluid and urea concentration in blood serum were determined as described by Silanikove *et al.* (1996).

A 6 × 6 Latin-square design was used (with repeated measuring in the case of rumen and plasma sampling) in which comparisons between treatments were made by two-way analysis of variance with goats and treatments as independent terms in the model. Statistical significance was assessed by Duncan's multiple range test, following finding a main effect, using Statistical Analysis Systems Institute (1982) procedures. In the case of repeated measurements of pH and VFA in rumen fluids, no interaction between PEG supplementation and a specific diet was found and therefore the results were pooled.

Results

Intake, apparent digestibility, and changes in body weight (Table 1)

Dry-matter intake (DMI) of oak was 664 g/day. PEG increased the DMI to 836 g/day, whereas CC supplementation reduced it to 565 g/day and the combination of PEG and CC increased the DMI of oak to 770 g/day. SC supplementation increased the DMI of oak to 844 g/day and SC + PEG increased it further to 1023 g/day; all the responses differed significantly from each other (except oak + SC = oak + PEG).

Goats given oak lost 275 g body weight per day and supplementation with CC to oak reduced the loss of body weight to 151 g/day ($P < 0·05$). All other treatments led to non-significant increases in body weight ($P > 0·05$).

OM apparent digestibility (OMD) of goats given oak alone was 260 g/kg. PEG increased the OMD to

Table 1 *Effects of either cereal concentrate (CC), or soya-bean concentrate (SC) supplementation, with or without polyethylene glycol (PEG) provision on oak dry matter intake (DMI), changes in body weight (BW), and apparent digestibility of organic matter (OM), crude protein (CP) and neutral-detergent fibre (NDF) in six goats given* Quercus calliprinos *(oak)*

Treatments	DMI (g/day)	BW (g/day)	Apparent digestibility (g/kg) OM	CP	NDF
Oak	664[d]	−275[d]	258[d]	113[c]	287[b]
Oak + PEG	836[b]	77[a]	602[a]	442[b]	606[a]
Oak + CC	565[e]	−151[b]	425[c]	33[d]	281[b]
Oak + PEG + CC	770[c]	91[a]	623[a]	537[a]	593[a]
Oak + SC	844[b]	35[a]	538[b]	453[b]	581[a]
Oak + PEG + SC	1023[a]	122[a]	661[a]	564[a]	598[a]
s.e.	30	32	12	15	14

[a, b, c, d] Values in columns marked with different superscript letters differ significantly ($P < 0.05$).

602 g/kg, CC supplementation increased it to 425 g/kg and the combination of PEG and CC raised OMD to 623 g/kg, which was higher ($P < 0.05$) than in goats given oak but not higher than in oak + PEG. SC supplementation increased OMD to 538 and oak + SC + PEG further increased OMD to 661 g/kg ($P < 0.05$), which did not differ from that of oak + PEG and oak + PEG + CC.

CP apparent digestibility (CPD) of goats given oak was 113 g/kg. CC supplementation reduced the CPD to 33 g/kg whereas PEG increased it to 442 g/kg and the combination of PEG + CC increased it further to 453 g/kg. SC supplementation increased the CPD to 537 g/kg and the combination with PEG increased it further to 564 g/kg; all responses differed significantly from each other, except for the CPD of oak + SC, which was similar to that of oak + PEG.

NDF apparent digestibility (NDFD) of goats given oak alone was 287 g/kg. CC supplementation did not change NDFD, whereas PEG and PEG + CC increased it to 606 g/kg and 593 g/kg, respectively ($P < 0.05$). SC and SC + PEG increased NDFD to 581 g/kg and 598 g/kg, respectively, which was higher ($P < 0.05$) than with oak and oak + CC.

Metabolites (Table 2)

Ammonia concentrations in rumen fluid of goats given oak were similar to those given PEG with oak. CC or SC supplementation increased significantly the ammonia concentrations in rumen fluid.

VFA concentration in rumen fluid was not affected by PEG with either type of supplemented food. CC or SC supplementation increased ($P < 0.05$ for CC) VFA concentrations. The proportions of acetic and propionic acid were not influenced by PEG. In goats given oak, addition of CC or SC decreased the proportion of acetic acid ($P < 0.05$) and, conversely, increased the proportion of propionic acid ($P < 0.05$), whereas SC supplementation increased the proportion of butyric acid over the other treatments ($P < 0.05$; Table 2). Consequently, CC supplementation in goats given oak increased the glycogenic ratio (C3/C2 + C4) above all other treatments (data not shown) ($P < 0.05$). The pH of rumen fluid was 6·92 to 6·98 in all treatments and was not influenced by sampling time.

The level of urea in blood serum was not affected by PEG, CC, or CC + PEG. SC and SC + PEG increased significantly the serum urea concentrations in goats given oak leaves.

Discussion

The results of this study confirmed our presumption that the content of tannin in some Mediterranean browse is so high that it may negatively affect the

Table 2 *Effects of either cereal concentrate (CC), or soya-bean concentrate (SC) supplementation, with or without polyethylene glycol (PEG) provision on the concentration of serum urea, rumen ammonia, rumen volatile fatty acids (VFA) and the proportions of individual VFA in six goats given* Quercus calliprinos *(oak)*

	Serum urea (mg/l)	Rumen NH_3 (mg N per l)	Rumen VFA (mmol/l)	Proportions of individual VFA Acetate	Propionate	Butyrate	Fraction of total valerate
Oak	241[b]	80[b]	27·5[b]	0·74[a]	0·15[c]	0·10[b]	0·02[c]
Oak + PEG	153[b]	76[b]	26·8[b]	0·73[a]	0·16[c]	0·11[b]	0·04[b]
Oak + CC	234[b]	186[a]	38·0[a]	0·59[b]	0·29[a]	0·12[b]	0·04[b]
Oak + PEG + CC	269[b]	194[a]	29·5[ab]	0·64[b]	0·19[b]	0·16[a]	0·08[a]
Oak + SC	422[a]	180[a]	34·5[ab]	0·62[b]	0·20[b]	0·17[a]	0·07[a]
Oak + PEG + SC	438[a]	172[a]	28·7[b]	0·63[b]	0·19[b]	0·17[a]	0·09[a]
s.e.	25	11	2·2	0·03	0·02	0·010	0·005

[a, b, c, d] Values in columns marked with different superscript letters differ significantly ($P < 0.05$).

utilization of protein in supplementary foods. Neutralizing the tannins by once-daily provision of PEG proved to be an effective means of preventing this negative effect of tannins. Therefore, supplementing tannin-rich leaves with concentrate food is recommended only if done in combination with PEG (Tables 1 and 2). The marked positive effect of tannin-rich food given with PEG may allow economies in the use of such high-cost foods due to the greater efficiency of protein utilization of the supplementary food and to increased intake and protein utilization of the basal leaf diet. Generally, the present findings are consistent with studies with cattle, sheep and goats in which supplementation of a low basal diet with low protein concentrates depressed the basal diet intake whereas high protein intake stimulated basal diet intake (Forbes, 1995).

The high pH and the low level of VFA in the rumen fluid indicated that the microbial activity was considerably depressed by ingestion of tannin-rich leaves in comparison with the microbial activity in goats consuming a typical medium quality roughage (Silanikove *et al.*, 1996). The increase in digestible OM and CP in the present study upon supplementation with concentrate or high-protein foods (with or without PEG) was much higher than the parallel increase in ruminal VFA and ammonia concentrations, as was found when only PEG was supplemented to the goats (Silanikove *et al.*, 1996). Two explanations are possible: (i) changes in food intake were associated with a parallel increase in rumen DM and fluid contents, and therefore any increase in VFA production rate was not reflected by a proportional increase in their concentration; and (ii) part of the improved apparent digestibility could be related to an increased proportion of food digested in the intestine. This is consistent with findings in sheep, where much of the food-protein that was bound to tannins passed the rumen undegraded (Barry *et al.*, 1986). The tannins, which probably were released before reaching the intestine greatly inhibit intestinal pancreatic enzyme activity in sheep (Silanikove *et al.*, 1994) and goats (Silanikove *et al.*, 1996). In goats supplemented with high-protein food, the larger increase in serum urea as compared with that in rumen ammonia concentration, further strengthens the possibility that the complex between tannins and proteins dissociated after leaving the rumen and increased the absorption of amino acids from the intestine.

Silanikove *et al.* (1996) supported the hypothesis of Provenza (1995) that intake regulation of tannin-rich leaves may be explained best by considering negative post-ingestive effects. Deficiency in protein (Forbes, 1995), energy (Provenza, 1995), and the ratio of protein : energy supplied to the tissues, relative to that required (Egan, 1977), could play a major rôle in controlling daily intake. According to these propositions, when metabolizable energy (ME) intake, protein and the ratio between digestible protein intake and ME intake was far below the optimal ratio (see Table 3), DMI of the leaves was lowest, and when the ratio approached the optimum, the increase in DMI was moderate. However, with high-protein supplementation, although ME intake, protein, and the ratio between digestible protein intake and ME intake exceeded the animal's requirements, there was still a positive response to supplementation, suggesting the involvement of additional factors, such as the interaction between tannins and rumen epithelium (Provenza, 1995; N. Silanikove *et al.*, unpublished data).

Table 3 *Effects of either cereal concentrate (CC) or soya-bean concentrate (SC) supplementation, with or without polyethylene glycol (PEG) provision on crude protein (CP) intake, digestible CP intake (DCP), metabolizable energy (ME) intake, and the ratio DCP/ME in six goats given* Quercus calliprinos *(oak)*

Treatments	CP intake (g/day)	Digestible CP intake		ME intake		Digestible CP intake/ME intake	
		(g/day)	Fraction†	(MJ/day)	Fraction†	(g/MJ)	Fraction†
Oak	42·5	4·8	0·12	2·44	0·42	1·97	0·28
Oak + PEG	53·5	23·7	0·59	7·31	1·26	3·24	0·47
Oak + CC	84·2	2·8	0·07	3·51	0·61	0·80	0·16
Oak + PEG + CC	97·3	52·3	1·31	6·98	1·21	7·49	1·08
Oak + SC	204	92·3	2·31	6·61	1·14	13·9	2·02
Oak + PEG + SC	216	122	3·01	9·83	1·70	12·41	1·79

† Fraction of the maintenance requirements, where the maintenance requirement for CP were taken as 40 g DCP per day, ME requirement as 5·78 MJ/day, and the ratio between them as 6·92 g/MJ (National Research Council, 1981). DCP was calculated from Table 1. ME intake was calculated from Table 1 assuming a caloric value of 18·2 MJ per g digestible organic matter and a conversion factor of 0·8

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The effect of different concentrations of protein and fat in milk replacers on protein utilization in kid goats

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Abstract

The efficiency of utilization of protein for retention was analysed in pre-ruminant kid goats of the Granadina breed. Sixty male kids were used. Six were slaughtered at birth and the remaining 54 were offered different protein and fat intakes using nine different milk replacers. The protein concentrations were 200, 240 and 280 g/kg dry matter (DM) and those of fat were 200, 240 and 280 g/kg DM. Animals were maintained on experiment until they were 60 days old. All were slaughtered on day 61. Nitrogen (N) balance trials were performed during the last 8 days of the 1st and 2nd months. Body composition of the animals slaughtered at birth and at 61 days were determined. Rates of energy retained as protein and as fat were determined (kJ/kg $M^{0·75}$ per day) and the corresponding rates of metabolizable energy intake as protein and as fat (kJ/kg $M^{0·75}$ per day) estimated.

Once the relationships between the rates of N retained and those of digestible N ingested had been established, it was evident that by increasing the protein content of the diet the efficiency of protein retention was decreased. In contrast, increasing the fat content of the milk replacer increased the efficiency of protein retention. The latter effect was noted for the milk replacers containing the high and medium levels of protein but not for those that contained the lowest level of protein, indicating that the level of protein was then the limiting factor. Having recorded this protein-sparing effect of the fat, the results obtained from the slaughter trials were used to develop generalized equations expressing the rates of energy retention in the form of protein or fat as a function of the rates of metabolizable energy intake achieved as both protein and fat. From the analysis of these equations conclusions are drawn about the variable contribution to protein retention in these animals of energy ingested as fat. This contribution depended on the energy intake achieved both in the form of protein and in the form of fat.

Keywords: *fat content, kids, milk replacers, protein content, protein utilization.*

Introduction

The determination of the ideal composition for milk replacers to be used for feeding different mammalian species is of great interest. Given that the protein component of these diets is the most expensive, there are obvious advantages from using only the amount which is strictly necessary. Such an analysis needs to take into account the effect on protein utilization of the amount and quality of the available energy. Fuller and Crofts (1977) indicated that in the analysis of protein utilization, three dietary variables should be considered: protein quality, protein quantity and the nature of the non-protein energy of the diet.

At the beginning of the 1970s, experimental results on possible 'improvements' in the composition of natural milks indicated that one of these variables was particularly interesting from the economic point of view. The amount of protein in the milk in relation to its energy content seemed excessive. This fact suggested that energy content would then be limiting. This point of view, described by Blaxter (1950), was shared by various others. Lodge and Lister (1973) increased the protein retention in suckling calves and obtained a better utilization of the protein ingested by adding different amounts of butter to whole cows' milk. These results gave rise to the hypothesis that by increasing the fat content of milk diets a better utilization of the protein of these diets could be obtained. In consequence and under different experimental conditions, attempts were made to design milk replacers for calves, with increasing amounts of fat. The results obtained were varied and at times contradictory (Raven, 1970; Roy *et al.*, 1970; Bouchard, 1977; Jenkins and Emmons, 1979; Stobo *et al.*, 1979; Henning, 1982).

Because the typical development of the pre-ruminant kid goat, gives rise to very lean carcasses, a great deal of attention has been paid to analysing the effects that dietary composition has on that development. In the present study, results obtained from kid goats of the Granadina breed are presented. The results obtained highlight the changes in protein utilization as a function of the protein and/or fat concentration of the milk replacers used. The manner in which these factors affect food intake, digestive and metabolic utilization of the nutrients and body composition have been analysed previously (Sanz Sampelayo *et al.*, 1995a).

Material and methods

Animals and experimental design

Sixty male kid goats of the Granadina breed were used. These were separated from their dams when they were 2 days old. An initial slaughter group of six animals was killed while the remaining 54 were given different milk replacers at different feeding levels, until they were 60 days old. All animals were slaughtered on day 61. Treatments consisted of three concentrations of crude protein in the milk replacer (low: 200 g/kg dry matter (DM), medium: 240 g/kg DM and high: 280 g/kg DM) and three concentrations of fat in the milk replacer (low: 200 g/kg DM, medium: 240 g/kg DM and high: 280 g/kg DM). Six animals were allocated at random to each of the nine treatments.

Experimental procedure

Animals were housed in individual cages in an animal house maintained at a temperature of 24 ±2°C and a relative humidity of 0·60 ±0·05. During the first 4 days of life all animals consumed colostrum *ad libitum*. Thereafter one of the nine milk replacers was offered until they were 60 days old. In order to obtain different growth rates, each animal within each treatment, received a different energy intake, from near maintenance to appetite. The energy maintenance requirements were estimated from those for milk-fed kid goats (Sanz Sampelayo *et al.*, 1988). The replacers were prepared at 170 g fresh powder per kg and were offered in containers fitted with teats. The containers were placed over a device designed to maintain the replacer temperature at 39 ±2°C and to keep it satisfactorily mixed (Ruiz Mariscal *et al.*, 1990). Because kid goats are very sensitive to cold, the milk replacer temperature was maintained at body temperature which also helped to prevent separation of the fat. The protein component of the milk replacers was obtained from three different protein sources: spray-dried skimmed milk powder, soya bean micronized for milk replacer, and casein (50 : 25 : 25 w/w). To bring the methionine and lysine contents up to those recommended for pre-ruminants (Walker, 1973; Patureau-Mirand, 1975; Williams and Hewitt, 1979) further quantities of these amino acids were added separately. To satisfy requirements for calcium and phosphorus (Sanz Sampelayo *et al.*, 1987), $CaHPO_4.2H_2O$ and $CaCO_3$ were added. NaCl and a mineral-vitamin supplement (Auromix Milk, Cyanamid Iberica, Madrid, Spain) were included in all replacers. The fat component of the replacers was obtained from purified pork lard. After melting the lard in a water bath, it was incorporated into the diets by homogenization using a mechanical mixer. To stabilize the emulsion, two emulsifiers were added (Keltrol F., Vedegsa, Barcelona, Spain and Tween 80, Acofar, Barcelona, Spain). Glucose was included in the nine replacers in different quantities to balance the remaining ingredients. To reduce bacterial growth and digestive disorders the replacers were acidified with 3 ml/l of lactic acid, as proposed by Havrevoll *et al.* (1991). The ingredient composition and DM composition of the milk replacers are shown in Table 1.

All animals were weighed every 3 days. Two balance trials of 8 days duration were performed, starting when the animals were aged 23 and 53 days respectively. During these trials total faeces and urine collections were made and samples were stored frozen. From the bulked samples subsamples were taken for the determination of DM, nitrogen (N) and energy contents of the urine and DM, N, and fat contents of the faeces. The day after the end of the final balance period, when the animals were aged 61 days, they were slaughtered by carotid section after anaesthetization using an intramuscular injection of xylacine (Rompum, Bayer, Barcelona, Spain). Once the animals were dead and completely bled, the skin, limbs, internal organs and head were removed. The rumen, reticulum, omasum and abomasum were washed of their contents. The blood, skin, alimentary canal, all internal organs and the carcass were considered as distinct body parts and were minced separately. Empty-body-weight (EBW) composition, i.e., DM, protein (g/kg EBW) and energy (MJ/kg EBW) were calculated. From the body composition of animals slaughtered at birth and at 61 days of life, protein retention (g/kg $M^{0.75}$ per day) and energy retention (ER) rates (kJ/kg $M^{0.75}$ per day) were also calculated.

The metabolizable energy ingested as fat (MEIf) was estimated as equal to the corresponding digestible energy ingested as fat. On an equal basis, the quantity of metabolizable energy ingested as protein (MEIp) was assessed as equal to the digestible energy ingested as protein minus that excreted in the urine. The digestible energy ingested as fat was calculated by multiplying the weight of digested fat

Table 1 *Composition of milk replacers according to protein concentration and fat concentration*

	Protein concentration								
	High			Medium			Low		
Fat concentration	High	Medium	Low	High	Medium	Low	High	Medium	Low
Ingredient (g/kg)									
Skimmed milk	394·6	394·6	394·6	339·7	339·7	339·7	284·8	284·8	284·8
Micronized soya bean	133·8	133·8	133·8	114·8	114·8	114·8	95·8	95·8	95·8
Casein	72·7	72·7	72·7	62·3	62·3	62·3	62·3	62·3	62·3
Pork lard	275·9	238·0	196·5	276·8	238·9	197·2	277·7	239·8	198·1
Glucose	59·6	97·5	139·2	138·8	176·7	218·4	220·1	258·0	299·7
Mineral-vitamin mixture	19·3	19·3	19·3	19·3	19·3	19·3	19·3	19·3	19·3
Sodium chloride	14·5	14·5	14·5	14·5	14·5	14·5	14·5	14·5	14·5
Dibasic calcium phosphate	14·5	14·5	14·5	19·4	19·4	19·4	22·2	22·2	22·2
Calcium carbonate	9·7	9·7	9·7	9·7	9·7	9·7	9·7	9·7	9·7
Lysine	2·9	2·9	2·9	2·5	2·5	2·5	2·1	2·1	2·1
Methionine	2·5	2·5	2·5	2·2	2·2	2·2	1·9	1·9	1·9
Dry matter (DM) in liquid diet (g/kg)†	181·3	186·1	189·5	173·7	180·8	191·9	174·2	181·3	187·5
DM composition (g/kg)†									
Protein (N × 6·25)	283·8	272·4	262·1	241·2	231·9	223·2	200·9	193·1	187·1
Fat	296·0	238·6	198·4	294·2	247·8	194·2	284·7	238·3	196·8
Carbohydrates‡	339·3	411·4	464·8	383·0	441·8	507·1	438·4	494·9	545·1
Ash	80·9	77·6	74·7	81·6	78·5	75·5	76·7	73·7	71·0
Gross energy (MJ/kg DM)	23·9	23·2	22·1	23·4	22·5	21·5	23·3	22·3	21·4
Digestible protein (g/kg DM)	249·0	248·4	236·1	212·1	202·2	192·9	162·2	163·9	159·0
Metabolizable energy (MJ/kg DM)	19·8	20·4	19·0	19·3	18·7	17·7	18·2	17·9	17·5

† Average values for the containers.
‡ Values obtained by difference.

by 39·8 (kJ/g fat) (Brouwer, 1965; Assendelf *et al.*, 1973). In the same way, the digestible energy ingested as protein and the energy retained as protein (ERp) were calculated by multiplying the weight of digested protein or retained protein by 23·8 (kJ/g protein) (Brouwer, 1965; Assendelf *et al.*, 1973). Finally, the energy retained as fat (ERf) was calculated as the difference between ER and ERp (ERf = ER – ERp).

Analytical procedures

DM and N analyses were performed on samples of the freshly minced parts. All other determinations were carried out on lyophylized samples. N was determined by the Kjeldahl method and energy using an adiabatic bomb calorimeter. The fat concentration of the different milk replacers was analysed by the Gerber method (Pearson, 1976) and that of the faeces by extraction with petroleum ether (boiling point 40-60°C) after HCl hydrolysis.

Statistical analysis

The results of the experiments were statistically analysed by means of standard regression techniques, using the least squares method (Steel and Torrie, 1984). Statistical analyses were performed using the Statgraphics statistical package (Statgraphics, 1991).

Results

Milk replacers composition and animal live weights

In relation to the nutritional utilization and the performance of animals, the protein, fat and carbohydrate sources employed (Table 1) are considered to have been adequate for lamb and calf milk replacers (Soliman *et al.*, 1979; Ørskov, 1982; Moulin, 1983), and also for kid goats (Sanz Sampelayo *et al.*, 1995a).

The mean weight at birth was 3·0 ±0·1 kg and the highest slaughter weights achieved within each treatment were: 9·4, 9·7 and 8·9 kg respectively for the high protein concentration at the high, medium and low fat concentration; 9·2, 8·9 and 8·4 kg respectively for the medium protein concentration at the high, medium and low fat concentration and, 7·9, 7·4 and 7·1 kg respectively for the low protein concentration at the high, medium and low fat concentration.

Relationships between the amounts of retained nitrogen and those of digestible nitrogen ingested

Table 2 shows the intervals of values obtained for the rates of digestible N intake and of retained N according to the milk replacer consumed. These values, expressed as g/kg $M^{0.75}$ per day, were obtained from the balance trials performed during the last 8 days of the 1st and 2nd months of the kids'

Table 2 *Intervals of values of rates of intake of digestible nitrogen (IDN) and nitrogen retention (NR) (g/kg $M^{0.75}$ per day) according to milk replacer protein concentration and fat concentration in kid goats*

Protein concentration	Fat concentration	IDN	NR
Low	Low	0·769-1·265	0·417-0·822
Low	Medium	0·859-1·235	0·442-0·939
Low	High	0·866-1·677	0·488-1·189
Medium	Low	0·960-1·718	0·528-0·986
Medium	Medium	0·998-1·855	0·414-1·167
Medium	High	0·986-1·719	0·426-1·094
High	Low	1·216-2·104	0·620-1·076
High	Medium	1·241-2·344	0·633-1·533
High	High	1·283-2·123	0·687-1·366

life and were used to determine the relationships between the N retention and the corresponding digestible N intake.

The relationships indicated were analysed using either the simple linear model or considering the possibility of including a quadratic term. Only those relationships which the model, as well as the different components of the corresponding equations, found significant ($P < 0·05$) were accepted. In accordance with these criteria the regressions derived fit the simple linear model. Table 3 shows the values for the corresponding independent terms, regressions coefficients, correlation coefficients and residual standard deviations. In each block the differences detected between the corresponding regression coefficients are indicated, these values being indicators of the efficiency with which the digested or absorbed N was retained.

From the results obtained for the medium and high concentrations of protein (Table 3: equations 4 to 6 and 7 to 9), increasing values for regression coefficients were estimated as the fat content of the diet increased. The difference between the extreme values for these coefficients were statistically significant ($P < 0·05$). At the same time it can be seen that lower coefficients were obtained on the higher protein concentration than on the medium protein concentration, even though the fat level remained the same. Once the relationships had been established for each of the protein concentrations independently of the fat (Table 3: equations 1 to 12), progressively lower regression coefficients were observed as the concentration of protein increased. The first of these values was significantly different ($P < 0·05$) from the other two. For the low concentration of protein at each of the fat concentrations (Table 3: equations 1 to 3), the values for the respective regression coefficients, in addition to being the highest obtained, did not vary with the concentration of fat, as was the case for the other two protein concentrations. Regression equations 13 to 15 (Table 3) correspond to each fat concentration at the medium and high concentrations of protein. The respective regression coefficients indicate the effect

Table 3 *Regression coefficients for relationships between nitrogen retention (NR) and the intake of digestible nitrogen (IDN) in kid goats*†

			a		*b*			
f(*x*)	P	F	Mean	s.e.	Mean	s.e.	*r*	Residual s.d.
(1)	Low	Low	–0·976	0·302	1·420	0·267	0·920	0·044
(2)	Low	Medium	–0·656	0·272	1·210	0·249	0·905	0·061
(3)	Low	High	–0·491	0·095	1·011	0·079	0·971	0·060
(4)	Medium	Low	–0·310	0·207	$0·742^{a}$	0·115	0·850	0·104
(5)	Medium	Medium	–0·473	0·116	$0·890^{ab}$	0·083	0·955	0·079
(6)	Medium	High	–0·585	0·162	$0·963^{b}$	0·058	0·935	0·091
(7)	High	Low	–0·198	0·161	$0·608^{a}$	0·095	0·915	0·079
(8)	High	Medium	–0·506	0·201	$0·788^{ab}$	0·110	0·915	0·104
(9)	High	High	–0·706	0·228	$0·940^{b}$	0·133	0·856	0·106
(10)	Low	Low + Medium + High	–0·500	0·091	$1·022^{a}$	0·074	0·925	0·071
(11)	Medium	Low + Medium + High	–0·478	0·087	$0·883^{b}$	0·063	0·923	0·089
(12)	High	Low + Medium + High	–0·447	0·162	$0·767^{b}$	0·092	0·820	0·144
(13)	Medium + High	Low	–0·110	0·110	$0·573^{a}$	0·072	0·870	0·096
(14)	Medium + High	Medium	–0·238	0·116	$0·674^{ab}$	0·071	0·891	0·125
(15)	Medium + High	High	–0·395	0·190	$0·788^{b}$	0·121	0·802	0·165

[a,b] Within each block, mean values with different superscript letter were significantly different: $P < 0·05$.
† Mean values with their standard errors according to milk replacer protein concentration (P) and fat concentration (F). Regression equation: $y = a + bx$, where y is NR (g/kg $M^{0.75}$ per day) and x is IDN (g/kg $M^{0.75}$ per day).

Table 4 *Intervals of values of metabolizable energy intakes as protein (MEIp) and as fat (MEIf) and retention rates as protein (ERp) and as fat (ERf) (kJ/kg $M^{0.75}$ per day) according to milk replacer protein concentration and fat concentration in kid goats*

Protein concentration	Fat concentration	MEIp	MEIf	ERp	ERf
Low	Low	94-120	196-248	49-77	26-78
Low	Medium	93-133	209-299	60-80	43-117
Low	High	99-132	264-352	86-94	37-108
Medium	Low	133-179	221-297	73-98	49-128
Medium	Medium	112-184	227-372	47-109	35-161
Medium	High	108-187	251-433	50-111	37-125
High	Low	140-214	203-323	73-99	49-186
High	Medium	186-256	255-430	65-119	49-216
High	High	145-223	294-452	61-101	44-194

of fat concentration which has already been described, the extreme values also being significantly different ($P < 0{\cdot}05$).

Metabolizable energy intakes as protein and as fat and rates of energy retention as protein and as fat

Table 4 shows the intervals for the values of intakes of metabolizable energy and retention of energy in the form of protein and of fat on the different milk replacers. From these values, the optimum relationships between the retention rates as protein or as fat and the corresponding metabolizable energy intakes, as both protein and fat, were derived. These values are expressed as kJ/kg $M^{0.75}$ per day. The following equations were obtained :

$$ERp = 0{\cdot}644 \text{ (s.e. } 0{\cdot}078) \text{ MEIp} - 0{\cdot}0014 \text{ (s.e. } 0{\cdot}0004) \text{ MEIp}^2 + 0{\cdot}0016 \text{ (s.e. } 0{\cdot}0001) \text{ MEIf}^2$$
$$(R^2 = 0{\cdot}980; \text{ residual s.d.} = 15{\cdot}48)$$

$$ERf = -136{\cdot}57 \text{ (s.e. } 10{\cdot}24) + 0{\cdot}83 \text{ (s.e. } 0{\cdot}06) \text{ MEIf} - 0{\cdot}0000017 \text{ (s.e. } 0{\cdot}0000007) \text{ MEIf}^2.\text{MEIp}$$
$$(R^2 = 0{\cdot}945; \text{ residual s.d.} = 31{\cdot}65).$$

The nature and values of the terms of the first of these equations suggests that the rate of protein retention depended on the energy ingested as protein, this intake leading to a progressively lower increase in protein retention. At the same time, the protein retention also depended on the energy ingested as fat, this parameter leading to a progressively higher increase in retention. The second of these equations indicates that the retention of fat depended on the energy ingested as fat, this leading to a progressively lower increase in fat retention. This diminishing returns in fat retention depended not only on the increasing energy ingested as fat but also on that ingested as protein.

Discussion

Efficiency of utilization of digestible nitrogen intake for retention

The different relationships between N retained and the intake of digestible N which have been established in the present study, were significantly explained by the simple linear model, non-significant coefficients being estimated using the quadratic function. Berschauer *et al.* (1983), analysing the principal aspects of protein metabolism in the growing pig in an attempt to estimate the efficiency of utilization of protein intake for retention, established quadratic equations relating the rates of N retained and those of the corresponding digestible N intakes. In these trials, the results for N intakes were between 0·8 and 4·0 g/kg $M^{0.75}$ per day. However, in the present study, and bearing in mind that the maximum food intake achieved in kid goats is considerably lower than that achieved in pigs, the intakes were between 0·8 and 2·3 g/kg $M^{0.75}$ per day.

Effect of the protein concentration of the milk replacer

The rate and efficiency with which the N consumed from a diet is retained depends on the amount of this element in the diet as well as on the corresponding energy availability. Thus, at any given N intake, as the available energy increases the amount of N retained increases. When non-protein energy is limiting, the protein component of the diet may be used as an energy source and, as a result, the efficiency of utilization of protein for retention diminishes. This implies that the protein in a diet is used optimally for protein synthesis only when there is sufficient available energy. This has been observed during the growth period in both lambs (Black and Griffiths, 1975) and in pigs (Fuller and Crofts, 1977). These findings explain why, when the protein level in the milk replacers used in the present study was increased, the efficiency of utilization of digestible N intake for retention decreased (Table 3: equations 1, 4, 7; 2, 5, 8; 3, 6, 9). This was true both when maintaining the fat content constant as well as independently of the fat content (Table 3: equations 10 to 12). Such findings are illustrated by the fact that at a digestible N intake equal to the mean from all of the trials conducted here, 1·413 g/kg $M^{0.75}$ per day, the mean amounts of N retained would be 0·944, 0·770 and 0·637 g/kg $M^{0.75}$ per day on the milk replacers with the low, medium and high concentrations of protein, respectively, irrespective of their fat concentrations.

Effect of the fat concentration in the milk replacer

The achievement of a better utilization of protein intake for protein retention when its level is decreased in the diet, has been an aspect considered in relation to the nutrient composition that milk replacers should have. In comparison with their

protein contents the composition of natural milks have been shown to be deficient in energy (Blaxter, 1950; Lodge and Lister, 1973). These findings led to the recommendations that milk replacers be designed to provide energy : protein ratios higher than those found in natural milks. This can be achieved easily by increasing the fat content. Such results led to experiments, especially with calves, in which the animals were given milk substitutes with increasing fat contents. The results initially obtained from such trials were discouraging (Raven, 1970; Roy *et al.*, 1970; Bouchard, 1977). Jenkins and Emmons (1979), however, studied how the method of incorporating the fat into the other components of the milk replacer could affect the results. These authors used two methods of incorporation, simple homogenization and vacuum dispersion. When they used the latter of the two methods to incorporate up to 400 g fat per kg milk replacer for calf feeding, they found a better utilization of the foodstuffs for growth as well as a better development of the animals. The authors suggested that the positive response obtained, at least in part, may have been due to the formation of an appropriate protein-fat coagulum at the abomasal level as a result of the method used to incorporate the fat into the diet. This coagulum, they suggested, being firmer than that otherwise formed, led to a better utilization of the nutrients because there was a slower liberation of its different components to the intestine.

The results obtained from the present study, demonstrate the beneficial effects on the efficiency of utilization of protein for retention of increasing the fat content of milk replacers used for feeding kid goats. With milk replacers containing medium and high levels of protein, increasing the fat content led to increasing efficiencies of protein utilization (Table 3: equations 4 to 6 and 7 to 9). This did not occur with the low level protein replacers (Table 3: equations 1 to 3). This suggests that the protein concentration was then the limiting factor. Because of this, the relationships between the retained N and the digestible N intakes were established for each fat concentration at the medium and high concentration of protein and these allowed the corresponding efficiencies to be estimated, illustrating the effect, already described, of the level of fat in the diet (Table 3: equations 13 to 15). These results were obtained using milk replacers in which the fat component of the diet was incorporated into the other components by simple homogenization using two different emulsifiers. It seems, therefore, that this method either gave rise to a sufficiently firm abomasal coagulum or that the firmness of the coagulum was not as important in the kid goats as in the calves used by Jenkins and Emmons (1979).

It is now well known that during growth an increase in the amount of fat or carbohydrates in the diet leads to a better utilization of protein together with a higher protein retention. This is the so-called protein-sparing effect of fats and carbohydrates. Fuller and Crofts (1977) and Fuller *et al.* (1977) suggested possible causes for this effect, citing the contribution that fats can make to the requirements for maintenance and for protein synthesis. From the results of various experiments on pre-ruminant kid goats, it can be seen that the dietary fat seems to participate in both of these processes. Sanz Sampelayo *et al.* (1995b), using the same class of animals as those used in the present study, demonstrated that in kid goats, protein retention occurs at a metabolizable energy intake much lower than the value estimated for maintenance. In contrast, for fat deposition, a metabolizable energy intake much higher than that for maintenance is necessary, both inferences showing that a lipid mobilization to protein retention would take place.

Analysis of the protein-sparing effect of the fat

Having demonstrated the protein-sparing effect of the fat in the pre-ruminant kid goats, using the results obtained from the slaughter trials, it was possible to derive generalized equations which related protein or fat retention rates with the intake of metabolizable energy as both protein and fat, independently of the type of diet fed. From the equation obtained and by calculation of its first partial, derivative with respect to the metabolizable energy intake as fat, the variations in protein retention with variations in fat intake could be analysed:

$$dERp/dMEIf = 0{\cdot}0032\ MEIf.$$

In accordance with this result, it can be deduced that under a constant protein intake, the increment in intake of energy as fat would determine a certain increment in protein retention dependent upon the amount of energy ingested as fat. This process would end at a certain protein retention, according to protein intake or protein retention capacity of the animal.

From the relationships established between the rates of fat retention and the energy intakes as protein and as fat, it is deduced that retention as fat depends, in these animals, on the quantity of metabolizable energy ingested as protein and as fat. The existence of an interaction between both kinds of intake is also deduced. In order to analyse the changes in fat retention arising from changes in protein intake, the first partial derivative of the equation established was calculated as a function of the energy ingested as protein:

$$dERf/dMEIp = -0{\cdot}0000017\ MEIf^2.$$

From this it was shown that at a constant intake of energy as fat, the variation in fat retention as a function of the variation in protein intake would always be negative and dependent on the corresponding fat intake. Thus the greater the protein intake under a constant fat intake, the lower the fat retention as a consequence of the greater need to use the energy ingested as fat for protein retention. Thus, the protein-sparing effect of the fat depends not only on the quantity of the fat ingested but also on the metabolizable energy ingested as protein. To quantify the effect of protein concentration (PC, g/kg DM) on the use of the energy ingested as fat for fat retention, the authors established a simple relationship between the intakes of metabolizable energy as fat and its retention as a function of the concentration of protein included *a priori* in the food:

PC = 200:

$$ERf = -190{\cdot}23\ (\text{s.e. } 10{\cdot}75) + 1{\cdot}58\ (\text{s.e. } 0{\cdot}13)\ MEIf - 0{\cdot}0022\ (\text{s.e. } 0{\cdot}0004)\ MEIf^2$$

($R^2 = 0{\cdot}983$; residual s.d. = 18·66)

PC = 240:

$$ERf = -151{\cdot}48\ (\text{s.e. } 15{\cdot}76) + 0{\cdot}98\ (\text{s.e. } 0{\cdot}16)\ MEIf - 0{\cdot}00095\ (\text{s.e. } 0{\cdot}00039)\ MEIf^2$$

($R^2 = 0{\cdot}946$; residual s.d. = 27·41)

PC = 280:

$$ERf = -134{\cdot}93\ (\text{s.e. } 16{\cdot}48) + 1{\cdot}04\ (\text{s.e. } 0{\cdot}17)\ MEIf - 0{\cdot}00087\ (\text{s.e. } 0{\cdot}00039)\ MEIf^2$$

($R^2 = 0{\cdot}962$; residual s.d. = 28·67).

From the equations obtained it is possible to calculate for each level of protein, the variation that would occur in fat retention when changing its intake:

$$PC = 200:\ dERF/dMEIf = 1{\cdot}58 - 0{\cdot}0044\ MEIf$$

$$PC = 240:\ dERF/dMEIf = 0{\cdot}98 - 0{\cdot}0019\ MEIf$$

$$PC = 280:\ dERF/dMEIf = 1{\cdot}04 - 0{\cdot}0017\ MEIf.$$

According to the form of the corresponding first derivative equations, it is possible to estimate the fat intake for each concentration of protein, that would determine a null variation in its retention. These would be the quantities of fat intake from which a higher intake would never determine a higher retention. These values would be: 359, 516 and 598 kJ/kg $M^{0.75}$ per day under intakes of the milk replacers with 200, 240 and 280 g/kg DM of protein, respectively. Diets containing higher proportions of protein therefore require greater energy intakes in the form of fat to achieve maximal fat deposition by virtue of the fact that the higher the dietary protein concentration, the greater the proportion of energy intake as fat being used for protein retention by the animal.

Finally, it is worth noting how the factorial calculation of the energy requirements of growing animals supposes that the energy expenditure associated with the deposition of one unit of protein and/or fat is a constant quantity, regardless of the nature of the diet. However, from the results obtained here, it is deduced that in pre-ruminant kid goats the contribution of the metabolizable energy ingested as fat to protein retention varies according to the metabolizable energy available as protein and as fat. This fact obliges one to consider as variable the partial efficiency with which an intake of metabolizable energy would be used for protein retention because of the rôle the origin of the energy plays in this respect.

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Prediction of voluntary intake and digestibility of maize silages given to sheep from morphological and chemical composition, *in vitro* digestibility or rumen degradation characteristics

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Abstract

Eleven maize silages with crude protein (CP) and neutral detergent fibre (NDF) ranging from 77 to 93 and 359 to 542 g/kg dry matter (DM) respectively, were used to study the relationship between ear content, chemical composition, fermentative characteristics, in vitro *DM digestibility and ruminal degradation characteristics, on the one hand, and the voluntary DM intake by sheep or* in vivo *organic matter apparent digestibility (OM digestibility), on the other.*

The silages were offered ad libitum *to mature ewes given a daily supplement of 85 g of soya-bean meal. DM intake varied from 41·1 to 68·6 g DM per kg* $M^{0.75}$ *daily. OM digestibility and NDF apparent digestibility were measured, using the same ewes in a period subsequent to that of voluntary intake measurement. The silages, in this case, were offered at a feeding level of 1·2 maintenance. OM digestibility and NDF apparent digestibility ranged from 0·622 to 0·757 and from 0·377 to 0·605, respectively. Rumen DM disappearance was measured by incubating samples in nylon bags in the rumen of three silage-fed rumen-cannulated wethers for 3, 6, 12, 24, 48 and 72 h and by fitting the exponential model* $y = a + b(1 - e^{-ct})$ *to the results. Potential degradabilities (defined as* a + b*) for DM ranged from 728 to 815 g/kg.*

Accurate prediction of DM intake ($r = 0{\cdot}93$; $P < 0{\cdot}01$; *residual s.d. = 3·9) and OM digestibility (*$r = 0{\cdot}86$; $P < 0{\cdot}01$; *residual s.d. = 0·022) was achieved using the soluble fraction (*a*) and the insoluble but fermentable matter (*B*) and the insoluble but potentially degradable fraction (*b*), respectively. However, looking for a compromise between accuracy and simplicity, reliability and inexpensiveness, ear content is proposed as a predictor of OM digestibility (*$r = 0{\cdot}85$; $P < 0{\cdot}01$*) and the pH and acetic acid concentration of the silages may be used as a predictor of DM intake (*$r = 0{\cdot}80$; $P < 0{\cdot}05$*).*

Keywords: *degradation, digestibility, food intake, maize silage, sheep.*

Introduction

Maize is probably one of the best plants to store as silage due to the high content of fermentable water-soluble carbohydrates, a low buffering capacity and a high dry matter (DM) content; that is to say, the characteristics of an ideal crop for preservation as silage (McDonald *et al.*, 1991). In spite of this, voluntary intake of maize silage is less than that of the same crop offered fresh (Andrieu and Demarquilly, 1974).

The difficulty of predicting the voluntary intake of silages has often been attributed to the effects of fermentation end products present in the silage, although these effects have not always been shown. For example, Wilkins et *al.* (1971) working with grass, cereal and legume silages, given to wethers, concluded that the voluntary intake is negatively correlated with the contents of acetic acid and ammonia, whereas Buchanan-Smith (1990) found that ammonia had no negative effect on intake of lucerne silage and Van Os *et al.* (1995) found that the addition of ammonia (NH_3) was not the causal factor in the negative correlations between silage NH_3-nitrogen content and intake observed by other authors. McLeod *et al.* (1970) concluded that the acids

produced during the normal grass silage fermentation can limit the intake in calves and wethers but Phillip *et al.* (1981) found that food intake was unaffected by intraruminal infusion of acetic acid. Finally, other products of protein degradation such as amines may also affect silage intake (Buchanan-Smith and Phillip, 1986) although Van Os *et al.* (1995) found that the amines tended to reduce DM intake only.

Several equations have been proposed to predict maize silage digestibility (Jones *et al.*, 1980; Andrieu, 1984) and DM intake of maize silages (Jones *et al.*, 1980) although none include cultivars with exotic germplasm in their pedigree. The aim of the present study was to assess the possibility of predicting voluntary DM intake and apparent organic-matter (OM) digestibility (OM digestibility) of maize silage, given to sheep, from morphological and chemical characteristics, *in vitro* digestibility and ruminal degradation characteristics.

Material and methods

Silages

Eleven maize (*Zea mays*) silages were made using plastic containers with a capacity of 1100 litres. Five adapted (S1 to S5) and six (S6 to S11) semiexotic materials (Table 1), obtained by crossing adapted and tropical populations provided by the International Maize and Wheat Improvement Centre (CIMMYT), were cultivated in Caldes de Montbui (north-east Spain) with irrigated conditions in the summers of 1993 and 1994. After harvesting, the plants were chopped with a precision-chop forage harvester, stored in containers, covered with plastic sheets and weighted with bricks. Two containers for each material were employed. Prior to ensilage, ear content, as a proportion of the contribution of the ear in the total yield, was measured and stover samples taken (except in the case of S9). The materials were harvested with an average DM concentration of 310 g/kg.

Voluntary intake of silages

The maize silages were offered to eight mature Manchega-bred ewes (average body weight 64·2 (s.d. 1·2) kg), which were neither lactating nor pregnant, kept in individual pens with free access to water and salt blocks. The eight ewes were randomly put into two groups each of four animals. Five experimental periods, each one lasting for 3 weeks, with 2 weeks of adaptation and 1 week of measurement, were used. During each period, two silages were selected at random, one of which was given to one group of four animals, and the other to the other group. The silage was offered every day, at the same time (09.00 h), *ad libitum* (proportionally 0·15 in excess of the previous day's consumption) together with 85 g soya-bean meal per animal to avoid a deficit of degradable protein. Prior to the experiment all sheep were given a commercial maize silage (AE703, Agrar Semillas S.A., Zaragoza, Spain), *ad libitum*, for 3 weeks. Silage and residue samples were taken daily and bulked over the last 7 days of each period for each treatment

Table 1 *Characteristics of cultivars*

Silage	Cultivar type	FAO maturity rate	Pedigree
S1	Adapted	700	Commercial hybrid AE703 (Agrar Semillas S.A., Zaragoza, Spain) of unknown pedigree
S2	Adapted	400	Commercial hybrid CARMINA (Pioneer Hi-Brand Intern., Inc., Johnston, USA) of unknown pedigree
S3	Adapted	700	Adapted open pollinated population Lancaster surecrop
S4	Adapted	700	Commercial hybrid A640 (Adour, Semillas Fitó S.A., Barcelona, Spain) of unknown pedigree
S5	Adapted	400	Commercial hybrid SABRINA (Pioneer Hi-Brand Intern., Inc., Johnston, USA) of unknown pedigree
S6	Semiexotic	900	(Mo17 × Brazil 1792) × B73†
S7	Semiexotic	1000	B73 × Brazil 1792†
S8	Semiexotic	1000	B73 × Across 8443‡
S9	Semiexotic	1000	B73 × V465§
S10	Semiexotic	1000	L696 × Across 8443‖
S11	Semiexotic	1000	B73 × Brazil 1741¶

† Mo17 B73: adapted public inbred; Brazil 1792: exotic open pollinated population with Cateto racial composition.
‡ Across 8443: exotic open pollinated population with Tuxpeño racial composition.
§ V465: exotic open pollinated population from Brazil of unknown racial composition.
‖ L696: experimental adapted inbred.
¶ Brazil 1741: exotic open pollinated population with Cateto Paulistic racial composition.

and stored at –20°C until analysis. Animals were weighed at the beginning and the end of each period, and the mean live weights were used to calculate the individual intakes per kg $M^{0.75}$ per day. The sheep were treated for nematoda (7·5 mg netobimin per kg live weight, Hapasil, Shering-Plough S.A., San Agustin de Guadalix, Spain) before starting the experimental period.

Apparent digestibility

In vivo apparent digestibility was recorded using the same design and the same ewes employed in the voluntary intake measurement, in a subsequent period. The silage was offered at a feeding level of 1·2 maintenance, together with 85 g soya-bean meal. Each period was of 3 weeks' duration, with 2 weeks of adaptation and 1 week of measurement. The ewes were housed in metabolism cages with free access to water and salt blocks. Faeces were collected daily and samples of silages and faeces were also taken and bulked over the last 7 days of each period and stored at –20°C. The *in vitro* DM digestibility (Tilley and Terry, 1963) was determined according to the modified method of Alexander and McGowan (1966).

DM degradation in situ

DM degradation was determined by incubating about 3 g dry sample in nylon bags in the rumen of the three cannulated wethers. The samples were dried for 72 h in a forced-air oven at 40°C and milled through a 1·5-mm screen. The bags measured 125 × 100 mm, with an average pore size of 50 µm. Duplicates of bags were withdrawn after 3, 6, 12, 24, 48 and 72 h, washed for 15 min in a washing machine and dried for 48 h at 60°C. Zero-time washing losses were estimated by soaking two bags per sample in warm tap water (39°C) for 30 min followed by washing and drying as before. The wethers were given a commercial maize silage (AE703, Agrar Semillas S.A., Zaragoza, Spain) *ad libitum* plus 85 g soya-bean meal.

Chemical analysis

Stover and faeces samples were dried in a forced air-oven at 60°C and milled through a 1 mm screen for chemical analysis. DM was determined at 103°C for 48 h. Ash content was measured gravimetrically by igniting samples in a muffle furnace at 550°C for 4 h. Crude protein (CP) was determined by Kjeldahl procedure (N × 6·25) on a Kjeltec Auto 1030 Analyser (Tecator, Höganäs, Sweden) and ether extract (EE) by the Soxhlet method (European Communities, 1984). Neutral-detergent fibre (NDF) was determined by the method of Van Soest *et al.* (1991), acid-detergent fibre (ADF) and acid-detergent lignin (ADL) according to the method of Goering and Van Soest (1970).

Silage samples were mixed thoroughly and two subsamples taken. One subsample was dried and milled for determination of DM, ash, EE, NDF, ADF and ADL content as described before. DM was corrected for volatile components lost (Dulphy and Demarquilly, 1981). The remaining wet subsample was analysed for concentration of total nitrogen (N) (Kjeldahl method) and NH_3-N (Association of Official Analytical Chemists, 1990) and used to obtain silage juice for the determination of pH and water-soluble N (Nsol) (Dulphy and Demarquilly, 1981) and volatile fatty acids (VFA). VFA were determined by gas chromatography. Samples were prepared according to the method of Guitart *et al.* (1996) with the modified amount of 2 ml of sample. Derivatized extract (0·5 µl) containing heptanoic acid as an internal standard was injected into a gas chromatograph equipped with a Stabilwax metallic capillary column of 60 m × 0·53 mm i.d. (Restek, Bellefonte, USA) using helium as a carrier gas at 14 m/min. The column was held at 50°C and then ramped at 2°C per min to 110°C. Injector and detector temperatures were 270°C.

Statistical analysis

The values for disappearance of DM with time were fitted to the exponential equation: $y = a + b(1 - e^{-ct})$ of Ørskov and McDonald (1979), where y is DM degradation at time t, a represents the immediately soluble material, b is the insoluble but potentially degradable material and c the rate of degradation. Potential degradability was defined as $(a + b)$. A was defined as the initial washing loss, while the insoluble but fermentable matter was defined as $B = (a + b) - A$. Data for DM intake and apparent OM digestibility of silages were submitted to a one-way variance analysis, using the general linear model procedure of Statistical Analysis Systems Institute (SAS, 1990). The comparison between adapted and semiexotic materials was also made by analysis of variance. A correlation matrix between the data for ear content, chemical composition, intake, *in vivo* and *in vitro* digestibility and *in situ* DM degradation was obtained. Stepwise regression was used to find the best fitting equation for DM intake and OM digestibilty using the stepwise procedure of SAS (1990).

Results

The ear content and the stover chemical composition are given in Table 2. At harvest, ear content ranged between 380 and 550 g/kg DM. Three cultivars, two adapted (S1, S4) and one semiexotic (S8) had an ear content higher than 500 g/kg DM. The stover NDF, ADF and ADL concentration, expressed as g/kg DM, varied from 570 to 696, from 311 to 403 and from 34 to 60, respectively.

Table 2 *Ear content of the whole plants and chemical composition of stover maizes*

Silages†	Ear content (g/kg DM)	Chemical constituents (g/kg dry matter (DM)) Ash	Crude protein	Neutral-detergent fibre	Acid-detergent fibre	Acid-detergent lignin
S1	550	92	63	639	354	46
S2	400	81	63	570	311	34
S3	460	85	63	586	333	48
S4	520	81	61	647	381	56
S5	400	81	64	571	312	34
S6	490	107	75	691	403	60
S7	420	106	76	642	372	56
S8	520	116	71	669	390	54
S10	380	102	62	696	328	39
S11	430	80	67	646	316	52
Mean	460	93	67	634	350	48
s.e.	18·8	4·3	1·8	14·5	10·9	3·0

† 59 was not measured.

The chemical composition and the fermentative characteristics of silages are given in Table 3. The CP concentration ranged from 77 to 93 g/kg DM. Cell wall concentration (as defined by NDF) varied from 359 to 542 g/kg DM and ADL ranged from 26 to 45 g/kg DM. The mean values of the fermentative characteristics (Table 3) showed that, in general, the silages were of good quality according to the classification of Dulphy and Demarquilly (1981). The best silage, in relation to the fermentative characteristics, was S10 and the worst S5, S6 and S7. In the case of S6, the high level of volatile fatty acids was due to a high level of propionic acid although the acetic acid content was low. The pH of silages ranged from 3·50 to 3·83.

The degradation characteristics are given in Table 4. The zero time washing loss of DM ranged from 242 to 415 g/kg DM. The insoluble but fermentable matter (*B*) varied from 331 to 515 g/kg DM, potential DM degradation from 728 to 815 g/kg DM and *c* values from 0·024 to 0·053.

The mean DM intake of the maize silages was 57·2 (s.e. 2·5) g DM per kg $M^{0.75}$ per day and ranged from 41·1 to 68·6 g DM per kg $M^{0.75}$ per day (Table 5). OM

Table 3 *Chemical composition and fermentative characteristics of the maize silages*

Silages	Chemical constituents (g/kg dry matter (DM)) Ash	Crude protein	Neutral-detergent fibre	Acid-detergent fibre	Acid-detergent lignin	Ether extract	Fermentative characteristics pH	Acetic acid (g/kg DM)	Volatile fatty acid (mmol/kg DM)	Ammonia nitrogen (% total N)	Water-soluble nitrogen (% total N)
S1	49	77	359	193	26	31	3·75	22	399	6·3	46·4
S2	77	93	449	254	37	31	3·56	25	437	6·9	60·2
S3	60	82	449	241	28	31	3·68	15	259	6·1	67·8
S4	51	77	420	234	28	29	3·71	25	437	5·4	51·6
S5	62	87	542	321	45	27	3·50	41	723	6·9	63·3
S6	55	84	436	246	37	32	3·83	18	984	8·4	56·3
S7	67	89	457	268	44	29	3·69	41	785	7·4	66·7
S8	66	85	464	273	43	27	3·59	23	403	6·5	65·1
S9	54	84	363	209	30	34	3·77	26	462	8·4	47·6
S10	72	78	507	249	40	25	3·73	13	237	4·7	49·7
S11	61	82	487	243	37	30	3·56	29	510	6·0	63·8
Mean	61	84	448	248	36	30	3·67	25	512	6·6	58·0
s.e.	2·6	1·5	16·6	10·1	2·1	0·7	0·03	2·7	65·5	0·3	2·4

Table 4 *Dry matter (DM) degradability (g/kg DM) of silages after 12, 24 and 48 h and their degradation characteristics obtained by fitting data of DM degradation over 72 h incubation to the equation* $y = a + b(1 - e^{-ct})$†

	DM degradation after:							
Silages	12 h	24 h	48 h	*A*	*B*	*a* + *b*	*c*	Residual s.d.
S1	476	536	660	415	331	745	0·025	2·4
S2	443	492	659	320	471	791	0·026	2·3
S3	456	527	621	321	439	759	0·027	1·3
S4	428	561	654	324	455	779	0·027	1·6
S5	490	614	674	320	419	739	0·053	2·3
S6	449	547	680	242	516	759	0·037	4·0
S7	441	557	658	304	511	816	0·026	1·6
S8	492	573	625	356	388	744	0·030	2·5
S10	427	515	616	248	480	728	0·034	2·0
S11	363	500	624	279	485	764	0·024	2·0
Mean	447	542	647	313	449	762	0·031	
s.d.	38	36	23	51	58	27	0·009	

† *A* is the washing loss (water soluble fraction) and $B = (a + b) - A$ is the insoluble but fermentable matter; *a*, *b* and *c* are the constants in the exponential equation $y = a + b(1 - e^{-ct})$; $a + b$ is the potential DM degradation and *c* is the rate of DM degradation. S9 was not measured.

digestibility and NDF apparent digestibility varied from 0·622 to 0·757 and from 0·377 to 0·605 respectively. The mean values were 0·678 (s.e. 0·012) and 0·491 (s.e. 0·021) for OM digestibility and NDF apparent digestibility, respectively.

Significant differences between adapted and semiexotic cultivars were only found in stover fibre components, whereas the silage chemical composition of the adapted and semiexotic materials was similar. The adapted and semiexotic materials

Table 5 *Dry matter (DM intake in g DM per kg* $M^{0.75}$ *per day),* in vivo *apparent digestibility of organic matter (OM digestibility),* in vivo *apparent digestibility of cell walls (NDF digestibility) and* in vitro *digestibility of dry matter* (in vitro *DM digestibility) of the maize silages*

	DM intake	s.e.	*In vivo* OM digestibility	s.e.	*In vivo* NDF digestibility	s.e.	*In vitro* DM digestibility
Silages							
S1	$60·1^{ab}$	4·48	$0·757^{a}$	0·011	$0·437^{de}$	0·023	0·736
S2	$54·8^{bc}$	3·32	$0·652^{cde}$	0·010	$0·485^{bcd}$	0·028	0·681
S3	$46·2^{cd}$	1·98	$0·695^{bc}$	0·005	$0·552^{ab}$	0·008	0·663
S4	$62·5^{ab}$	1·71	$0·722^{ab}$	0·013	$0·538^{abc}$	0·023	0·685
S5	$41·1^{d}$	0·85	$0·680^{bcd}$	0·016	$0·605^{a}$	0·024	0·669
S6	$68·4^{a}$	5·14	$0·679^{bcd}$	0·008	$0·475^{cd}$	0·014	0·690
S7	$68·6^{a}$	4·25	$0·622^{e}$	0·015	$0·446^{de}$	0·030	0·668
S8	$51·4^{bcd}$	2·56	†		†		0·635
S9	†		$0·680^{bcd}$	0·023	$0·377^{e}$	0·043	0·692
S10	$57·2^{bc}$	3·36	$0·648^{de}$	0·018	$0·497^{bcd}$	0·005	0·639
S11	$56·9^{bc}$	3·63	$0·672^{cd}$	0·007	$0·536^{abc}$	0·006	0·619
Mean	57·2		0·678		0·491		0·671
s.d.	7·9		0·039		0·065		0·031
Adapted cultivars							
Mean	52·9		0·701		0·524		0·687
s.d.	9·1		0·040		0·066		0·029
Semiexotic cultivars							
Mean	60·5		0·660		0·467		0·657
s.d.	7·7		0·025		0·060		0·030

^{a,b,c,d,e} Means in the same column with different superscripts differ significantly ($P < 0·05$).
† Not measured.

Table 6 *Correlation matrix (r) between ear content, fibre components, ruminal degradation constants and* in vivo *and* in vitro *measures*†

	Ear content	Stover fibre components			Silage fibre components			Fermentative characteristics					Ruminal degradation constants			*In vivo* digestibility of			
		NDF	ADF	ADL	NDF	ADF	ADL	pH	Acetic acid	VFA	NH_3-N	Nsol	*a*	*b*	*c*	DM intake	OM	NDF	*In vitro* DM digestibility
Ear content	1·00																		
Stover:																			
NDF	0·27	1·00																	
ADF	0·69*	0·63*	1·00																
ADL	0·60	0·60	0·83**	1·00															
Silage:																			
NDF	–0·77**	–0·14	–0·47	–0·41	1·00														
ADF	–0·55	–0·32	–0·21	–0·32	0·87***	1·00													
ADL	–0·58	0·07	–0·08	–0·18	0·77**	0·84***	1·00												
pH	0·42	0·64*	0·63*	0·53	–0·64*	–0·67*	–0·51	1·00											
Acetic acid	–0·25	–0·38	–0·13	–0·09	0·26	0·52	0·51	–0·49	1·00										
VFA	–0·04	0·10	0·40	0·35	0·12	0·36	0·42	0·11	0·52	1·00									
NH_3-N	0·09	–0·10	0·38	0·28	–0·35	–0·01	0·08	0·25	0·31	0·69*	1·00								
Nsol	–0·31	–0·42	–0·14	0·08	0·58	0·66*	0·51	–0·61*	0·35	0·21	0·06	1·00							
a	0·87***	0·12	0·55	0·42	–0·78**	–0·50	–0·44	0·28	–0·10	–0·15	0·09	–0·16	1·00						
b	–0·84**	–0·22	–0·44	–0·26	0·61	0·46	0·41	–0·26	0·29	0·28	0·08	0·30	–0·90***	1·00					
c	–0·32	–0·15	–0·14	–0·39	0·63*	0·73**	0·52	–0·25	0·30	0·41	0·22	0·05	–0·45	0·20	1·00				
DM intake	0·25	0·63*	0·62*	0·64*	–0·51	–0·49	–0·13	0·70*	–0·04	0·41	0·27	–0·37	–0·37	0·19	0·05	1·00			
OM apparent digestibility	0·85**	–0·04	0·19	0·13	–0·54	–0·53	–0·77**	0·24	–0·29	–0·26	–0·18	–0·49	0·69*	–0·86**	–0·10	–0·11	1·00		
NDF apparent digestibility	–0·29	–0·46	–0·47	–0·31	0·76**	0·67*	0·29	–0·64*	0·11	–0·03	–0·49	0·54	–0·52	0·33	0·56	–0·77**	0·04	1·00	
In vitro DM digestibility	0·49	–0·17	0·25	–0·02	–0·72**	–0·50	–0·56	0·50	–0·01	0·19	0·36	–0·57	0·50	–0·40	–0·03	0·28	0·62*	–0·46	1·00

† NDF = neutral-detergent fibre; ADF = acid-detergent fibre; ADL = acid-detergent lignin; VFA = volatile fatty acid; NH_3-N = ammonia nitrogen; Nsol = water-soluble nitrogen; OM = organic matter; DM = dry matter; for ruminal degradation constants see Table 4 footnote.

were no different for DM intake (52·9 *v.* 60·5, $P > 0·05$), or OM digestibility (0·701 *v.* 0·660, $P > 0·05$) (Table 5).

The simple correlation coefficients between the most relevant variables and a series of DM intake and OM digestibility prediction equations are given in Tables 6 and 7, respectively. The correlation matrix shows the negative correlation between NDF of silage and ear content ($r = -0·77$, $P < 0·01$). The constants *a* and *b* were significantly correlated with the ear content ($r = 0·87$; $P < 0·001$, and $r = -0·84$; $P < 0·01$, respectively) and with silage NDF concentration ($r = -0·78$; $P < 0·01$ and $r = 0·61$; $P < 0·10$, respectively). The correlations between the constant *c* and the NDF and ADF concentration were positive and significant ($r = 0·63$; $P < 0·05$ and $r = 0·73$; $P < 0·01$, respectively), as were those between *c* and the amount of digestible cell walls (NDF × NDF digestibility) ($r = 0·70$; $P < 0·05$; not tabulated).

There was no correlation between DM intake and *in vivo* OM digestibility, but DM intake was correlated ($P < 0·01$) with the amount of digestible cell wall ($r = -0·73$; not tabulated). On the other hand, DM intake was positively and significantly ($P < 0·05$) correlated with the stover fibre components ($r = 0·63$, $r = 0·62$ and $r = 0·64$ for NDF, ADF and ADL, respectively) and with the pH of the silage ($r = 0·70$; $P < 0·05$). When the acetic acid concentration was added to the silage pH the equation accounted for 0·64 of the variability in DM intake (Table 7). The correlations between DM intake and the potential DM degradation ($a + b$) and the constant *c* were $r = 0·50$ and $r = -0·45$, respectively and neither were significant ($P > 0·05$).

OM digestibility was correlated positively with the ear content ($r = 0·85$; $P < 0·01$) and negatively with silage ADL concentration ($r = -0·77$; $P < 0·01$). *In vitro* DM digestibility was also significantly correlated with *in vivo* OM digestibility ($r = 0·62$; $P < 0·05$). The constants *a* and *b* accounted for proportionately 0·48 and 0·74 of the variability in OM digestibility respectively when considered individually.

Discussion

Ear content was a good predictor of OM digestibility. This variable accounted for proportionately 0·72 of the variability in OM digestibility, in agreement with Andrieu (1984) who found a good correlation between both OM digestibility and energy value of maize silages, and the grain content. The high positive correlation ($r = 0·87$; $P < 0·001$) between ear content and the constant *a* can be attributed to the high level of non-structural carbohydrates linked to a high grain content. On the other hand, the similar potential DM degradation of the silages could explain the high and negative correlation between ear content and the constant *b* ($r = -0·84$; $P < 0·01$) and the negative and significant correlation between *a* and *b* ($r = -0·90$; $P < 0·001$).

The DM intake predictive value of the chemical composition of the silage has been reported by several authors. Laforest *et al.* (1986), working with legume and grass silages, found that the intake of DM was correlated positively with CP ($r = 0·92$) and negatively with NDF ($r = -0·87$). Jones *et al.* (1980), with maize silages, hay crop silages and mixtures of both, also found the same kind of relationships. In

Table 7 *Multiple stepwise regressions between DM intake (g DM per kg $M^{0·75}$ per day) or OM digestibility and ear content, fibre components, fermentative characteristics,* in vitro *DM digestibility and* in situ *DM degradation*†

Y	Technique	Equation	*r*	Significance	Residual s.d.
DM intake					
	Stover fibre components	28·09 + 0·60 (ADL)	0·641	0·046	7·2
	Silage fibre components	70·50 – 0·13 (NDF) + 329·2 (ADL/ADF)	0·757	0·051	6·6
	Fermentative characteristics	–249·34 + 80·74 (pH) + 0·42 (acetic)	0·802	0·027	6·0
	In situ DM degradation	–81·21 + 0·12 (*a*) + 0·22 (*B*)‡	0·932	0·002	3·9
	All four techniques	–188·66 + 0·12 stover NDF + 1·98 NH_3-N –0·51 Nsol + 0·24 ($a + b$)	0·990	0·001	1·7
OM digestibility					
	Ear content	0·4163 + 0·0006 (ear content)	0·849	0·004	0·023
	Silage fibre components	0·8305 – 0·0043 (ADL)	0·769	0·009	0·025
	In vitro DM digestibility	0·1728 + 0·75 (*in vitro* DM digestibility)	0·624	0·054	0·032
	In situ DM degradation	0·9828 – 0·0007 (*b*)	0·862	0·003	0·022
	All four techniques	0·9828 – 0·0007 (*b*)	0·862	0·003	0·022

† For abbreviations see Table 6.
‡ Equation obtained removing the silage S1.

our case, the DM intake was significantly correlated with the stover fibre components ($P < 0.05$). The stover NDF, ADF and ADL accounted for proportionately 0·40, 0·38 and 0·41 of the variability in DM intake respectively when considered individually. The best predictive equation using only the stover fibre components is shown in Table 7 and it was through the stover ADL. In relation to the fibre components of the silages, when an index of lignification (ADL/ADF) was added to the cell wall concentration (NDF), the DM intake predictive value of the multiple regression equation was improved significantly ($P < 0.05$) and the equation then accounted for 0·57 of the variability. On the other hand, the silage ADL concentration was a good predictor of OM digestibility whereas the other fibre components did not increase significantly the predictive value.

DM intake increased as pH of the silage increased. The linear equation obtained to predict DM intake was: $y = -164.2 + 60.4$ (pH) ($r = 0.703$: $P < 0.023$; residual s.d. = 6·7). Shaver *et al.* (1984) found that the silage pH was a factor that affects voluntary consumption of maize silage. They developed a quadratic equation to predict OM intake, that is to say, that intake increases if silage pH rises from 3·6 to 5·6 but falls after this point. In our case, the quadratic equation did not improve the prediction, probably because of the short range of pH values. On the other hand, the silage pH was positively related to the DM percentage of the silage ($r = 0.73$; $P < 0.01$; not tabulated) in accordance with the stability criterion proposed by Dulphy and Demarquilly (1981).

There was no correlation between DM intake and *in vivo* OM digestibility. Our results confirm what has been indicated by Ørskov *et al.* (1988); that is, when intake is voluntary, *in vivo* apparent digestibility of forages given to ruminants at maintenance is an inadequate measure of nutritive value. On the other hand, many regression equations have been published relating *in vivo* to *in vitro* digestibility. With the two-stage fermentation procedure of Tilley and Terry (1963), Aerts *et al.* (1977) found, with silages of different species, that the *in vitro* OM digestibility could explain 0·73 of the variability of *in vivo* OM digestibility. We also found a positive and significant correlation ($r = 0.62$, $P < 0.05$) between OM digestibility and *in vitro* DM digestibility.

The insoluble but potentially degradable fraction (b) allowed us to predict the OM digestibility. Potential DM degradation ($a + b$) provided an inaccurate DM intake ($r = 0.50$; $P > 0.05$) in agreement with Carro *et al.* (1991). In the same way, Khazaal *et al.* (1995) obtained a low and non-significant coefficient of correlation with grass and legume hays. In our case, some silages differing in DM intake had similar degradabilities. When S1, with a practically linear DM degradation was removed, the accuracy of DM intake, through the degradation components, increased and the correlation between DM intake and the insoluble but fermentable matter (B) was significant ($r = 0.79$; $P < 0.01$). In fact, when constant a from the exponential equation and B were employed together we obtained an acceptable prediction of DM intake when a single technique was used. The precision of the equation to predict DM intake was improved using ruminal degradation characteristics (residual s.d. = 3·9) compared with chemical composition (residual s.d. = 6·6) or fermentative traits (residual s.d. = 6·0). Nevertheless, the best DM intake equation was obtained when all four techniques were employed together ($r = 0.99$; $P < 0.001$; residual s.d. = 1·7). On the other hand, the insoluble but potentially degradable fraction (b) allowed us to predict *in vivo* OM digestibility, though with a similar precision to ear content. The use of the four techniques together did not improve the OM digestibility prediction given by the *in situ* DM degradation technique (Table 7).

In conclusion, prediction of DM intake and OM digestibility of maize silages was slightly more accurate using degradability characteristics than the other single techniques used, although the best predictive DM intake equation was obtained using stover NDF, NH_3-N and water-soluble N and the potential DM degradation. However, given the complexity of using the four techniques together, and with the aim of striking a balance between accuracy and simplicity, reliability and inexpensiveness, it is possible to propose ear content as a predictor of OM digestibility, and fermentative traits, pH and acetic acid concentration, as a DM intake predictor.

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The effect of nutrition on fibre growth in the alpaca

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Abstract

Twelve adult male alpacas were given either 0·67 (low) or 2·0 (high) × assumed maintenance requirements for a period of 6 weeks after which time each was transferred to the alternative level of nutrition for a further 6 weeks. Fibre samples were taken from two 10-cm^2 areas on the mid-side position of each animal at 2, 6, 8 and 12 weeks, and measurements of fibre weight, yield (clean fibre weight/raw fibre weight), fibre diameter and fibre length made on the samples collected at weeks 6 and 12. The higher level of feeding resulted in higher clean fibre weight (low = 0·42 (s.e. 0·03); high = 0·53 (s.e. 0·04)mg/cm^2 per day, P < 0·001) and fibre growth rate (low = 186 (s.e. 10); high = 223 (s.e. 14) μm/day, P < 0·05). Changes in yield (low = 0·917 (s.e. 0·006); high = 0·929 (s.e. 0·009)) and mean fibre diameter (low = 31·4 (s.e. 1·9); high = 32·1 (s.e. 1.6) μm) were not statistically significant. Calculations showed that the increased weight of fibre attributed to the higher level of nutrition could be explained in terms of the observed increases in fibre-length and diameter but that, unlike the sheep in which the ratio fibre length : diameter remains relatively constant under varying nutritional regimes, the effect of nutrition in the alpaca has a proportionally larger effect on fibre length than on fibre diameter.

Keywords: *alpacas, fibre quality, nutrition.*

Introduction

Wool production has been shown by many workers to be sensitive to nutrition (see reviews by Russel, 1992; Sumner and Bigham, 1993). It is known that changes in fleece weight attributable to nutrition are a consequence of changes in both fibre length (L) and fibre diameter (D) (Sumner and Wickham, 1969) and Reis (1992) noted that, within individual animals, the changes in these components of fleece weight were such that the ratio L : D remains relatively constant.

There are few reports in the literature on the effects of nutrition on fibre production in the alpaca which, being a single-coated species growing fibre continuously, might be expected to respond in terms of fibre growth to nutritional manipulation in a similar manner to sheep. Newman and Paterson (1994) reported a positive relationship between food intake and the weight of fibre produced but were unable to demonstrate statistically significant effects of nutrition on either fibre length or fibre diameter, the principal components of fibre weight.

Wuliji (1993) observed changes in the weight and diameter of the fibre grown by alpacas in different seasons. These changes may have been a consequence of seasonal variation in the level of nutrition. Although the rate of extension in fibre length was not measured, Wuliji (1993) concluded by inference that fibre length was proportionately more affected than fibre diameter by the presumed influence of nutrition.

Other reports of variation in alpaca fibre characteristics attributed to season may also have a nutritional component (e.g. Pumayalla and Leyva, 1988).

This paper reports the results of an experiment designed to study the responses in terms of fibre weight, length and diameter to sub- and super-maintenance levels of nutrition in the alpaca.

Material and methods

The experiment, which was conducted over the period May to August, 1994, employed a cross-over design involving two contrasting levels of nutrition. Twelve adult male alpacas (mean live weight 73·7 (s.e. 3·0) kg) were allocated, by restricted randomization taking account of live weight and age, to levels of nutrition calculated to supply either 0·67

(low) or 2·0 (high) × assumed maintenance requirements based on a value of 0·44 MJ metabolizable energy per kg $M^{0.75}$ per day commonly used for sheep (Agricultural Research Council (ARC), 1980). Animals on the low treatment were offered individually only grass hay (8·0 MJ ME and 97·7 g crude protein (CP) per kg dry matter); those on the high treatment received 0·17 of their energy intake in the form of hay and 0·83 as a dried grass-sugar beet pulp pelleted diet (12·2 MJ ME and 160 g CP per kg dry matter). The daily CP intakes of animals on the low and high treatments were 3·6 and 11·4 g CP per kg $M^{0.75}$ per day.

After a period of 6 weeks each animal was transferred to the alternative level of nutrition for a further 6 weeks. This design allowed treatment comparisons to be made within animals.

Fibre samples were taken from two 10-cm^2 areas on the mid-side on each animal, using small animal surgical clippers to cut the fibre at skin level and calipers with a width of 10 cm to define the margins of the sample area. The samples were collected at 2, 6, 8 and 12 weeks. The second and fourth samples were of fibre grown during the final 4 weeks on each nutritional treatment.

The first and third samples were taken primarily to remove the fibre produced below the level of the skin surface prior to the imposition of the current nutritional treatment. Measurements made on material collected on these occasions were also used in the statistical analysis to examine the possible effect of the order in which the treatments were imposed.

Weight of clean fibre was determined following washing in petroleum ether (60-80°C) and yield expressed as a proportion of raw weight. Mean fibre diameter and fibre length were measured by two operators using standard procedures (International Wool Textile Organisation, 1989).

Statistical analyses showed no significant operator effects. Means of measurements made on the two samples collected on each occasion, and of the diameter and length measurements made by two operators, were calculated. A preliminary analysis of variance, employing as covariates measurements of fibre diameter and fibre weight made on the samples at 2 and 6 weeks, showed that there was no significant treatment × period interaction, i.e. there was no effect of the order in which the nutritional treatments were imposed and that the within-animal differences could be analysed without regard to whether the nutritional regime had changed from low to high or high to low. The significance of the effects of the nutritional treatment was assessed using paired *t* tests.

Table 1 *Means (± s.e.) of dry matter intake, live-weight change, clean fibre weight, yield (clean weight as a proportion of raw weight), mean fibre diameter and fibre growth rate in alpacas given food at 0·67 (low) or 2·0 (high) × maintenance*

	Low		High	
Level of feeding	Mean	s.e.	Mean	s.e.
Dry-matter intake ($g/kg\ M^{0.75}$ per day):				
hay	36·9		18·7	
pelleted diet			59·9	
total	36·9		78·6	
Live-weight change (g/day)	–69·1	17·4	70·3***	16·8
Clean fibre weight (mg/cm^2 per day) (W)	0·42	0·003	0·53***	0·04
Yield (clean/raw weight)	0·917	0·006	0·929	0·009
Mean fibre diameter (μm)(D)	31·4	1·9	32·1	1·6
Fibre growth rate (μm/day)(L)	186	10	223*	14
L : D	5·92	0·37	6·95*	0·39
$(W : D^3) \times 10^{-6}$	13·6	1·4	16·0*	1·5

Results

The results presented in Table 1 show that the contrasting nutritional treatments produced a substantial and very highly significant difference in live-weight change, the submaintenance level of nutrition causing a daily live-weight loss of 69·1 g, while the twice maintenance level resulted in a daily increase of 70·3 g.

With respect to fibre, the effect of the higher level of nutrition was to increase clean fibre weight by 0·11 mg/cm^2 per day ($P < 0·001$) and fibre growth rate by 37 μm/day ($P < 0·05$). These represent proportional increases of 0·25 and 0·20 in fibre weight and length extension respectively. The increases in yield of 0·012 and mean fibre diameter of 0·7 μm were not statistically significant.

The results also show that the ratios of fibre length to diameter (L : D) and fibre weight (W) to diameter^3 ($W : D^3$) were higher in the samples from the animals on the higher level of nutrition ($P < 0·05$).

Discussion

The results confirm the implied conclusion from the study of Wuliji (1993) and the finding of Newman and Paterson (1994) that nutrition has a positive effect on the weight of fibre produced in the alpaca.

Table 2 *Contributions of fibre length (L) and diameter (D) to increased fibre volume resulting from higher level of nutrition*

	Nutritional treatment		Increased volume	Increased volume due to		
	Low	High		L	D	D (L)
Volume $\times 10^{-3}$ (μm/day)	144·0	180·4	36·4	28·7	6·5	1·2
Proportional increase in volume			0·253	0·788	0·179	0·033

In sheep, wool production is known to be sensitive to the influence of nutrition: changes in feeding are reflected in changes in both fibre length and diameter such that the ratio L : D remains relatively constant within individual animals (Reis, 1992). Because fibre weight (W) varies directly as LD^2, the ratio $W : D^3$ would also be expected to remain relatively constant.

Examination of the results presented here indicates, however, that in the alpaca the relative contribution of the increments in L and D appear to be different from those operating in sheep. The ratios L : D and $W : D^3$ calculated from the data relating to the higher level of nutrition are both greater, by a proportion of about 0·18, than those for the lower level. This suggests that the increase in fibre weight attributable to the higher level of nutrition is due more to an increase in L than in D.

Reis and Sahlu (1994), in a consideration of the relative contributions of L and D to changes in the volume of fibre produced, point out that the increased diameter of the extra fibre length (which they term D (L)) must also be taken into account. The figures in Table 2, based on the calculations described by Reis and Sahlu (1994), indicate that in this experiment the contribution of increased length to the additional volume of fibre grown by the animals on the high treatment was substantially greater than that attributable to that of diameter. The respective proportional contributions of L, D and D (L) were 0·788, 0·179 and 0·033.

The step from fibre volume to fibre weight involves the incorporation of fibre density, in the gravimetric sense, in the calculation. If $W = f\ L\pi(d/2)^2$, then f, a function of density, can be shown to have the same value ($2{\cdot}93 \times 10^{-6}$) in the data relating to both levels of nutrition. Thus, the relative contributions of L and D to the increased fibre weight resulting from the higher level of nutrition are of the order of 0·8 and 0·2 respectively.

It is difficult to compare the results of this study with reports in the literature. Newman and Paterson's (1994) work appears to be the only example of an experiment in which nutrition and fibre weight, length and diameter were all measured. They reported that increased food allowances resulted in increased fibre weights, but that there were no significant effects of nutrition on either fibre length or diameter. Examination of their results shows, however, that nutrition affected fibre weight in summer and fibre diameter in winter. Although their lower level of nutrition was designed to supply only maintenance needs, animals on this treatment gained live weight in the course of the experiment, and it may be that the imposition of more extreme nutritional regimes would have revealed clearer effects on the length and diameter components of fibre weight.

This experiment was not designed to study the energy requirements for maintenance and live-weight change in the alpaca but the live-weight losses of the animals on the lower level of feeding (which supplied 0·295 MJ ME per $kgM^{0.75}$ per day) indicate that maintenance requirements were substantially greater than the values of 0·276 MJ ME per $kgM^{0.75}$ per day reported by Newman and Paterson (1994) in the alpaca and of 61·2 kcal/$kgM^{0.75}$ per day (0·256 MJ ME per $kgM^{0.75}$ per day) calculated by von Engelhardt and Schneider (1977) in the llama. Making reasonable assumptions regarding the efficiency of use of dietary ME for live-weight gain and of body tissue for maintenance, it can be calculated that the maintenance requirement was of the order of 0·44 MJ ME per $kgM^{0.75}$ per day used by ARC (1980) for sheep and adopted in this experiment for alpacas. This apparent wide discrepancy in maintenance requirements may be a consequence of differences in diet quality. Newman and Paterson (1994) quote references to work indicating that while camelids digest poor quality forages more efficiently than do sheep and cattle, there is little difference between species with respect to their efficiency of digestion of higher quality diets, such as used in this experiment.

The conclusions drawn from the results of this study, in which the lower level of nutrition was unequivocally submaintenance, are that in the alpaca fibre production is sensitive to nutritional manipulation and that the effect of nutrition on fibre weight acts more through changes in fibre length

than in fibre diameter. This is in contrast to the sheep in which changes in fibre length have generally been shown to account proportionately for only 0·2 to 0·3 of the observed increases in fibre volume and weight (Reis and Sahlu, 1994). Therefore it can be argued that the balance between the gains to be made from increased fibre weight and the penalties resulting from increased fibre diameter as a consequence of higher levels of nutrition will differ between sheep and alpacas. In the alpaca higher food allowances designed to improve production are likely to be associated with smaller penalties in terms of fleece quality than is the case in sheep.

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The effect of the introduction of the Thoka gene for fecundity on lamb production from Cheviot ewes

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Abstract

A flock of Cheviot ewes carrying the Thoka gene for fecundity was established in southern Scotland through the importation of frozen semen from two Icelandic rams. Ewes considered to be probable carriers of the Thoka gene were identified on the basis of measurements of ovulation rate as juveniles at 1·5 years of age and again as adults. Analysis of 3 years' records of 8926 Cheviot lambings and lambings of 351 Icelandic × Cheviot ewes considered to be probable carriers showed that fecundity increased with age in both genotypes, the number of lambs born per ewe mated being 1·07, 1·19, 1·31 and 1·41 in the Cheviots and 1·55, 1·74, 2·33 and 2·30 in the Icelandic-Cheviot crosses at first to fourth lambings respectively. The apparent effect of the crossbreeding on fecundity thus also increased with age, values at first to fourth lambings being 0·48, 0·55, 1·02 and 0·89 lambs per ewe mated, giving a weighted mean value of 0·61 (1·20 v. 1·81 lambs per ewe mated in Cheviot and Icelandic × Cheviot ewes respectively). There was a positive effect of the proportion of Icelandic ancestry on the number of lambs born per ewe mated and thus the observed increases in fecundity, although attributed principally to the effects of the Thoka gene, are also due in part to other genetic factors introduced from the Icelandic sheep in the crossbreeding programme. Lamb mortality to 6 weeks of age was only marginally higher in Icelandic × Cheviots (0·10) than in Cheviots (0·08). The increase in fecundity was achieved without an excessive proportion of large litters (<0·02 of probable carriers gave birth to quadruplets). The results demonstrate that the introduction of the Thoka gene to breeds such as the Cheviot can increase fecundity and hence the potential for more efficient lamb production.

Keywords: *ewes, fecundity, lamb production.*

Introduction

Potential fecundity in sheep is genetically determined. The extent to which that potential is realized depends on previous and contemporary nutrition and other environmental factors. In breeds with a low potential fecundity there could be substantial biological and economic advantages to be gained by the introduction of a single gene for increased fecundity, provided that the ewe is capable of rearing additional lambs and that the nutritional regime in which the higher output is sought is adequate.

The best known and most intensively researched example of highly prolific sheep is the Booroola Merino, in which the existence of a single gene for fecundity was first hypothesized by Piper and Bindon (1980). Single genes for increased fecundity have since been identified and studied in other breeds, including Icelandic sheep in which the gene has been named 'Thoka' after the ewe in which it was first identified (Jonmundsson and Adalsteinsson 1985; Adalsteinsson, *et al.*, 1989).

This paper presents the results of preliminary work on the introduction of the Icelandic Thoka gene to Cheviot sheep in the United Kingdom. Some of the earlier data have been summarized by King *et al.* (1990).

Material and methods

Frozen semen from two Icelandic rams (nos 81001 and 83002) known to be carrying the Thoka gene was used to inseminate 30 North Country Cheviot (NCC) ewes in November 1985 at the Macaulay Land Use Research Institute's Sourhope Research Station in south Scotland.

Of the progeny born in 1986, four rams (one from 81001 and three from 83002) and seven ewes (five from 81001 and two from 83002) were retained for further breeding. The seven ewe lambs were mated to NCC rams later that year and again the following year.

The four ram lambs were mated to 120 NCC × South Country Cheviot (SCC) ewes in 1986. No male progeny were retained for breeding. The 76 ewe lambs subsequently born in 1987 were examined laparoscopically following oestrus induction (using vaginal pessaries and a low dose (250 IU) of PMS) in November 1988 to measure ovulation rate. Because this measurement was made early in the breeding season, PMS was used to facilitate the oestrus induction. The dose rate was considered to be insufficient to override the endogenous variation in ovulation rate. Animals with ovulation rates of 2 or greater were mated to the four Icelandic × NCC rams, in such a way that no ewe was mated to a son of its paternal grandsire.

The Icelandic × NCC rams were also mated to 48 SCC ewes in November 1988.

Subsequently a flock of between 50 and 60 ewes was maintained, female replacements with varying proportions of Icelandic, NCC and SCC ancestry being retained on the basis of ovulation rate determined by laparoscopic examination of ovaries following synchronization of oestrus at approximately 1·5 years of age. Ewes with ovulation rates of 2 or more were considered to be probable carriers of the Thoka gene and were retained for breeding; those with 0 or 1 ovulation were not retained. The ewes kept as possible carriers were again examined laparoscopically at 2·5 years of age and those with fewer than two ovulations were discarded. Ewes were mated at oestrous cycles following the laparoscopic examination.

Rams were retained for breeding on the basis of their dams' lambing performance; no single-born rams were selected for breeding.

Statistical methods

Statistical analyses were carried out on two different data sets collected over the same 3 years. The aim of both analyses was to summarize the effects of genotype and age of ewe on the number of lambs born (Efron, 1979).

The first data set comprised 8926 lambings for the three Cheviot genotypes (SCC, NCC × SCC and NCC) and is typical of their lambing rates in the absence of any new genetic material. This data set contained information on mean lambing rate and numbers of ewes, classified by genotype, age of ewe and year, although not on the performance of individual ewes in successive years.

The second data set, collected from the breeding flock established from the original two Icelandic rams, comprised 351 records from 163 ewes in which the reproductive history of each animal could be traced. For this data set, ewes were classified according to the proportion of Icelandic genes (0·125, 0·25, 0·325 or 0·5) which each carried.

Data from the three Cheviot genotypes were examined by analysis of variance of the means, weighted by the number of ewes within each combination of genotype, age and year. The significance of age, genotype and age by genotype interaction was assessed against the residual variation. Although this was not considered to be ideal, in that it took no account of the variability between individual animals, it was the best that could be achieved with the available records.

With the Icelandic × Cheviot data, a three-way table of counts, classified by age, proportion of Icelandic genes and number of lambs born, was constructed. This table was modelled to predict the proportion of ewes in each age by genotype group which would give birth to each of the number of lambs observed (0 to 4). These proportions were then used to estimate the mean number of lambs born per ewe according to age and genotype. The model fitted to the table of counts was log-linear with Poisson variation, and contained all factorial terms except the third order interaction. As with the three Cheviot genotypes, the fitted model did not take direct account of the variation between animals. However, because individual animal records were available, it was possible in this case to estimate prediction errors. These were calculated by a bootstrapping procedure in which individuals were resampled at random, but with replacement from each genotype group to form new samples, each containing the records from the same number of individuals as in the original sample. The same model was refitted to each new sample, the summary statistics re-estimated and the standard deviations of the predicted values used to estimate the standard errors of the original means. The difference between lambing rates in older and younger ewes was demonstrated by calculating the

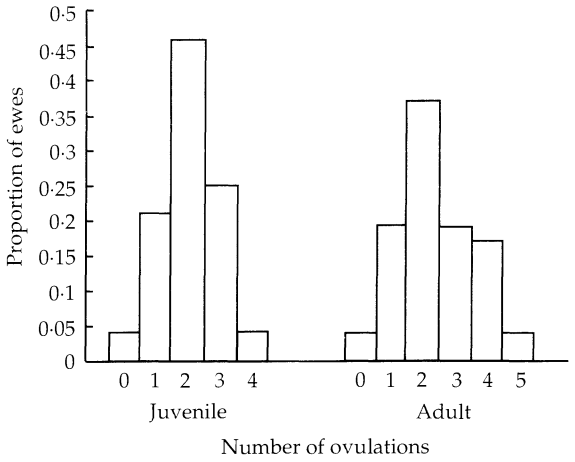

Figure 1 Distributions of ovulation rate in juvenile and adult ewes.

ratio of year 4 to year 2 values. T-tests were used to assess any consistent differences in this ratio between the four Icelandic × Cheviot groups and the three Cheviot genotypes.

Results

The programme of crossbreeding and retaining females on the basis of ovulation rate has led to the establishment of a flock of 55 breeding ewes and 16 rams with between 0·125 and 0·50 Icelandic ancestry.

Distributions of ovulation rate measured in ewes as juveniles at approximately 1·5 years, and as adults, are illustrated in Figure 1. The distribution referring to the younger age includes both the presumed carriers of the Thoka gene, which were retained and subsequently mated and those assumed to be non-carriers which were discarded from the flock. The mean ovulation rate of these younger ewes was 2·21 (s.e. 0·08). The distribution referring to the older ewes is based on the ovulation rate as adults of those ewes selected at 1·5 years as probable carriers of the Thoka gene. Their mean ovulation rate was 2·48 (s.e. 1·16).

The number of lambs born to the different Cheviot genotypes and to Icelandic × Cheviot ewes probably carrying the Thoka gene are presented in Table 1. These data show that the number of lambs born per ewe mated in the Cheviot genotypes was highly significantly affected by genotype ($P < 0·001$), age ($P < 0·001$) and genotype × age interaction ($P < 0·01$). The probability of the Iceland × Cheviot ewes falling into a particular lamb number class was dependent on both the proportion of Icelandic ancestry in the cross ($P < 0·001$) and age ($P < 0·001$), whilst the remaining variation appeared to be random.

In all genotypes the older animals generally gave birth to more lambs than did the younger animals. In the three Cheviot genotypes this was most marked in the NCC ewes. In the Icelandic × Cheviot ewes the number of lambs born per ewe increased as the proportion of Icelandic ancestry increased from 0·125 to 0·5 and, as with the Cheviots, the older ewes tended to give birth to more lambs. The ratio of mean number of lambs born to 4-year-old ewes to number born to 2-year-old ewes showed that the Cheviots (with ratios of 1·23, 1·23 and 1·34 for SCC, NCC × SCC and NCC respectively) exhibited less change between age classes than the Icelandic × Cheviot ewes (with ratios of 1·45, 1·41, 1·67 and 1·53 for the classes with 0·125, 0·25, 0·325 and 0·5 Icelandic

Table 1 *Mean number (with s.e.) of lambs born per ewe mated to Cheviot (North Country (NCC), North × South and South Country (SCC)) ewes not carrying the Thoka gene and to Cheviot-cross ewes with varying proportions of Icelandic ancestry and considered as probable carriers of the Thoka gene (No. = number of observations over 3 years)*

	Age of ewe (years)											
	2			3			4			5		
Breed	No.	Lambs born	s.e.	No.	Lambs born	s.e.	No.	Lambs born	s.e.	No.	Lambs born	s.e.
SCC	725	1·02	0·02	525	1·11	0·02	346	1·25	0·02	208	1·18	0·03
NCC × SCC	2154	1·06	0·01	1602	1·21	0·01	1129	1·30	0·01	685	1·37	0·02
NCC	621	1·16	0·02	444	1·34	0·02	300	1·55	0·03	187	1·63	0·03
0·125 Icelandic	87	1·55	0·06	52	1·75	0·11	31	2·25	0·10	22	2·27	0·16
0·25 Icelandic	24	1·55	0·10	13	1·70	0·16	10	2·19	0·13	10	2·07	0·20
0·325 Icelandic	43	1·50	0·14	21	1·63	0·22	9	2·50	0·19			
0·5 Icelandic	9	1·76	0·25	8	2·00	0·28	7	2·69	0·25	5	2·87	0·36

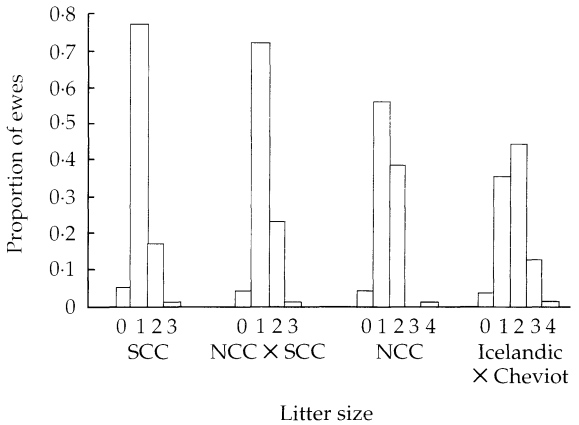

Figure 2 Distributions of litter size in Cheviot ewes (South Country (SCC), North Country × South Country, North Country (NCC)) and Icelandic × Cheviot ewes.

ancestry respectively). The difference between the Cheviot and Icelandic × Cheviot genotypes in this ratio was significant ($t = 3{\cdot}3$; d.f. = 5; $P = 0{\cdot}02$).

The distributions of litter size illustrated in Figure 2 show that the three Cheviot genotypes all produced substantially more single than multiple births, whereas the presumed Thoka-carriers produced more multiples than singles. The proportions of single births were: Icelandic × Cheviot, 0·36; SCC, 0·77; NCC × SCC, 0·72; NCC, 0·56.

Lamb mortality, measured as the difference between numbers born (including stillbirths) and those alive at 6 weeks of age, was marginally greater in the Icelandic × Cheviot ewes (0·18 lambs per ewe mated) than in the Cheviot genotypes (0·07, 0·12 and 0·115 lambs per ewe in the SCC, NCC × SCC and NCC respectively) but, when expressed as a proportion of lambs born, lamb mortality in the Icelandic-cross ewes (0·10) was no greater than in the other genotypes (0·06, 0·10 and 0·10).

Discussion

Evidence presented by King *et al.* (1990) based on data from the flock referred to in this report indicated that one of the two original Icelandic sires (no. 81001) was homozygous for the Thoka gene and that the other (no. 83002) was probably heterozygous. This supposition has since been confirmed (J. V. Jonmundsson, personal communication). The analysis of King *et al.* (1990) also indicated that three of the four Icelandic × NCC sires used in the crossbreeding programme outlined above carried the Thoka gene.

The effect of crossbreeding Icelandic sheep carrying the Thoka gene with the Cheviot genotypes was to increase the fecundity of the Cheviots by approximately 0·6 lamb per ewe mated. Although this is of the same order as the values of 0·64 and 0·57 reported in Icelandic sheep by Jonmundsson and Adalsteinsson (1985) and Eythorsdottir *et al.* (1991) the increased fecundity noted in this study cannot be wholly attributed to the effect of the Thoka gene. The measurement of ovulation on only one occasion at 1·5 years of age and on a second occasion a year later is likely to have resulted in the retention of some non-Thoka carriers in the small high fecundity flock. This would have the effect of underestimating the effect of the gene on fecundity in Cheviot sheep. The imperfect identification of animals carrying the Thoka gene is likely to have been offset, however, by other genetic factors introduced by the selection involved in the crossbreeding programme. Nevertheless, the results indicate that the proportion of Icelandic ancestry in the crossbred ewes had a positive effect on fecundity, although the small number of records available in each age and ancestry class make it difficult to quantify the magnitude of this effect with confidence.

The distributions of litter size in the three Cheviot genotypes and in the ewes probably carrying the Thoka gene show that the major part of the greater fecundity of the latter ewes stems from a decrease in the proportion of single lambs born. There is a consequent increase in the proportion of multiple births but the number of large litters is few: less than 0·02 of the ewes produced quadruplets. An increase in fecundity in Cheviot sheep could theoretically be an advantage in many situations, but if this were to be achieved by a significant proportion of ewes having large litters, the consequent increases in lamb mortality and management difficulties could more than offset the potential advantages. It appears from the results presented here that this would not be the case and that the introduction of the Thoka gene to breeds such as Cheviots could increase fecundity, thereby improving the efficiency of production, without excessive numbers of triplets and quadruplets.

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Predicting the effects of animal variation on growth and food intake in growing pigs using simulation modelling

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Abstract

All pig nutrition models to date predict growth responses of either an individual animal or the average animal of a given population over time. Translating the predicted nutrient requirements from the average animal to the population introduces a number of errors as the cause-and-effect response of the average animal is different from the population response. To overcome the problem of estimating the requirements for a given population using models it is necessary to simulate a number of individuals representative of a population and then average these results. This approach however, requires a knowledge of those animal characteristics that vary between individuals and the nature of their distribution. In this paper a scaled growth rate constant (B), protein weight at maturity (Pm) and the ratio of lipid to protein at maturity (LPRm) are the parameters used to define an individual animal. As no data existed from which the nature of the distribution of B*, Pm and LPRm can be estimated for pigs of different strains and sexes, and due to the impracticality of determining this variability by experimentation, a simulation model was used to estimate the variations within each parameter. In addition this paper quantifies the subsequent effects these distributions have on the genetic variability of average daily gains (ADG) and daily food intake (FI) over a live-weight range of 25 to 90 kg. Comparisons were made between the genetic variation determined by modelling and those published in the literature. The results indicated coefficients of variation for B*, Pm and LPRm of 0·01, 0·05 and 0·10, respectively. An increase in the variability of all three parameters resulted in an increase in the variation in ADG whilst only an increase in the variation of B* and LPRm affected the distribution of FI.*

Keywords: *genetic variation, growth, nutrition, pigs, simulation models.*

Introduction

All pig growth models to date (Roux, 1976; Whittemore and Fawcett, 1976; Phillips and MacHardy, 1982; Bridges *et al.*, 1986; Black *et al.*, 1986; Moughan *et al.*, 1987; Pomar *et al.*, 1991; Ferguson *et al.*, 1994) have been designed to simulate the growth of an individual animal over time, so the description of the genotype, although defined in different ways by the above researchers, has not taken into account the variability that can be expected in a population of animals. It is the purpose of this paper to consider the population response to a given food rather than the response of the individual.

However, to define the nutrient requirements of a population over time, it is important to understand first how an individual animal within the population will respond, at a time, to increasing dietary concentrations of the nutrient (Emmans and Fisher, 1986; Whittemore, 1993). The reason being that there is a marked difference in the response of the average individual in the population, and the mean population response, which is an average of all individuals. The average animal can be defined as that individual which has the average animal characteristics of the population. The cause-and-effect response of the average animal to increasing dietary nutrient concentrations will always be different from the population response, even though this difference may be small in some cases (Curnow, 1973; Emmans and Fisher, 1986). As a consequence, it is a mistake to believe that the deterministically simulated response of an

individual animal is representative of the population mean. The extent of the errors introduced by translating requirements from the average animal to the population will depend either on the genotype or the variations in genetic parameters within a population, and the extent of the correlation between the various parameters. It is for this reason that estimates of the nutrient requirements of a population of animals are more meaningful than those of individuals when formulating diets to optimize the performance of a group of animals.

The models described by Black *et al.* (1986) and Moughan *et al.* (1987) attempt to overcome the problem of predicting amino acid requirements for a given population by assuming a curvilinear response by the average individual to nutrient input. This is done by continually reducing the availability of the amino acids as the maximum rate of protein deposition is approached. However, this approach does not permit the modelling of individual within-population variation in the response to amino acid intakes, particularly when environmental conditions are not ideal. The estimate of within-population variation is essential for the accurate determination of the optimum amino acid concentration of the population (Curnow, 1973). More recently Black *et al.* (1989) questioned the precision of the results obtained with this approach.

The only sensible approach to predicting the nutrient requirements of a population, using simulation models, is to repeat the simulation for a number of individuals representative of the population and then determine the mean and within-population variation from these results. This approach requires a knowledge of the parameters in the model that vary between animals, the nature of their distribution and the possible correlations that may exist between parameters (Emmans and Fisher, 1986). Of the current pig models the model described by Ferguson *et al.* (1994) can most readily include variations in animal characteristics, because only three parameters are required to describe the animal and these parameters are both biologically meaningful and can be readily measured (Ferguson and Gous, 1993a and b). The three parameters used to describe an animal are a rate of maturing (B), the mature protein weight (Pm) and the lipid : protein ratio at maturity (LPRm). It would not be possible to introduce stochastic elements, at the animal level, into most of the existing models because either the parameters have little biological meaning and it would therefore be difficult to measure the differences between and within genotypes, or the description of the animal is inadequate, such that the effect of variation would have little impact on the response of the animal. If B, Pm and LPRm can be used to describe an individual animal, and the nature of the distributions of these parameters is known, then it would theoretically be possible to simulate a population.

It is also important to know if any correlations exist between these parameters. If there are, this will affect the nature and the description of the variation of the correlated parameters. Within or between a genotype there is likely to be a strong, negative correlation between B and Pm (Emmans, 1988). Animals with a high Pm will have a low B value. Axiomatic to this is that larger animals will have a lower growth rate relative to body size (Taylor, 1968). The implications for modelling are that the variability of B or Pm cannot be modelled separately and there needs to be an estimate of the co-variability between the two parameters. Errors of prediction will result if this correlation is ignored. Emmans and Fisher (1986) describe a scaled rate parameter ($B^* = B \times Pm^{0.27}$) that holds B and Pm in a fixed relationship and would, therefore, prevent such errors from occurring.

Currently, no data are available from which the distributions of B*, Pm and LPRm can be estimated for pigs of a given sex and strain, although Emmans and Fisher (1986) and Emmans (1988) did speculate, in the case of broilers, general coefficients of variation (CV) for B* of 0·02 to 0·04, and 0·06 to 0·10 for Pm. It would be impractical to determine experimentally the CVs and correlations between B*, Pm and LPRm as a large number of widely different populations of pigs would have to be used and these would be required to be grown under similar conditions. As the population means, standard deviations and correlation coefficients would be estimated from samples of different populations, the sample size or number of pigs required to test whether the hypothesis is true, is related to the probability of incorrectly rejecting the null hypothesis (*Ho*) that there is no correlation between the parameters (type I error or α-error), the probability of incorrectly accepting *Ho* (type II error or β-error) and the value of the true correlation coefficient (ρ) if it is not equal to zero. It is the latter error (type II) that needs to be minimized (Groebner and Shannon, 1989).

To be conservative in the test, let $\alpha = 0{\cdot}2$ such that there would be a 20 percent chance of incorrectly rejecting *Ho*. This is a conservative estimate because if *Ho* is rejected at $\alpha = 0{\cdot}2$ there is still a 20 percent chance that *Ho* is true. Since confirmation is required that there is no correlation between the parameters, the chance of incorrectly accepting *Ho* must be minimized. This is done by setting $\beta = 0{\cdot}05$ and assuming that the true population correlation coefficient (ρ) equals 0·15. The low value of ρ (0·15) was used to ensure that if there were any correlations between parameters then they would be detected. If ρ

was found to be greater than 0·15 between two parameters then it would be possible to test for significant correlations with fewer animals. In the case of this paper none of the correlations between parameters was known and therefore a low estimate of ρ was used.

The minimum number of animals (n) required to test whether there are any correlations between the parameters is determined from Groebner and Shannon (1989) as follows:

$$n = [(Z_\alpha - Z_\beta)/0{\cdot}5 \times \log_e[(1 + \rho)/(1 - \rho)]]^2 + 3$$

where Z_α = standard normal variate associated with a = 0·2; Z_β = standard normal variate associated with β = 0·05; ρ = rho, the true population correlation coefficient > 0.

Since the correlations could be negative or positive a two-tailed test is required, which means that Z_β could be either a positive or a negative value. Substituting into this equation the probability values from Z tables of Z_α = 1·282, Z_β = –1·960 and ρ = 0·15, a sample size of 463 animals per population is required in order to test whether there are significant correlations between the parameters across different pig populations. Clearly, this is an impractical and costly exercise. In addition the data collected by direct measurement are prone to many errors, including the effect of weighing errors (differential gut fill, balance/reading errors), food spillage, disease and inappropriate environmental effects. To circumvent the problems of experimentation, simulation models are a viable alternative for estimating parameter variability. However, when using simulation modelling to estimate distributions of genetic parameters some basis for comparison must be available in order to determine whether the values are realistic or not.

In this paper the model described by Ferguson *et al.* (1994) was used with simulated values of B*, Pm and LPRm to measure the effect that these distributions have on the variability of average daily gain (ADG) and daily food intake (FI).

Method

To make sense of the results it is important to understand the basic theory behind the model proposed by Ferguson *et al.* (1994). The model describes an animal in terms of Pm, B and LPRm and uses a Gompertz growth function to determine the potential protein gain, and allometry to determine the body-weight gain of the animal. From this is calculated the amount of a given food that would need to be consumed in order that the animal can grow at its potential (desired food intake). The actual food intake, and hence the gain in component weights, would be dependent on the capacity of the animal to consume bulk and to lose heat to the environment, and on the environmental conditions. Because only three parameters are needed to account for differences between genotypes, variation around the mean of each of these three parameters should describe all the individuals within a population. The estimates of normal independent distributions of these parameters suggested by Emmans and Fisher (1986) were used as the basis of the values chosen for this investigation.

Three coefficients of variation of both Pm and LPRm and four of B* were chosen in a factorial arrangement (Table 1). The values of the coefficients of variation of the three parameters were chosen to be less than, equal to and greater than the values speculated by Emmans and Fisher (1986).

The three parameters B*, Pm and LPRm were generated independently for a normally distributed population, to ensure that no correlations existed between them. If any interactions were found to exist between the parameters it would be extremely difficult to quantify the relationship between the variation of these parameters and the mean ADG and FI. To predict the means of either FI or ADG would involve selecting a specific combination of B*, Pm and LPRm appropriate to the desired prediction. This would clearly not be very practical. For this reason the parameters were forced to be uncorrelated in the simulation by generating 465 independent values of B*, Pm and LPRm for each genotype. To check that there were no strong correlations between the parameters correlation coefficients were computed. The results of the computed correlation coefficients are shown in Table 2 with there being a weak correlation between B* and LPRm (r = –0·200) and between Pm and LPRm (r = –0·066). This does not mean that no correlations between the parameters actually exist in a real population of pigs.

For each genotype a total of 465 individuals was generated, using the same population means over all genotypes (μB* = 0·0294 per day; μPm = 38·0 kg and μLPRm = 2·5 g/g; average values for a typical, Large White × Landrace entire male (Ferguson and Gous, 1993a)), but using the CVs appropriate to each genotype in turn (Table 1). The replicates (individual pigs) within each genotype, thus defined, were then used to estimate the population response using the model to provide estimates of FI and ADG, and their respective standard errors, at 20, 40, 60 and 90 kg, and between 25 and 90 kg live weight. The simulations were started at a body weight of 10 kg. The reason for including data between 25 and 90 kg

Table 1 *The effect of different levels of the coefficient of variation (CV) of three model parameters (B*, Pm, LPRm)† on the simulated mean, standard deviation (s.d.) and genetic CV (CV_g) of average daily gain (ADG) and food intake (FI) between 25 and 90 kg body weight. The mean values of the model parameters were μPm = 38·0 kg, μB* = 0·0294 per day and μLPRm = 2·5 g/g and 465 individuals were simulated for each genotype*

	CV			ADG			FI		
Genotype	B*	Pm	LPRm	Mean (g/day)	s.d.	CV_g	Mean (g/day)	s.d.	CV_g
1	0·01	0·05	0·10	880	34·7	0·0394	2088	122·2	0·0588
2	0·01	0·05	0·15	878	37·0	0·0421	2070	142·6	0·0689
3	0·01	0·05	0·20	886	46·3	0·0523	2095	183·4	0·0875
4	0·01	0·10	0·10	881	47·1	0·0535	2080	124·6	0·0599
5	0·01	0·10	0·15	883	49·8	0·0564	2078	153·0	0·0736
6	0·01	0·10	0·20	885	57·5	0·0650	2101	190·4	0·0907
7	0·01	0·15	0·10	879	61·0	0·0694	2063	125·1	0·0606
8	0·01	0·15	0·15	881	66·6	0·0756	2086	161·9	0·0776
9	0·01	0·15	0·20	881	67·0	0·0760	2085	197·8	0·0949
10	0·02	0·05	0·10	882	40·4	0·0458	2084	129·6	0·0622
11	0·02	0·05	0·15	883	46·6	0·0528	2079	160·5	0·0772
12	0·02	0·05	0·20	883	49·4	0·0559	2090	186·6	0·0892
13	0·02	0·10	0·10	883	53·9	0·0610	2093	143·1	0·0684
14	0·02	0·10	0·15	882	56·2	0·0637	2081	171·9	0·0826
15	0·02	0·10	0·20	882	62·6	0·0710	2094	201·3	0·0961
16	0·02	0·15	0·10	876	66·0	0·0753	2076	139·3	0·0667
17	0·02	0·15	0·15	882	68·0	0·0771	2087	171·8	0·0823
18	0·02	0·15	0·20	871	70·5	0·0809	2061	193·3	0·0938
19	0·03	0·05	0·10	882	46·4	0·0560	2082	147·0	0·0706
20	0·03	0·05	0·15	882	53·6	0·0608	2085	176·5	0·0847
21	0·03	0·05	0·20	885	57·1	0·0645	2091	199·1	0·0952
22	0·03	0·10	0·10	880	62·7	0·0713	2079	164·1	0·0789
23	0·03	0·10	0·15	881	65·1	0·0739	2085	189·6	0·0909
24	0·03	0·10	0·20	885	68·3	0·0772	2101	207·4	0·0988
25	0·03	0·15	0·10	883	72·8	0·0824	2084	161·1	0·0773
26	0·03	0·15	0·15	875	74·5	0·0851	2067	184·9	0·0894
27	0·03	0·15	0·20	876	77·0	0·0879	2077	204·2	0·0983
28	0·06	0·05	0·10	893	84·8	0·0950	2113	231·4	0·1095
29	0·06	0·05	0·15	881	91·5	0·1038	2081	264·0	0·1269
30	0·06	0·05	0·20	880	93·9	0·1067	2077	278·3	0·1340
31	0·06	0·10	0·10	883	89·8	0·1006	2091	230·7	0·1103
32	0·06	0·10	0·15	880	88·8	0·1021	2077	246·4	0·1186
33	0·06	0·10	0·20	880	91·5	0·1040	2083	262·9	0·1262
34	0·06	0·15	0·10	878	93·9	0·1069	2084	233·1	0·1118
35	0·06	0·15	0·15	885	97·7	0·1104	2093	248·3	0·1186
36	0·06	0·15	0·20	880	104·5	0·1188	2086	269·9	0·1294

† B* = scaled growth rate constant; Pm = protein weight at maturity; LPRm = ratio of lipid to protein at maturity.

Table 2 *The correlation coefficients (r) between the genetic parameters B, B*, Pm and LPRm in the populations simulated*†

	B*	Pm	LPRm
Pm	0·000		
LPRm	–0·200	–0·066	
B	0·815	–0·570	0·015

† B = rate of maturing. Also see Table 1.

was that this weight range was the average body weight over which Standal and Vangen (1985), Cameron *et al.* (1988), Ellis *et al.* (1988), Cameron (1990) and Mrode and Kennedy (1993) investigated growth performances in different populations of pigs. It was possible, therefore, to compare the results obtained in this exercise with those in the literature. The composition of the food was the same in all simulations. This contained 13·50 MJ digestible energy per kg, 160·0 g crude protein per kg and 10·0 g total lysine per kg (assumed the first limiting amino acid), this being similar to the food used in those experiments from which results were compared.

The ADGs and FIs at 20, 40, 60 and 90 kg live weight were used to determine the effects of variation on the estimation of the genetic parameters. The mean and standard deviation of both traits were determined for each genotype, from which coefficients of variation were calculated. As there was no variation in the non-genetic or environmental component of the model, the calculated variations in ADG and FI are a result of the genetic variation in B*, Pm and LPRm only. Hence, the CVs of the two predicted sole traits are estimates of genetic variation (CV_g) and not phenotypic variation.

The variation in the data from real experiments comprises both genetic and environmental variations and therefore it is necessary, for meaningful comparisons with predicted values, to determine the genetic component only. As the heritability (h^2) of a trait is an estimate of the genetic proportion of the phenotype variation, it will be used to calculate the CV_g of ADGs and FIs from the real experiments. In the literature h^2 values for ADG vary between 0·14 and 0·46 while for FI they vary between 0·19 and 0·62 (McPhee *et al.*, 1979; Wyllie *et al.*, 1979; Stewart and Schinckel, 1989; Mrode and Kennedy, 1993; Whittemore, 1993; Cameron and Curran, 1994). According to Stewart and Schinckel (1989), who reported weighted average values from over 175 research papers, the h^2 of ADG and FI are closer to 0·30 and 0·24, respectively. However, it is not known whether the h^2 estimates reported in the 175 papers were from experiments where pigs had been provided free and continuous access to food ('true' *ad-libitum* food intake) or given food to appetite twice a day ('assumed' *ad-libitum* food intake). According to Standal and Vangen (1985) h^2 estimates of FI are higher on real *ad-libitum* feeding compared with appetite feeding twice a day. To accommodate a range of heritabilities the CV_g values from the real experiments will be calculated using h^2 values of 0·15 and 0·50 for ADG and 0·20 and 0·60 for FI, as well as an average h^2 value of 0·30 for both ADG and FI .

The effects of the various simulated levels of the CV of B*, Pm and LPRm on the average value and variability (CV_g) of ADG and FI were tested by four separate analyses of variance over the four different body weights, each using the following model of three main effects and their two-way interactions:

$$y_{ijk} = \mu + CV(B^*)_i + CV(Pm)_j + CV(LPRm)_k + [CV(B^*) \times CV(Pm)]_{ij} + [CV(B^*) \times CV(LPRm)]_{ik} + [CV(Pm) \times CV(LPRm)]_{jk} + e_{ijk}$$

Table 3 *A summary of the tests of significance (from the analyses of variance) of the main effects and the interactions of the different coefficients of variation (CV) of B*, Pm and LPRm on the mean and CV_g of ADG and food intake (FI) at 20, 40, 60 and 90 kg live weight for simulated populations of 465 individuals*

		Level of significance (P)			
Weight (kg)	Variable	ADG	CV_g(ADG)	FI	CV_g(FI)
20	CV(B*)		<0·001		<0·001
	CV(Pm)		<0·01		
	CV(LPRm)				
	CV(B*) × CV(Pm)	<0·05	<0·05	<0·05	<0·05
	CV(B*) × CV(LPRm)				
	CV(Pm) × CV(LPRm)				
40	CV(B*)		<0·001	<0·05	<0·001
	CV(Pm)		<0·001		
	CV(LPRm)		<0·001	<0·01	<0·001
	CV(B*) × CV(Pm)	<0·01	<0·01	<0·001	<0·05
	CV(B*) × CV(LPRm)				
	CV(Pm) × CV(LPRm)				
60	CV(B*)		<0·001		<0·001
	CV(Pm)	<0·05	<0·001		<0·05
	CV(LPRm)		<0·001		<0·001
	CV(B*) × CV(Pm)	<0·001	<0·001	<0·001	<0·01
	CV(B*) × CV(LPRm)			<0·01	<0·01
	CV(Pm) × CV(LPRm)			<0·001	
90	CV(B*)		<0·001		<0·001
	CV(Pm)	<0·05	<0·001	<0·05	<0·001
	CV(LPRm)		<0·001	<0·05	<0·001
	CV(B*) × CV(Pm)	<0·001	<0·001	<0·001	<0·001
	CV(B*) × CV(LPRm)			<0·05	<0·05
	CV(Pm) × CV(LPRm)			<0·01	<0·05

Table 4 *The effects (estimated from the analyses of variance) of different coefficients of variation (CV) of B*, Pm and LPRm on the mean, s.d. and CV_g of ADG at 20, 40, 60 and 90 kg live weight for simulated populations of 465 individuals*

		ADG				ADG				ADG		
Weight (kg)	B* CV	Mean (g/day)	s.d.	CV_g	Pm CV	Mean (g/day)	s.d.	CV_g	LPRm CV	Mean (g/day)	s.d.	CV_g
20	0·01	543	30·5	0·0562	0·05	545	36·6	0·0671	0·10	545	37·1	0·0681
40		787	40·5	0·0514		789	53·0	0·0672		787	54·5	0·0693
60		952	59·6	0·0626		953	64·9	0·0681		952	70·1	0·0736
90		1070	85·3	0·0797		1070	70·7	0·0661		1070	88·6	0·0828
20	0·02	544	30·6	0·0563	0·10	544	37·0	0·0680	0·15	545	37·7	0·0692
40		787	46·3	0·0588		789	56·9	0·0721		788	57·1	0·0725
60		950	65·2	0·0686		953	75·3	0·0790		952	75·3	0·0791
90		1066	88·1	0·0826		1068	92·8	0·0869		1070	93·1	0·0870
20	0·03	544	35·4	0·0650	0·15	544	38·5	0·0708	0·20	544	37·4	0·0687
40		787	54·5	0·0693		786	61·2	0·0778		789	59·5	0·0754
60		951	73·2	0·0770		949	86·6	0·0913		951	81·4	0·0856
90		1067	93·0	0·0872		1063	118·3	0·1113		1064	100·7	0·0946
20	0·06	545	56·2	0·1032								
40		790	86·8	0·1099								
60		953	104·4	0·1095								
90		1064	109·6	0·1030								

Table 5 *The effects (estimated from the analyses of variance) of different coefficients of variation (CV) of B*, Pm and LPRm on the mean, s.d. and CV_g of food intake (FI) at 20, 40, 60 and 90 kg live weight for simulated populations of 465 individuals*

		FI				FI				FI		
Weight (kg)	B* CV	Mean (g/day)	s.d.	CV_g	Pm CV	Mean (g/day)	s.d.	CV_g	LPRm CV	Mean (g/day)	s.d.	CV_g
20	0·01	1050	57·1	0·0544	0·05	1053	74·8	0·0710	0·10	1053	73·7	0·0700
40		1709	124·6	0·0729		1713	157·8	0·0921		1709	141·5	0·0828
60		2332	190·1	0·0815		2337	224·8	0·0962		2252	184·7	0·0820
90		3010	256·1	0·0851		3011	264·4	0·0878		3015	242·4	0·0804
20	0·02	1052	62·3	0·0592	0·10	1052	74·2	0·0705	0·15	1052	75·2	0·0715
40		1712	134·2	0·0784		1716	158·9	0·0926		1711	157·4	0·0920
60		2333	202·5	0·0868		2339	231·6	0·0990		2331	229·6	0·0985
90		3005	265·0	0·0882		3009	283·1	0·0941		3001	282·7	0·0942
20	0·03	1052	70·6	0·0671	0·15	1052	74·9	0·0712	0·20	1051	74·8	0·0712
40		1713	149·7	0·0874		1713	155·7	0·0909		1722	178·4	0·1007
60		2334	220·1	0·0943		2245	221·6	0·0987		2338	264·7	0·1132
90		3005	276·8	0·0921		2993	304·4	0·1017		2997	327·0	0·1091
20	0·06	1054	108·6	0·1030								
40		1721	221·5	0·1287								
60		2230	287·7	0·1290								
90		2997	338·4	0·1129								

where y_{ijk} = average value of the 'observed' ADG or FI of simulated genotype *ijk* with levels i = (1,2,3,4), j = (1,2,3) and k = (1,2,3) for the CV values of B*, Pm and LPRm, respectively; e_{ijk} = the three-way interaction of $[CV(B^*) \times CV(Pm) \times CV(LPRm)]_{ijk}$.

This analyses of variance also provided estimates of the y variables for each of the classes of CV(B*), CV(Pm) and CV(LPRm). All analyses were performed with the GLM procedure of Minitab (1994). Although this comparative technique is crude it does provide an estimate of the expected genetic variation of ADG and FI in a population of pigs from which the distribution of B*, Pm and LPRm can be deduced.

Results

The genetic variability in ADG and FI was examined within the simulated genotypes to determine to what extent the simulated variation in each of the three model parameters influenced the genetic variation in

ADG and FI, and whether any trends were evident in the relative contributions of each of these parameters with increasing body weight. The average values, standard deviations and CV_g values of ADG and FI (25 to 90 kg body weight) of the 36 simulated genotypes are in Table 1. A summary of the results of the significance tests from the analysis of variance are shown in Table 3.

There were no significant interactions between the effects of the different CV levels of B* and LPRm and, Pm and LPRm, on ADG and FI at any of the body weights evaluated. There were, however, significant interactions between the effects of the different CV levels of B* and Pm on the distribution of both ADG and FI, irrespective of body weight.

The effects (estimated from the analyses of variance) of the different CV levels of B*, Pm and LPRm on the average values, standard deviations and CV_g values of ADG and FI at 20, 40, 60 and 90 kg body weight are in Tables 4 (ADG) and 5 (FI).

The distribution of the variability in FI followed different trends to that of the variability in ADG. No significant differences were measured in the variation of FI as a result of varying the spread of Pm until 60 kg live weight.

To determine which of the 36 evaluated genotypes most closely approximated real-life genetic variation in ADG and FI, the simulated results were compared with data from studies on various populations of growing pigs published by Standal and Vangen (1985), Cameron *et al.* (1988), Ellis *et al.* (1988), Cameron (1990) and Mrode and Kennedy (1993). Table 6 provides a summary of the comparisons between the range of CV_gs from real experiments and the genotypes associated with the best estimated CV_gs from the populations simulated.

Discussion

As B* is used in the model to determine the potential rate of growth, it is expected that an increased variation in this parameter will affect the variation in daily gains and subsequent food intake. The implications from Tables 3 to 5 are that different combinations of the CV of B* and Pm, in particular, are likely to have different effects on the distribution of ADG and FI.

As body weight increases, the effect of variation in Pm on the distribution of FI remains constant. This is in contrast to the response in ADG where CV_g is increased with an increasing CV of Pm already at 20 kg live weight. When the CV of Pm is increased there is a concomitant increase in the range of potential FI but this increased range is not expressed because of the thermal environmental constraints. Given that the environment remains constant, those individuals which have the potential to consume more food (of which there will be more as the CV of Pm increases) will be constrained by the amount of heat they can lose. Hence, the narrow range of FIs. Whilst the hot environment may result in similar FIs for both fast- and slow-growing animals, the ADG will be significantly different because individuals have different predispositions to depositing lean and fat tissue as a means of reducing the environmental heat demand. Hence, the greater the variation in Pm, the higher the probability of obtaining very divergent genotypes and therefore larger variations in ADGs particularly at higher body weights where the environment is limiting to a larger number of individuals in the population.

From Table 4 there are indications that the genetic variability in ADG is dependent on body weight, as the effect of the CV levels of the parameters on the CV_g of ADG differed over the considered range of body weights. When the CV of B* was increased from 0·01 to 0·06 the CV_g of ADG increased by 0·84 at 20 kg live weight, but only by 0·29 at 90 kg live weight. Conversely, virtually no variation in ADG occurred at 20 kg live weight when the CVs of either Pm or LPRm were increased, yet significant increases occurred at 90 kg live weight. At a low CV of B*, individual growth rates will be similar and therefore the observed range of ADG would be expected to be narrower, particularly in young animals where the growth rate is less likely to be constrained by the thermal environment than when the animal is growing at its maximum rate. At a high CV of B* there is a greater probability of generating some potentially very fast and very slow growing animals, which will result in a wider distribution of potential ADGs in a population. It would be expected therefore, that by increasing the CV of B* from 0·01 to 0·06, the spread of ADG will be considerably wider across all body weights. Although a wider range of potential ADG is produced at all body weight levels when the CV of B* is increased, the increase of the range of actual ADG is smaller at higher body weights because of the constraining effect of the thermal environment. For example, those animals with the potential to grow fastest would require a lower environmental temperature than the slower-growing individuals, and, because in the model all pigs are subjected to only one temperature, these pigs would not be able to achieve their potential and hence the smaller increase of the range of actual ADG.

The genetic variation in FI largely followed that in live-weight gain: as with ADG, the CV_g of FI at 20 kg

Table 6 *A comparison between the range of CV_gs of average daily gain (ADG) and daily food intake (FI) in the literature and the best simulated estimates from the model, with the associated CVs of B*, Pm and LPRm*

Reference	Published range of CV_g (%)		Best simulated genotype(s)	CV			Best simulated CV_g	
	ADG†	FI‡		B*	Pm	LPRm	ADG	FI
Standal and Vangen (1985)								
Danish Landrace	0·015-0·049 (0·030)	0·019-0·058 (0·029)	1	0·01	0·05	0·10	0·0394	0·0588
Norwegian Landrace	0·013-0·043 (0·026)	0·019-0·056 (0·028)	1	0·01	0·05	0·10	0·0394	0·0588
Cameron *et al.* (1988)								
British Landrace	0·017-0·056 (0·034)	0·025-0·066 (0·038)	1, 10‖	0·01, 0·02	0·05, 0·05	0·10, 0·10	0·0394, 0·0458	0·0588, 0·0622
Large White	0·017-0·055 (0·033)	0·024-0·072 (0·036)	1, 10‖	0·01, 0·02	0·05, 0·05	0·10, 0·10	0·0394, 0·0458	0·0588, 0·0622
Ellis *et al.* (1988)								
Large White	0·016-0·053 (0·032)	0·025-0·075 (0·038)	1, 10‖	0·01, 0·02	0·05, 0·05	0·10, 0·10	0·0394, 0·0458	0·0588, 0·0622
Cameron (1990)								
Duroc/British Landrace§	0·017-0·057 (0·034)	0·024-0·072 (0·036)	1, 10‖	0·01, 0·02	0·05, 0·05	0·10, 0·10	0·0394, 0·0458	0·0588, 0·0622
Mrode and Kennedy (1993)								
Yorkshire/Landrace/Duroc§	0·016-0·054 (0·033)	0·022-0·067 (0·033)	1, 10‖	0·01, 0·02	0·05, 0·05	0·10, 0·10	0·0394, 0·0458	0·0588, 0·0622

† The range of CV_g for ADG is calculated by multiplying the published phenotypic variation by 0·15 and 0·50 h^2 values. The figure in brackets is the estimate using the average h^2 of 0·30.
‡ The range of CV_g for IF is calculated by multiplying the published phenotypic variation by 0·20 and 0·60 h^2 values. The figure in brackets is the estimate using the average h^2 of 0·30.
§ The values obtained from published results are from means of combining all given breeds.
‖ These genotypes had CV_gs of ADG and FI within the range of published CV_gs. The simulated CVs for each respective genotype are shown in adjacent columns.

live weight increased significantly as the CV of B* was increased, with smaller differences also being evident at 90 kg (Table 5); whilst the increase in CVs of both Pm and LPRm had a curvilinear response on the CV_g of FI, with differences between the chosen CVs occurring mainly at the heavier live weights (60 and 90 kg) (Table 5). Because of the close relationship between FI and growth rate (r = 0·853, calculated from data in Table 1) it is not surprising that the CV_g values of ADG and FI showed similar relations with the CV levels of the model parameters. The considerable increase in the CV_g of FI from 20 to 40 kg body weight is a result of there being a greater opportunity for individuals within the population to attempt to express their genetic potential by the time they reach 40 kg than at 20 kg, where the differences in potential food intake are likely to be considerably smaller.

To prevent a reduction in the genetic variability of FI across all live weights, the CV of LPRm should not be reduced. However, in young animals or animals with a live weight of less than 40 kg, preventing a reduction in the CV of B* would be more effective. Animals that have a high LPRm are more predisposed to having a higher appetite than those with a low LPRm, hence this parameter has a greater effect on variations in food intake, especially at heavier body weights, than does B*. The reason LPRm influences FI more at heavier body weights is because as the animal increases in size the energy requirements for lipid deposition become higher than for protein deposition (Campbell and Dunkin, 1983; Rinaldo and Le Dividich, 1991). The inherent fatness of the animal (as defined by LPRm) will, therefore dominate the animal's requirements for energy and subsequent energy (food) intake. The greater the variability of LPRm the greater the genetic variability in FI in older animals (Table 5).

An important point to consider when comparing the results of this exercise with those reported in the literature is that the CVs for ADG and FI of the different genotypes, shown in Table 1, reflect the potential growth characteristics of populations of pigs more closely than could have been achieved by experimentation because of extraneous random variation. The response of a real population of pigs to a given food and environment is not only subject to variations in animal characteristics but also to variations in nutrient quality and quantity, environmental changes and sample size. Additional sources of variation could result from biological variations associated with factors other than B*, Pm and LPRm, such as net efficiencies of amino acid and energy utilization, which are assumed constant in the model.

Standal and Vangen (1985) provide population estimates for Danish and Norwegian Landrace pigs. The calculated range of CV_gs for ADG and FI for Danish Landrace pigs were 0·0148 to 0·0493 and 0·0194 to 0·0583, respectively, whilst for Norwegian Landrace pigs they were 0·0128 to 0·0425 and 0·0188 to 0·0563, respectively. Comparing these results with those shown in Table 1, it is evident that the results produced by genotype 1 (with CV values for B*, Pm and LPRm of 0·01, 0·05 and 0·10, respectively) provided the closest match of the genetic variability of ADG and FI in these Danish and Norwegian Landrace pig populations.

The data from Cameron *et al.* (1988), Ellis *et al.* (1988) and Cameron (1990) showed considerably more genetic variation than those of Standal and Vangen (1985), with the data of Mrode and Kennedy (1993) somewhere in between. The differences are the result of combining entire males and females and the use of very different pig breeds, such as the British Landrace and the Duroc, in providing estimates of the population mean and standard error, and the standard errors given by Standal and Vangen (1985) were estimated and not calculated values. The genotypes that produced CV_gs within the range of CV_gs for ADG and FI for the British Landrace and Large White pigs of Cameron *et al.* (1988) and Cameron (1990) were genotypes 1 and 10 with CV values of B*, Pm and LPRm of 0·01 to 0·02, 0·05 and 0·10, respectively. In both the genotypes the CVs of Pm and LPRm were 0·05 and 0·10, respectively with the only difference being an increase in the CV of B* from 0·01 to 0·02.

As discussed previously, the assumptions concerning B*, Pm and LPRm appear to have different effects on the genetic variation in ADG and FI. However, most of the simulated genotypes within the range of the published CV_gs of both ADG and FI, were similar within and between breeds.

Conclusion

The results indicate that across a live-weight range of 25 to 90 kg most, if not all, genetic variation in ADG and FI can be accounted for when the CV of B* is between 0·01 and 0·02 (0·01 likely to be optimal), the CV of Pm is 0·05 and the CV of LPRm is 0·10.

The CV_gs of both ADG and FI tend to increase with increasing variation in all three inherent parameters, the only exception being that the CV_g of FI does not increase with increasing Pm. This trend is apparent across all body weights, but particularly so at heavier weights. The parameter that has the most profound effect on the genetic variation in FI is LPRm and in ADG it is Pm.

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Variation of heart size and its correlation with growth performance and vascular space in domestic pigs

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Abstract

Heart size of purebred boars (13 Landrace, 12 Yorkshire, and 14 Duroc) and crossbred (Landrace × Yorkshire × Duroc) boars (no. =18) and gilts (no. = 24) was studied. Purebred boars were raised from 30 kg for 110 days and slaughtered. Crossbred pigs of various body weights (30 to 110 kg) were selected and their plasma and blood volume were measured before sacrifice. The variation of heart size of pigs was studied and its correlation with growth performance or to vascular space was investigated. According to the principal component analysis heart size was best expressed by its weight, followed by the thickness of the anterior ventricle septum or the thickness of the left ventricle (LV) wall. The food/gain ratio of boars during testing period was significantly correlated with some size characteristics of their heart including weight, width, and LV thickness. The back fat thickness at 100 kg was significantly negatively correlated with heart weight, heart/body weight ratio, and LV thickness. Thus, selection for growth performance would result in a bigger heart in domestic pigs. In the crossbred study, an allometry fitting of $H = 12{\cdot}18B^{0{\cdot}73}$ *(r = 0·96) was obtained between heart weight (H, g) and body weight (B, kg). The fittings of heart weight to blood/plasma volume generated values of r of between 0·79 and 0·75 in allometry models or between 0·84 and 0·80 in linear models. Thus, vascular space is no better than fractional body weight as the basis to express relative heart weight in pigs. It is suggested that the normal exponent relating heart size to body weight in growing pig is effectively 0·75, similar to the exponent of metabolic size, 0·734 or 0·75. Therefore, the size of the heart of domestic pigs varies in size proportionally with the changes of metabolism seen in terms of growth or maybe even reproduction. The wild boar, the ancestor of domestic pigs, has a heart proportionately about 0·5 bigger than modern pigs when scaled according to* $M^{0{\cdot}75}$*. The attribution of metabolic difference to the bigger heart of wild boar is uncertain and needs further elucidation. The trend to bigger hearts in domestic pigs under current selection pressure for leanness should not necessarily be interpreted as returning to a natural form but may reflect a pathophysiological change.*

Keywords: *growth rate, heart girth, pigs.*

Introduction

Heart size of domestic pigs (*H*, g) varies with body weight (*B*, kg). Best fit equations include those of $H = 16{\cdot}2B^{0{\cdot}63}$ (Brody, 1945); or $H = 9{\cdot}68B^{0{\cdot}736}$ (Engelhardt, 1966); or *H* (male) = $6{\cdot}756B^{0{\cdot}833}$, *H* (female) = $7{\cdot}607B^{0{\cdot}785}$ (Alaku and Steinbach, 1984). These equations all indicate that the relative heart weight decreases with increasing body weight, reaching proportionately only about 0·003 of body weight around adolescence (110 kg) with the proportional ratio even less at maturity. The European wild boar, the ancestral form of domestic pigs, however, has a heart/body weight ratio of 0·007 (40 kg) to 0·006 (90 kg) (Gschwend, 1931).

The small heart in relation to body weight in the domestic pig, as Engelhardt (1966) suggested, appears to be the result of selective breeding for growth rate; hence, heart growth has failed to keep up with the rate of body growth. If this assumption is correct, then the pig heart of today should support an even bigger body mass, or similarly, the domestic pigs of today should have an even smaller heart than 30 years ago since the promotion of growth has been a continuous practice in pig husbandry ever since the beginning of this century.

However, recent evidence indicates that modern domestic pigs might have bigger rather than smaller

hearts when compared with their ancestors. Cliplef and McKay (1993) reported that in Yorkshire and Hampshire pigs, both commonly used breeds for pork production, selection for reduction in back fat thickness and promotion in growth rate for seven to eight generations resulted in concurrent increases in visceral weights including heart, lung, spleen, liver, and kidney. In their report, 90 kg control Yorkshire pigs had heart weights of 305 to 323 g, while performance selected pigs of the same body size had significantly bigger hearts weighing 340 to 371 g. In comparison, published regressions of heart to body weight in pigs, suggest that a 90-kg pig should have a heart weighing less than 290 g (Brody, 1945; Engelhardt, 1966).

Heart size and its relationship to back fat thickness and growth rate in modern domestic pigs needs to be further clarified in a bigger and more varied sample size. The association of heart size to plasma or blood volume in domestic pigs is also worthwhile investigating since vasculature relates to body lean mass. Therefore this study was designed to provide a better understanding of variation of heart size in domestic pigs.

Material and methods

Heart size and growth performance

A total of 39 purebred boars (13 Landrace, 12 Yorkshire, and 14 Duroc) with minimum disease was selected from the test station. The animals were given food *ad libitum* from 30 kg for 110 days to reach around 120 kg on a diet containing 176 g/kg crude protein throughout the study. Fresh food was abundantly offered every morning after trough cleaning for the measurement of individual food consumption of the previous day. Tap water was provided freely through a self-supply nipple in which a valve was opened by the pigs' nose pressure.

When the boars reached 98 to 112 kg, their back fat thickness was monitored by an ultrasonic device at the fourth rib, the last rib, and the last lumbar vertebra of the right side. The mean value of these three locations was used to indicate the thickness. The animals then were allowed to grow further until slaughter.

Prior to being sent to market, boars were fasted for 16 to 24 h, weighed, then group transported to an abattoir. Animals were slaughtered by captive-bolt stunning and exsanguination. There was a holding period of about 4 h between arriving at the abattoir and stunning.

Hearts then were quickly removed within 30 min *post mortem* by dissection and pericardiectomy. Remaining blood inside and outside was washed away by generous flushing of 4°C saline. After trimming, towel drying, and weighing, hearts were laid on a clean gauze so that length (coronary groove to apex) and greatest width of base could be measured. Hearts then were immersed into buckets filled with 4°C saline and later taken back to the laboratory. After arrival, they were opened according to the inflow-outflow method (Liu, 1983) and examined. Those showing any sign of pericarditis or endocarditis, or any other morphological abnormality were discarded. Recording of the septum width was then conducted at the anterior (ventral), median, and posterior (dorsal) site at about 3 cm below the origin of the pulmonary artery. The measurement of thickness of the left ventricle wall was followed from the point between papillary muscles to outside. The thickness of the right ventricle wall was detected at the site near the opening of the pulmonary valve. The thinnest measurement was used to indicate the thickness of apex. The exsanguination sharply damaged the heart but little hindered the measurement of size characteristics. The weight, length, and width were recorded within 45 min *post mortem*; other measurements were completed within 4 h *post mortem*.

Plasma volume, blood volume, and heart size

Ordinary crossbred (Landrace × Yorkshire × Duroc) pigs for commercial production, including 18 males and 24 females ranging from 15 to 110 kg, were randomly selected from a production unit. They were control animals for other growth and carcass studies and received exactly the same food and feeding management as pigs for commercial practice.

Once chosen, pigs were individually moved to the operating theatre and anaesthetized. Catheters were introduced into jugular veins through an ear vein in pigs bigger than 35 kg. For smaller animals, the catheter was directly placed and secured into the vessel required. After sampling of 10 ml blood for blank reference, T-1824 in saline at a dosage of 0·3 mg/kg was injected through a catheter and flushed with 10 ml of saline to ensure the complete introduction of the dye. Blood samples were withdrawn from catheter 20, 40, 60, 80, and 120 min after the dye injection. Pigs then were sacrificed by exsanguination while still under anaesthesia, and hearts were removed and treated as in the method mentioned above. The plasma volume was estimated according to the method of Zweens and Frankena (1981) and the blood volume was obtained from the calculation of the mean pack cell volume and plasma volume. Data were statistically analysed by using the

Statistical Analysis Systems Institute (1988) package program.

Results

Heart size is usually indicated by weight. Other size characteristics, e.g. length and width, are also applicable but we do not yet know their appropriateness. Several size characteristics were measured in the present study; a chief one among them best representing overall size had to be identified first. The statistical method of principal component analysis was then applied to test the importance of each size characteristic measured in order to determine the chief one.

The results together with actual measurement values (mean ± s.d.) are shown in Table 1. It can be seen that, among all the size characteristics, weight generated the highest eigenvalue (0·456), suggesting it is the best single characteristic to represent heart size. The second best indicator of heart size in boars would be the thickness of anterior ventricle septum (AVS) or the thickness of the left ventricle (LV) wall. Further calculation by the step-wise regression method can generate index formulae that include several size characteristics and could more precisely represent heart size. However, only weight was used in the present study since the data can be compared easily with other reports. The eigenvalues in Table 1 are of importance for *in vivo* estimation of heart size (e.g. by echocardiography) in pigs when weight information cannot be obtained and an alternative measurement is needed to represent it.

Table 1 *Heart size characteristics and their principal component (PC) analysis in 39 purebred boars*

Characteristics	First PC	Second PC	Measurement	
			Mean	s.d.
Age (days)	0·255	0·313	209	14
Body weight (kg)	0·089	0·469	120	11
Heart measurements				
Weight (g)	0·456	–0·008	456	60
(g/kg body weight)	0·391	–0·329	3·8	0·5
Width (mm)	0·244	0·193	96	7
Length (mm)	0·163	–0·522	109	9
Thickness (mm)				
Anterior ventricle septum	0·366	0·082	23·4	3·1
Median ventricle septum	0·240	0·317	17·8	4·0
Posterior ventricle septum	0·305	0·178	24·3	4·1
Left ventricle	0·364	0·178	17·8	3·4
Right ventricle	0·207	–0·283	6·6	1·3
Apex	0·141	–0·164	2·8	1·3
Total eigenvalue	3·818	2·301		
Variation	0·318	0·192		
Total variation	0·318	0·510		

Table 2 *Correlation coefficients of cardiac morphological characteristics and growth performance in purebred boars (no. = 39)*

Characteristics	Growth performance†		
	Daily gain	Food/gain	Back fat
Age	0·302	–0·372**	–0·517**
Body weight	0·528	–0·232	0·255
Heart weight	0·123	–0·348*	–0·444**
Heart/body weight	–0·188	–0·202	–0·559**
Width	0·078	–0·353*	–0·247
Length	0·001	0·262	0·005
Thickness			
Anterior ventricle septum	0·006	–0·111	–0·167
Median ventricle septum	–0·070	–0·310	–0·095
Posterior ventricle septum	–0·061	–0·111	–0·267
Left ventricle	0·118	–0·460**	–0·449**
Right ventricle	0·165	0·195	–0·046
Apex	–0·089	0·061	–0·162

† *Ad libitum* feeding for 110 days from 30 kg.

The correlation coefficients between growth performance and heart size in boars studied are shown in Table 2. Significant correlation was found between conversion efficiency (food/gain) and some heart size characteristics including weight ($r = -0{\cdot}35$, $P < 0{\cdot}05$), width ($r = -0{\cdot}35$, $P < 0{\cdot}05$), and thickness of LV wall ($r = -0{\cdot}46$, $P < 0{\cdot}01$). Back fat thickness was also highly significantly negatively correlated with heart weight ($r = -0{\cdot}44$, $P < 0{\cdot}01$), heart/body weight ratio ($r = 0{\cdot}56$, $P < 0{\cdot}01$), and thickness of LV wall ($r = -0{\cdot}45$, $P < 0{\cdot}01$). The daily gain, however, did not correlate with any size characteristics measured.

Results of heart size and its correlation to plasma or blood volume are summarized in Figure 1 and

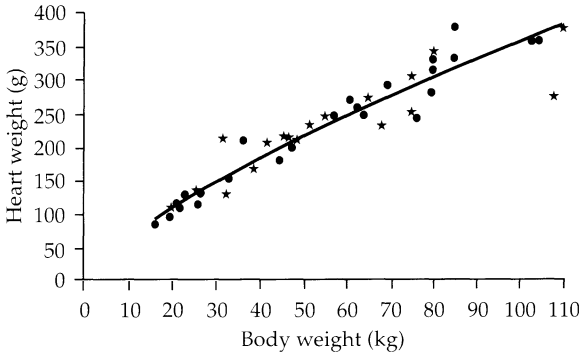

Figure 1 Allometry plotting of heart weight (g) against body weight (kg) in growing boars (no. = 18, ★) and gilts (no. = 24, ●) of Landrace × Yorkshire × Duroc crossbred. $y = 12{\cdot}18x^{0{\cdot}73}$ ($r = 0{\cdot}96$).

Table 3 *Allometry and linear regression between heart weight (HW, g), body weight (BW, kg), plasma volume (PV, l), and blood volume (BV, l) and their coefficients in growing pigs (no. = 42)*

Variables		$y = ax^b$			$y = ax + b$		
y	*x*	*a*	*b*	*r*	*a*	*b*	*r*
HW	BV	68·717	0·798	0·79	40·724	48·293	0·84
HW	PV	91·409	0·774	0·75	56·663	48·267	0·80
PV	BW	0·315	0·571	0·78	0·037	1·060	0·83
BV	BW	0·356	0·623	0·82	0·057	1·195	0·87
BV	PV				1·443	–0·157	0·99

Table 3. The allometry fitting of heart weight to body weight of growing crossbred pigs studied generated a regression of $H = 12{\cdot}18B^{0{\cdot}73}$ with a correlation coefficient of 0·96 on the pooled data of either sex. Difference between males and females was not found (Figure 1). It is of interest to note that, according to the fitted regression in Figure 1, a 120-kg crossbred pig of either sex should have a heart weighing 407 g. This is smaller than the data in Table 1 showing that a 120-kg purebred boar has on average a heart of 456 g.

The other correlations between heart weight and blood volume or plasma volume, between blood or plasma volume and body weight, and between blood and plasma volume, in either allometry or linear models together with their regression coefficients, are listed in Table 3. It should be noticed that none of the regression coefficients of heart weight to blood, or to plasma volume, in either allometry or linear models, was close to or could be compared with the strong allometry regression of heart weight to body weight illustrated in Figure 1 (r = 0·96). It is evident, therefore, that the variation of heart weight of crossbred pigs is more associated with weight changes than with volume changes of blood or plasma during the growing period. Heart weight in growing pigs, in other words, is more precisely expressed, using the allometry model, on a body-weight basis than on vasculature.

Data in Table 3 also provide information about the expansion of blood or plasma volume during the growing period of domestic pigs. The relative plasma volume of a 20-kg growing pig was 90 ml/kg decreasing to 47 ml/kg at around adolescence (110 kg). The blood volume decreased from 117 ml/kg to 68 ml/kg during the same period.

Discussion

Several factors are known to have an effect on heart size in domestic pigs. Straub *et al.* (1976) showed that in boars, heat (35°C) reduced heart weight as well as appetite, daily gain and back fat thickness, but had no effect on food conversion ratio. On the other hand, Alaku and Steinbach (1984) suggested that pigs reared in a tropical climate appeared to have higher heart weights than those reared in a temperate region.

Nutritional manipulations evidently cause variations in heart size in domestic pigs. By offering diets with different protein and energy content, Davey and Bereskin (1978) reported that heart weight in market pigs was significantly correlated with their carcass protein content and protein gain but not with fat gain. Food allowance also plays a rôle in determining heart size. If rations are reduced to 0·80 to 0·85 of *ad libitum*, marketing pigs can maintain a similar level of food conversion efficiency and carcass composition as under *ad libitum*, but have significantly smaller hearts along with slower weight gain (Pekas, 1993).

Selection for thinner back fat and a better growth rate in domestic pigs would also result in a concurrent increase in relative heart weight (Cliplef and McKay, 1993). This is supported partially by the results presented that show (Table 2) growing boars of various genetic backgrounds have a bigger heart if they exhibit a better food conversion efficiency or less back fat thickness, but not weight gain, under *ad-libitum* conditions.

The above mentioned evidence clearly indicates that a bigger heart correlates with better growth performance in growing pigs, although growth parameters may differ and do not agree in all aspects. It also suggests that body lean mass and/or energy turn-over direct heart size in domestic pigs. Body lean represents vasculature directly. Therefore, it is tempting to postulate that heart size may more closely vary with plasma or blood volume than with body weight. However, this is not proven in the present findings (Figure 1 and Table 3). The concept of heart size and its correlation to vascular space or lean mass is defied by the comparison between Yorkshire and Chinese Meishan pigs. Meishan pigs are known to be very prolific and attain sexual maturity early but have fat carcasses, poor food conversion efficiency, and low growth rates. Despite the great differences in these performance traits, Meishan pigs have a heart weighing the same as Yorkshire pigs on an equal body-weight basis (White *et al.*, 1995).

Alternative thinking on energy turn-over or metabolism and heart weight may provide a useful insight into understanding the variation of heart size in domestic pigs during the growing stage. In Figure 1, the best fitting of heart weight to body weight

Table 4 *Cardiac output of pigs expressed on the basis of absolute body weight† and cardiac exponent weight‡*

	Body weight (kg)	Cardiac output ml/kg per min	Cardiac output ml/kg $M^{0.75}$ per min	Reference
Domestic pigs				
	7·2	167	274	Widdowson and McCance, 1955
	20	144	305	Wachtel, 1963
	43	133	341	Wachtel, 1963
	91	72	222	Wachtel, 1963
Wild boar				
	41	82	207	Wachtel, 1963
	75	66	194	Wachtel, 1963

† Data taken directly from the references.
‡ Calculated from the reference data by using cardiac exponent (0·75) of growing pigs (see text).

generated an allometry model yielding an exponent of 0·73. In the literature, exponents relating heart size to body weight in pigs at different stages of maturity range from 0·63 (Brody, 1945), 0·74 (Engelhardt, 1966), to 0·79 or 0·83 (Alaku and Steinbach, 1984). All these are very close to the exponent of metabolic size, the fractional expression preferred being 0·75 (Kleiber, 1975) or 0·734 (Brody, 1945). It can thus be suggested that, the normal exponent relating heart size to body weight in growing pig is effectively 0·75. Since metabolic size also remains a satisfactory first approximation for comparing growth rates and energy exchanges during growth (Webster, 1989), a constant relationship between heart size and metabolism or energy turn-over may exist during the growth of pigs. An interesting piece of evidence is shown in Table 4 in which cardiac output of growing pigs and wild boars are expressed on a body-weight basis as in the collected references, and also calculated by using the conventional expression for metabolic body size ($M^{0.75}$) as the cardiac exponent. It can be seen that the exponent narrowed the variation to a small range and this may indicate that there is a constant ratio of heart size and cardiac output in pigs of various sizes. The low correlation coefficient between heart weight and blood or plasma volume obtained from the present study (Table 3) suggests that the vascular space accounted less than cardiac output for the variation of heart size in pigs.

The heart exponent of 0·75 of growing pigs should also be applied to other heart size characteristics in addition to weight. Results in Table 1 clearly show that the thickness of AVS or LV is the second choice after weight to represent heart size. Thus, should echocardiography be used to estimate heart size in live pigs of different size by measuring thickness of AVS or LV or other parts of the heart, the exponent of 0·75 should also be taken into consideration to express or interpret the data.

The heart size-metabolism relationship may explain the similarity in heart size of Yorkshire and Meishan pigs. The similarity appears to reflect that Yorkshire and Meishan pigs share an equal metabolic burden, although they are selected for different performance (growth and lean *v.* prolificacy and early maturity). It is known that the selection of growth performance in pigs of occidental breeds will result in a bigger heart (Cliplef and McKay, 1993; also results in Table 2). It is not known but is of great interest to learn whether the heart size of Chinese Taihu pigs (Meishan and six other strains) is also correlated with their selection traits, i.e. reproductive performance.

Comparison of heart size between wild boars and domestic pigs raises a difficulty in further understanding the variation of heart size in pig. The heart weight and body weight regression of wild boars (about 40 to 90 kg) is parallel to but higher than that of domestic pigs (Engelhardt, 1966). Thus the cardiac exponent of 0·75 in domestic pigs can also be extended to wild boars. By doing so, it is calculated that the heart size of those wild boars would be around 17 to 18 g/kg $M^{0.75}$, which is much higher than 11 to 12 g/kg $M^{0.75}$ shown by contemporary domestic pigs including the Meishan. It is widely acknowledged that the domestic pig is descended from the wild boar (*Sus scrofa*) and, during the past 10 000-year contact with humans, a marked change in body conformation has occurred (Meyer, 1992). Anatomically, the great reduction in heart weight is evident although the origin remains obscure. Low levels of locomotor activity due to genetic selection to conserve energy for economic production (Robert *et al.*, 1987) and perhaps for easier management during domestication, as well as the limited space allowance in the production environment, are characteristics of domestic pigs. However, the smaller heart in domestic pigs cannot entirely be attributed to these characteristics and other factors should also figure in this anatomical adaption.

Pig husbandry was rather extensive until recently. Today's intensive systems have a relatively short history compared with the history of the pig as a domestic animal. The smaller heart (relative to $M^{0.75}$) resulting from the long use of extensive systems during the past may be viewed as an anatomical and physiological adaptation to domestication and selection. The bigger relative heart size caused by recent intensive selection for leanness therefore should be examined from a different perspective,

and certainly should not be interpreted as returning to a natural form. Heavy selection pressure under the current intensive system of pig production may have exceeded innate physiological limits; this unfitness leading the animal to exhibit pathophysiological changes, perhaps including hypertrophic cardiomyopathy. Comparative studies in clinical cardiology and energy metabolism between those intensively selected for performance (growth or prolificacy) and those control pigs not selected is required to support the above view. This information will be of great value not only for designing future breeding programmes in domestic pigs but also for the understanding of the attributes of heart growth and its association with metabolism.

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Free amino acid concentrations in plasma, muscle and liver as indirect measures of protein adequacy in growing chickens

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Abstract

An experiment was carried out to study the effect of changes in either the quality or the quantity of dietary protein intake on the free essential amino acid profiles in plasma, muscle or liver of growing chickens. Following a randomized paired-feeding design based on metabolic body weight (kg $M^{0.75}$), White Rock male broilers were allocated to one of three isoenergetic (14·5 kJ metabolizable energy per g dry matter (DM)) semisynthetic diets containing different levels of protein (60, 120, 180 or 240 g/kg DM). All diets were based on soya-bean meal, as the sole source of protein, either unsupplemented (diets S) or supplemented with 20 g/kg L-lysine (diets SL) or 2 g/kg DL-methionine (diets SM). Samples of blood, biceps muscle and liver were taken and amino acid analysis was determined by high performance liquid chromatography. Plasma was only adequate to detect the effect of the supplementation with methionine to balance the dietary amino acid (AA) profile. Plasma concentrations of free methionine significantly increased as a result of the supplementation with methionine ($P < 0·001$) and remained unaffected by the amount of protein ingested. When lysine was added to diets S to induce an AA imbalance, a significant increase in muscle concentrations of free valine, isoleucine and phenylalanine ($P < 0·05$) and a significant decrease in liver concentrations of free arginine and phenylalanine were observed ($P < 0·05$). The supplementation with DL-methionine gave rise to a significant fall in the concentrations of free histidine, glycine and threonine ($P < 0·05$) and increased those of isoleucine and methionine in the skeletal muscle ($P < 0·05$), while in liver it significantly lowered the concentrations of free arginine, valine, leucine, phenylalanine and methionine ($P < 0·001$ to $P < 0·05$) and raised that of free glycine ($P < 0·001$). The ratios lysine : phenylalanine, lysine : valine and methionine : glycine in muscle together with that of methionine : glycine in liver seem to be appropriate indexes of the adequacy of the dietary AA profile to requirements.

Keywords: *fowls, free amino acids, protein content, protein quality.*

Introduction

Attempts to establish a direct relationship between the free amino acid (AA) concentrations in tissues and the AA profile of the protein ingested, as a reliable predictor of the nutritional value of dietary proteins, have yielded conflicting results. On theoretical grounds, AA concentrations in plasma and tissues should reflect an equilibrium between the AA supply and the rate of use of AAs in protein synthesis and gluconeogenesis or catabolism. Regulatory mechanisms involved in protein turnover rate interact to achieve the maintenance of the free AA pools within relatively narrow, although fluctuating, limits. A relationship between AA dietary supply and free AA concentration has been demonstrated in blood by several authors, more often in portal blood (Galibois *et al.*, 1987; Rérat *et al.*, 1988) than in systemic blood (Zimmerman and Scott, 1965; Kunachowicz *et al.*, 1983; Jansen *et al.*, 1986). However, other studies found no evidence for such a relationship (Tasaki and Ohno, 1971; Waterlow and Fern, 1981; Hagemeister *et al.*, 1990). In a previous experiment (Fernández-Fígares *et al.*, 1993) carried out in growing chickens given isoenergetic and isonitrogenous high protein diets of a widely different essential AA (EAA) profile, we identified by the so-called plasma AA ratio (Longenecker and Hause, 1959; Hill and Olsen, 1963) the most limiting AAs in the dietary proteins and the results were in agreement with those provided by the protein score approach. For the most limiting AAs in the diets the observed concentrations in plasma, muscle and liver paralleled their rank in the dietary protein, with minor exceptions. However this work failed to build

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up a clear link between free AA changes in tissues and AA intake, except that a significant relationship between lysine supply and its concentration in muscle was observed. Concentrations of free AAs in muscle have been postulated to be better indicators for the estimation of availability of specific AAs than AA concentrations in blood (Pion, 1976; Jansen *et al.*, 1986). Also, Pion (1976) reported direct relationships between the free AA concentrations in liver and AA intake in the case of diets imbalanced or deficient in one or more specific AAs. Jansen *et al.* (1986) found increases in the concentrations of essential AAs in liver of lactating rats as a result of increases in either protein level or protein quality. Skeletal muscle is the largest tissue in the body and is the major pool in the homeostasis of amino acids. Growth in response to AA intake is primarily dependent on the relative rates of protein synthesis and protein degradation, mainly in muscle. In a previous trial we studied the effect of supplementing with L-lysine or DL-methionine a diet containing soya-bean meal, as the sole protein source, on fractional rates of protein synthesis and degradation in the skeletal muscle and liver of growing chickens (Nieto *et al.*, 1994). We showed that the provision of a reduced intake or an AA imbalance affected growth by changing protein degradation rates, especially in muscle, while protein synthesis remained unaffected. A reduction of intake causing cessation of growth was also accompanied by changes in the fractional degradation rate in muscle and liver. Changes in protein degradation rates in muscle and liver would alter tissue free AA profiles and consequently the present work was carried out to test the hypothesis that AA concentrations in skeletal muscle and liver are better indicators of AA intake than are plasma concentrations.

Material and methods

Experimental design and feeding

Forty-eight White Rock male broilers (1-day-old) were raised in batteries at 30°C and given a commercial starter diet for 10 days and then randomly divided into 12 groups of four chicks each on a weight basis (mean live weight: 178 (s.e. 1·9) g) and individually kept in metabolism cages in a room maintained at 26±1°C. Following a randomized-block design (Figures 1a and b), each group of birds was given, above maintenance level (684 kJ metabolizable energy (ME) per kg $M^{0.75}$; Aguilera and Prieto, 1987), successively and throughout a period of 16 days, each of four levels of protein (60, 120, 180 or 240 g/kg dry matter (DM)) in three groups of isoenergetic (14·5 kJ ME per g DM) and semisynthetic diets. Soya-bean meal was the sole source of protein (known to be first limiting in methionine) and the diets were either unsupplemented (diets S), supplemented with 20 g/kg L-lysine (diets SL) or supplemented with 2 g/kg DL-methionine (diets SM). The supplementation with lysine or methionine was made at the expense of a concomitant reduction in the amount of soya-bean protein. The composition of diets is given in Table 1.

Table 1 *Composition of the experimental diets (g/kg)*†

	SL60	S60	SM60	SL120	S120	SM120	SL180	S180	SM180	SL240	S240	SM240
Ingredients (g/kg)												
Soya-bean meal‡	88·8	133·2	128·7	221·9	266·3	261·9	358·0	399·5	398·3	492·3	532·6	532·6
Maize oil	55·0	55·0	55·0	60·0	60·0	60·0	65·0	65·0	65·0	70·0	70·0	70·0
Mineral and vitamin pre-mix	61·3	61·3	61·3	61·3	61·3	61·3	61·3	61·3	61·3	61·3	61·3	61·3
L-lysine (L-lys)	20·0			20·0			20·0			20·0		
DL-methionine (DL-met)			2·0			2·0			2·0			2·0
Maize starch§	641·8	641·8	641·8	535·6	535·6	535·6	429·5	429·5	429·5	322·9	322·9	322·9
Cellulose	133·0	108·6	110·7	79·2	77·7	101·8	69·0	44·6	47·1	37·4	13·0	15·5
Chemical composition												
Dry matter (DM, g/kg)	897	893	897	901	900	904	907	911	908	912	910	916
Crude protein (CP) (N × 6·25 g/kg DM)	69·4	62·6	65·7	136·0	135·7	138·6	209·5	204·4	208·8	257·0	258·8	261·5
Gross energy (kJ/g DM)	17·60	17·36	17·78	18·07	18·03	18·28	18·37	18·37	18·62	19·00	18·79	19·00

† Soya, 60 g/kg DM CP (S60); Soya + 20 g/kg L-lys, 60 g/kg DM CP (SL60); Soya + 2 g/kg DL-met, 60 g/kg DM CP (SM60); Soya, 120 g/kg DM CP (S120); Soya + 20 g/kg L-lys, 120 g/kg DM CP (SL120); Soya + 2 g/kg DL-met, 120 g/kg DM CP (SM120); Soya, 180 g/kg DM CP (S180); Soya + 20 g/kg L-lys, 180 g/kg DM CP (SL180); Soya + 2 g/kg DL-met, 180 g/kg DM CP (SM180); Soya, 240 g/kg DM CP (S240); Soya + 20 g/kg L-lys, 240 g/kg DM CP (SL240); Soya + 2 g/kg DL-met, 240 g/kg DM CP (SM240).

‡ Chemical composition (g/kg DM): crude protein, 489·3; ether extract, 26·2; neutral-detergent fibre, 181·6; total minerals, 71·6.

§ Chemical composition (g/kg DM): crude protein, 8·3; ether extract, 10·2; crude fibre, 2·28; total minerals, 1·36.

Within diets of the same protein quality and after an adaptation period of 4 days, one chicken from each group of four was killed at random, between 1 and 4 h after the morning meal (9.00 h), and samples of blood, muscle and liver were taken for AA analysis, whereas the remaining three animals were allocated to the following treatment for another period of 4 days. A restriction was imposed to the experimental design so that within the same period only one experimental animal was killed at each protein level treatment. The same schedule was followed until the end of the experiment so that four replicates of each level of protein per diet were obtained, each one differing in the order by which previous dietary protein levels had been offered. Diets S and SM were given 1 day later than diet SL, according to a similar paired-feeding design, based on daily food intake scaled to a component of body weight (M) (g food per kg $M^{0.75}$), in order to eliminate the effect of differences in food intake among diets and to give all animals an equivalent intake. The amino acid requirements for growing chickens and the AA composition of the experimental diets are shown in Table 2.

Sampling and analytical procedures

The animals were anaesthetized with ketamine-rompun (10·0 and 3·0 mg/kg; Warner Lambert Co. Morris Plains, NJ, USA; Bayer, Laverkusen, Germany) and blood was drawn by heart puncture. Once the animals were killed by cervical dislocation, right biceps muscle and liver were removed immediately, rinsed with saline, frozen in liquid nitrogen and then stored at –20°C until analysis. Heparinized blood samples were taken and plasma was obtained by centrifugation. Muscle and liver samples were freeze-dried (Dura-Dry MP, FTS Systems, Inc., NY, USA) and then homogenized in hydrochloric acid (0·1 mol/l) (Omni 2000, Connecticut, USA) and centrifuged. Norleucine (40 mmol in 0·1 mol/l HCl) was used as internal standard. Samples were then deproteinized by ultrafiltration (Ultrafree MC microcentrifuge device, Millipore P/N SK 1P44V7). Amino acid analysis was determined by high performance liquid chromatography (HPLC), according to a method involving pre-column derivatization with phenylisothyocyanate (PITC) (Cohen *et al.*, 1989). The phenyl-thio-carbamyl (PTC) amino acid derivatives were separated on a Pico.Tag reverse-phase column and the separation employed a binary gradient at a flow-rate of 1·0 ml/min. Derivatized AAs were detected on-line spectrophotometrically at 254 nm. A Waters System Interface Module and Maxima 820 Chromatography software (Dynamic Solution, Division of Millipore) were used for gradient control and data processing. Each analysis presented is the result of one simple analysis.

Table 2 *Amino acid requirements (g/kg) of growing chickens and amino acid composition of the experimental diets (g/kg)*†

	Requirements‡		Diets											
Amino acid	INRA	ARC	SL60	S60	SM60	SL120	S120	SM120	SL180	S180	SM180	SL240	S240	SM240
Lysine	10·2	11·0	22·9	4·3	4·2	27·2	8·6	8·5	31·5	13·0	12·8	35·8	17·3	17·1
Arginine	10·6	10·3	3·3	4·9	4·7	8·2	9·8	9·7	13·1	14·7	14·6	18·0	19·6	19·5
Histidine	4·1	4·8	1·1	1·7	1·6	2·8	3·4	3·3	4·5	5·1	5·0	6·2	6·8	6·7
Valine	8·9	9·8	1·6	2·4	2·4	4·1	4·9	4·8	6·5	7·3	7·2	8·9	9·7	9·6
Tryptophan	2·0	2·1	§	§	§	§	§	§	§	§	§	§	§	§
Leucine	14·2	14·7	3·1	4·6	4·5	7·7	9·3	9·1	12·4	13·9	13·8	17·0	18·6	18·4
Isoleucine	8·0	8·5	2·3	3·4	3·3	5·7	6·8	6·7	9·1	10·2	10·1	12·5	13·6	13·5
Glycine + serine	16·9	14·0	3·5	5·2	5·0	8·7	10·4	10·2	13·9	15·6	15·4	19·1	20·8	20·6
Phenylalanine + tyrosine	13·5	15·8	3·6	5·4	5·2	9·0	10·8	10·7	14·4	16·2	16·0	19·8	21·5	21·4
Threonine	6·1	7·4	1·6	2·4	2·3	4·0	4·8	4·8	6·5	7·3	7·2	8·9	9·7	9·6
Methionine	4·4	4·8	0·7	1·1	3·1	1·8	2·2	4·1	2·9	3·3	5·2	4·0	4·3	6·3
Cystine	3·3	4·4	0·7	1·1	1·1	1·9	2·2	2·2	3·0	3·3	3·3	4·1	4·5	4·4
Ratio met/CP‖			0·01	0·02	0·05	0·01	0·02	0·03	0·02	0·02	0·03	0·02	0·02	0·03
Ratio lys/CP‖			0·38	0·07	0·07	0·22	0·06	0·07	0·18	0·07	0·07	0·15	0·07	0·07

† See Table 1.
‡ Chickens of 0 to 4 weeks (from INRA, 1984 and ARC, 1975).
§ Not analysed.
‖ Methionine/crude protein; lysine/crude protein.

Statistical analysis

The experimental data were analysed as a 3 (diet: SL, S and SM) × 4 (level of protein: 60, 120, 180 and 240 g/kg DM) factorial design. Body weight (equivalent to age) and the ratios methionine/crude protein and lysine/crude protein acted as covariates to account for the proportional diminishing effect of adding a fixed quantity of a single AA to diets with increasing protein content and also in order to try to avoid the effect of minor differences in composition among diets. The data were subjected to analysis by a two-way ANOVA followed by multiple range tests by means of a computer package (Statistical Analysis Systems Institute, 1985).

Results

Mean values of food intake and growth performance are presented in Table 3. The factorial analysis 3 (diet) × 4 (protein level) of the data showed that, with respect to the quality of dietary protein, DM intake and nitrogen (N) intake, both expressed per kg $M^{0.75}$ per day, remained unchanged; however, daily body gain and protein efficiency ratio (PER) were significantly affected ($P < 0.01$), increasing as the protein quality was improved (SL < S < SM). Significant differences appeared only between diet SM and the other two diets (S and SL) ($P < 0.05$). For the level of protein in the diet daily body gain increased on increasing the amount of protein consumed ($P < 0.001$), whereas PER increased from 60 to 120 g/kg DM and then remained unchanged. There were no significant interactions between diet and level of dietary protein.

Free EAA concentrations in plasma, muscle and liver are shown in Tables 4 and 5. The data showed significant interactions between diet and level of protein for most individual AAs, mostly in plasma (all EAAs, except methionine) but also, to a lesser extent, in muscle (lysine, arginine and leucine) and liver (lysine, histidine and tryptophan), indicating the dependence of the effect of diet (AA composition of ingested protein) upon the level of dietary protein and *vice versa*. Plasma was only adequate to detect the effect of the supplementation with 2 g/kg DL-methionine to balance the dietary AA profile, as this treatment gave rise to a significant increase in free plasma methionine ($P < 0.05$) irrespective of the amount of protein ingested. Plasma concentrations of methionine remained unaffected by the amount of protein eaten by the growing chickens. In muscle, free isoleucine, phenylalanine, glycine and methionine were significantly affected by the AA supplementation of the dietary protein ($P < 0.001$ to $P < 0.05$) but not by the level of protein in the diet, while the concentrations of histidine, valine and threonine were influenced by both factors ($P < 0.001$). Amino acid imbalance induced by feeding the growing chickens with the diet supplemented with L-lysine caused a significant rise in the concentrations of valine, isoleucine and phenylalanine ($P < 0.05$), irrespective of the level of dietary protein. The supplementation with DL-methionine significantly decreased the concentrations of free histidine, glycine and threonine and increased those of isoleucine and methionine ($P < 0.05$). The ratios lysine : phenylalanine and lysine : valine sharply decreased (on average 48·4, 21·8 and 6·15; and 37·4, 13·3 and 5·49, for diets SL, S and SM, respectively) and that of methionine : glycine increased (0·047, 0·025 and 0·186) on improving the protein quality of the diet. In liver, the concentrations of free arginine, valine, leucine, phenylalanine and methionine were significantly affected by the AA imbalance ($P < 0.001$ to $P < 0.05$) or balance caused by supplementation of the dietary protein with lysine or methionine, but they remained unaltered by the quantity of protein

Table 3 *Food intake and growth performance in broilers given diets based on soya bean meal unsupplemented (S) or supplemented with 20 g/kg L-lysine (SL) or 2 g/kg DL-methionine (SM) (mean values for the factors diet (no. = 16) and level of protein (no. = 12) of the factorial analysis 3 × 4; for details, see text)*

	Diet (D)†			Pooled s.e.	Protein level (g/kg DM; PL)				Pooled s.e.	Significance of main effects		
	SL	S	SM		60	120	180	240		D	PL	D × PL
Dry matter intake (g/kg $M^{0.75}$ per day)	95·5^{a}	88·5^{a}	89·4^{a}	4·52	76·4^{a}	90·1^{ab}	95·1^{ab}	103·1^{b}	5·09		**	
Nitrogen intake (g/kg $M^{0.75}$ per day)	2·61^{a}	2·54^{a}	2·50^{a}	0·12	0·83^{a}	1·89^{b}	3·12^{c}	4·37^{d}	0·13		***	
Body-weight gain (g/day)	17·2^{a}	18·8^{a}	21·9^{b}	1·15	2·7^{a}	15·2^{b}	24·5^{c}	35·0^{d}	1·17	**	***	
Protein efficiency ratio	2·0^{a}	2·3^{a}	2·8^{b}	0·15	1·2^{a}	2·9^{b}	2·8^{b}	2·7^{b}	0·17	**	***	

^{a,b,c,d} Within the same row and factor, values bearing different superscript differ significantly ($P < 0.05$).

† See Table 1.

Table 4 *Free essential amino acid (EAA) concentrations (μmol/l) in plasma, muscle and liver of growing chickens given semisynthetic diets based on soya bean meal unsupplemented (S) or supplemented with 20 g/kg L-lysine (SL) or 2 g/kg DL-methionine (SM) (mean values for the factors diet (no. = 16) and dietary protein level (no. = 12) of the factorial analysis 3 × 4; for details, see text)*

	Diet (D)†			Pooled	Protein level (PL) (g/kg DM)				Pooled	Significance of main effects		
EAA‡	SL	S	SM	s.e.	60	120	180	240	s.e.	D	PL	D × PL
(a) Plasma												
LYS	1110[a]	621[b]	262[c]	94	680[ab]	940[a]	581[ab]	454[b]	110	***	*	**
ARG	71[a]	132[b]	173[b]									
HIS	36[a]	38[a]	39[a]	12·3	69[a]	175[b]	29[a]	147[b]	13·8	***	***	***
VAL	110[a]	129[a]	128[a]	8·1	80[a]	146[b]	88[a]	175[b]	9·4			***
LEU	77[a]	118[b]	107[ab]	9·2	72[a]	134[b]	83[a]	122[b]	11	**	***	***
ILE	48[a]	63[a]	62[a]	5·1	34[a]	76[a]	44[a]	77[a]	5·9		***	***
PHE	50[a]	68[b]	67[b]	4·6	52[ab]	85[c]	42[a]	67[bc]	5·3	*	***	***
TRP	14[a]	33[b]	38[b]	17	18[a]	28[ab]	24[a]	44[b]	4·7	***	**	**
GLY§	172[a]	230[a]	203[a]	17	190[ab]	260[b]	144[a]	203[ab]	20		**	***
THR	224[a]	261[a]	132[b]	15	121[a]	264[b]	190[ac]	251[bc]	18	***	***	***
MET	13·2[a]	7·1[a]	50[b]	3·1	21[a]	26[a]	20[a]	25[a]	3·6	***		
(b) Muscle												
LYS	19910[a]	4654[b]	1544[c]	680	2221[a]	7860[b]	9864[c]	14870[c]	791	***	***	***
ARG	821[a]	633[a]	682[a]	74	172[a]	911[bc]	633[b]	1151[c]	85		***	**
HIS	222[a]	222[a]	141[b]	12	153[a]	182[a]	182[a]	262[b]	14	***	***	
VAL	533[a]	351[b]	281[b]	25	274[a]	353[a]	341[a]	583[b]	29	***	***	
LEU	524[a]	250[a]	1672[b]	141	310[a]	454[a]	2120[b]	394[a]	171	***	***	***
ILE	3740[a]	1944[b]	3553[a]	273	3071[a]	3360[a]	3141[a]	2743[a]	271	***		
PHE	411[a]	213[b]	251[ab]	50	162[a]	351[a]	282[a]	362[a]	58	*		
TRP	252[a]	82[a]	103[a]	76	73[a]	262[a]	113[a]	142[a]	88			
GLY§	4073[a]	4031[a]	1312[b]	382	2474[a]	3793[a]	3234[a]	3051[a]	433	***		
THR	1494[a]	1310[a]	582[b]	92	560[a]	1074[b]	1333[bc]	1553[c]	112	***	***	
MET	190[ab]	101[a]	244[b]	32	221[a]	180[a]	132[a]	152[a]	37	*		
(c) Liver												
LYS	3250[a]	2321[b]	1501[b]	250	1801[a]	2981[b]	2854[ab]	1790[a]	290	***	**	**
ARG	521[a]	852[b]	241[c]	79	392[a]	602[a]	573[a]	571[a]	91	***		
HIS	732[a]	953[a]	1542[b]	81	1513[a]	613[b]	1102[c]	1072[c]	94	***	***	***
VAL	803[ab]	1084[a]	533[b]	92	602[a]	754[ab]	831[ab]	1033[b]	110	***		
LEU	1354[a]	1020[ab]	864[b]	111	8311[a]	1110[a]	1200[a]	1164[a]	130	*		
ILE	494[a]	451[a]	440[a]	36	372[a]	431[ab]	491[ab]	550[b]	42		*	
PHE	123[a]	652[b]	81[a]	42	303[a]	262[a]	282[a]	361[a]	48	***		
TRP	472[a]	53[b]	112[b]	24	170[a]	233[a]	234[a]	210[a]	28	***	**	*
GLY§	4661[a]	5554[a]	7063[b]	330	4250[a]	5584[ab]	6044[bc]	7151[c]	382	***	***	
THR	2612[a]	2863[a]	2984[a]	200	1643[a]	2490[a]	3531[b]	3604[b]	231		***	
MET	353[a]	332[a]	113[b]	41	172[a]	251[ab]	282[ab]	351[b]	47	***		

[a,b,c] Within the same row and factor, values bearing different superscript differ significantly ($P < 0.05$).
† For diets see Table 1.
‡ For amino acids see Table 2.
§ Semiessential.

ingested. On the contrary, the concentration of free glycine was significantly influenced by both the AA composition and content of dietary protein ($P < 0.001$). Concentrations of free arginine and phenylalanine were significantly decreased in chickens given excess lysine ($P < 0.05$). The addition of 2 g/kg DL-methionine led to a significant fall in the concentrations of arginine, valine, leucine, phenylalanine and methionine ($P < 0.001$ to $P < 0.05$) and to an increase of free glycine ($P < 0.001$). There was a sharp fall in the ratio methionine : glycine (on average 0·076, 0·060 and 0·016 for diets SL, S and SM, respectively) in liver when methionine was added to improve the quality of the dietary protein. This effect was independent of the amount of protein ingested. Free levels of isoleucine and threonine significantly increased with the level of protein in the diet ($P < 0.05$ and $P < 0.001$, respectively).

Discussion

In our experiment an adaptation to diet period of 4 days was considered sufficient to eliminate the

Table 5 *Mean values (no. = 4) of free essential amino acid (EAA) concentrations (μmol/l) in plasma, muscle and liver of growing chickens given semisynthetic diets based on soya-bean meal unsupplemented (S) or supplemented with 20 g/kg L-lysine (SL) or 2 g/kg DL-methionine (SM)*

	Protein level (g/kg DM)												
	60			120			180			240			Pooled
Diet†	SL	S	SM	SL	S	SM	SL	S	SM	SL	S	SM	s.e.
EAA‡													
(a) Plasma													
LYS	1040^{ab}	845^{a}	165^{a}	1888^{b}	730^{a}	188^{a}	999^{ab}	487^{a}	265^{a}	504^{a}	414^{a}	432^{a}	187
ARG	38^{a}	84^{ab}	57^{ab}	136^{abc}	238^{cd}	144^{abc}	52^{ab}	56^{ab}	167^{bc}	51^{ab}	135^{abc}	294^{d}	25
HIS	24^{ab}	18^{ab}	16^{a}	84^{e}	60^{cde}	20^{ab}	21^{ab}	23^{ab}	43^{abc}	15^{a}	49^{bcd}	78^{de}	6·6
VAL	79^{a}	98^{ab}	63^{a}	196^{cd}	179^{c}	63^{a}	79^{a}	66^{a}	119^{abc}	85^{a}	172^{bc}	268^{d}	16
LEU	69^{a}	90^{ab}	62^{a}	134^{abc}	180^{bc}	78^{a}	51^{a}	78^{a}	106^{abc}	53^{a}	125^{abc}	182^{c}	18
ILE	27^{a}	47^{ab}	29^{a}	80^{bcd}	106^{cd}	42^{ab}	44^{ab}	29^{a}	58^{abc}	40^{ab}	71^{abcd}	119^{d}	10
PHE	53^{ab}	61^{ab}	43^{a}	90^{bc}	113^{c}	53^{ab}	33^{a}	34^{a}	60^{ab}	25^{a}	65^{ab}	111^{c}	9·2
TRP	10^{a}	24^{a}	19^{a}	21^{a}	40^{a}	24^{a}	14^{a}	31^{a}	29^{a}	12^{a}	38^{a}	82^{b}	8·2
GLY§	196^{abcd}	218^{abcd}	145^{abc}	264^{bcd}	354^{d}	166^{abc}	133^{abc}	105^{ab}	189^{abcd}	88^{a}	231^{abcd}	280^{cd}	35
THR	155^{ab}	162^{abc}	41^{a}	307^{cd}	442^{d}	45^{a}	256^{bc}	174^{abc}	127^{ab}	155^{ab}	277^{bc}	309^{cd}	30
MET	19^{abc}	$7·2^{a}$	38^{bcd}	18^{abc}	$8·3^{ab}$	53^{d}	$8·2^{ab}$	$9·4^{ab}$	44^{cd}	$5·6^{a}$	$4·1^{a}$	66^{d}	6·2
(b) Muscle													
LYS	3826^{a}	2029^{a}	790^{a}	16419^{b}	6572^{a}	591^{a}	24371^{c}	3653^{a}	1554^{a}	35039^{d}	6341^{a}	3222^{a}	1364
ARG	147^{a}	198^{a}	157^{a}	845^{ab}	1020^{bc}	861^{ab}	585^{ab}	514^{ab}	784^{ab}	1720^{c}	792^{ab}	9260^{b}	147
HIS	136^{ab}	217^{abc}	113^{ab}	233^{bc}	203^{abc}	104^{a}	212^{abc}	176^{abc}	144^{ab}	296^{c}	273^{c}	206^{abc}	25
VAL	353^{ab}	269^{ab}	193^{a}	510^{b}	383^{ab}	162^{a}	485^{b}	270^{ab}	270^{ab}	770^{c}	488^{b}	492^{b}	51
LEU	390^{a}	268^{a}	270^{a}	869^{a}	263^{a}	219^{a}	308^{a}	191^{a}	5854^{b}	527^{a}	284^{a}	346^{a}	288
ILE	4307^{c}	2206^{abc}	2701^{abc}	4001^{bc}	2172^{abc}	3896^{abc}	3517^{abc}	1668^{a}	4243^{c}	3117^{abc}	1733^{ab}	3371^{abc}	468
PHE	180^{a}	135^{a}	183^{a}	627^{a}	251^{a}	168^{a}	365^{a}	191^{a}	272^{a}	476^{a}	257^{a}	363^{a}	100
TRP	67^{a}	58^{a}	86^{a}	615^{a}	114^{a}	63^{a}	129^{a}	73^{a}	137^{a}	194^{a}	92^{a}	126^{a}	152
GLY§	2214^{abc}	2956^{abc}	2248^{abc}	5386^{c}	4771^{bc}	1219^{ab}	4510^{bc}	4073^{abc}	1105^{ab}	4162^{abc}	4328^{abc}	656^{a}	751
THR	714^{abcd}	752^{abcd}	199^{a}	1531^{de}	1370^{cde}	311^{ab}	2012^{e}	1368^{cde}	599^{abc}	1710^{e}	1733^{e}	1215^{bcde}	186
MET	327^{a}	102^{a}	237^{a}	160^{a}	120^{a}	274^{a}	102^{a}	59^{a}	230^{a}	158^{a}	98^{a}	203^{a}	64
(c) Liver													
LYS	2277^{ab}	1996^{ab}	1123^{a}	4961^{c}	2645^{abc}	1344^{a}	4314^{bc}	2068^{ab}	2175^{ab}	1458^{a}	2572^{abc}	1344^{a}	501
ARG	327^{abc}	640^{abc}	212^{a}	568^{abc}	995^{bc}	237^{ab}	698^{abc}	742^{abc}	285^{abc}	474^{abc}	1019^{c}	216^{ab}	158
HIS	680^{a}	918^{ab}	2928^{c}	829^{ab}	1005^{ab}	602^{a}	850^{ab}	829^{ab}	1616^{b}	562^{a}	1034^{ab}	1605^{b}	162
VAL	616^{ab}	831^{ab}	365^{a}	799^{ab}	956^{ab}	493^{a}	870^{ab}	1060^{ab}	557^{ab}	932^{ab}	1463^{b}	689^{ab}	184
LEU	1143^{ab}	807^{ab}	541^{a}	1725^{b}	894^{ab}	726^{ab}	1475^{ab}	1004^{ab}	1114^{ab}	1041^{ab}	1388^{ab}	1040^{ab}	220
ILE	368^{a}	449^{a}	297^{a}	491^{a}	444^{a}	348^{a}	549^{a}	418^{a}	514^{a}	567^{a}	509^{a}	590^{a}	73
PHE	146^{ab}	540^{bc}	55^{a}	86^{a}	602^{c}	80^{a}	89^{a}	650^{c}	102^{a}	156^{ab}	826^{c}	93^{a}	83
TRP	229^{a}	32^{a}	76^{a}	547^{b}	57^{a}	110^{a}	567^{b}	61^{a}	143^{a}	527^{b}	72^{a}	130^{a}	48
GLY§	3314^{a}	4201^{ab}	5229^{ab}	4481^{ab}	5325^{ab}	6936^{bc}	5072^{ab}	6271^{abc}	6765^{bc}	5753^{ab}	6390^{abc}	9318^{c}	651
THR	1437^{a}	1897^{abc}	1594^{ab}	2289^{abc}	2968^{abcd}	2222^{abc}	3746^{cd}	3091^{abcd}	3745^{cd}	2960^{abcd}	3488^{bcd}	4342^{d}	396
MET	193^{a}	228^{a}	82^{a}	365^{a}	293^{a}	101^{a}	380^{a}	322^{a}	140^{a}	440^{a}	476^{a}	136^{a}	82

a,b,c,d,e Within the same row and factor, values bearing different superscript differ significantly ($P < 0·05$).

† For diets see Table 1.

‡ For amino acids see Table 2.

§ Semiessential.

carry-over effect between different levels of protein. In this respect, in a previous experiment with growing chickens performed in our laboratory (Fernández-Fígares *et al.*, 1996), following a similar design, this adaptation period proved to be adequate to show significant changes in the excretion of uric acid-N and ammonia-N as a result of supplementing with DL-methionine a semisynthetic diet based on soya-bean meal as a single source of protein. The excretion of nitrogenous compounds significantly decreased on improving the biological quality of the dietary protein and increased with the intake of protein. Moreover, in rats given low-protein or adequate-protein diets based on casein supplemented with high quantities of individual branched-chain amino and α-ketoacids, Block and Harper (1991) found that the patterns of plasma and tissue (brain) amino acid concentrations were similar

at 2, 6 and 10 days of consumption of the experimental diets.

There is evidence suggesting that a severe AA imbalance has a primary effect on food intake which, in turn, can affect growth rate (Solberg *et al.*, 1971; Tasaki *et al.*, 1976; Okumura and Mori, 1979; Summers and Leeson, 1985). AA excesses can result in impaired growth performance (Katz and Baker, 1975; Han and Baker, 1993). It is clear, therefore, that careful matching of intakes is required to study the effects of AA supplementation *per se* so that the effects of protein quality and quantity are not confounding factors. In our experiment, the paired-feeding design (Figures 1a and b) allowed us to equalize feeding between treatments.

Period

I	II	III	IV
60 no. = 1	240 no. = 1	180 no. = 1	120 no. = 1
120 no. = 1	180 no. = 1	240 no. = 1	60 no. = 1
180 no. = 1	60 no. = 1	120 no. = 1	240 no. = 1
240 no. = 1	120 no. = 1	60 no. = 1	180 no. = 1

Figure 1a The randomized-block design used in chickens paired-fed semisynthetic diets based on soya bean meal unsupplemented (diet S) or supplemented with L-lysine (diet SL) or DL-methionine (diet SM). Each group of four birds was given each of four levels of protein (g/kg).

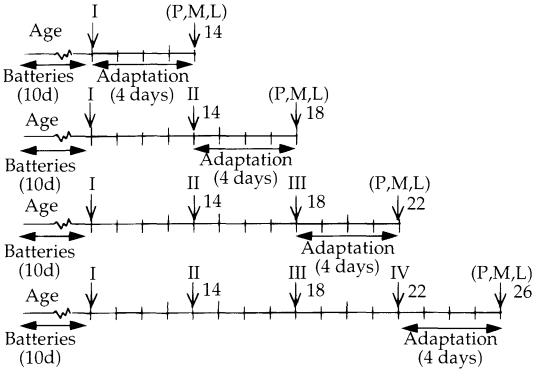

Figure 1b The experimental scheme followed to sample plasma (P), muscle (M) and liver (L) in chickens given, throughout four successive periods (I, II, III, IV) semisynthetic diets based on soya-bean meal unsupplemented (diet S) or supplemented with L-lysine (diet SL) or DL-methionine (diet SM). Diets S and SM were offered one day later than diet SL to achieve a paired-feeding design.

Compared with the AA requirement recommended (Table 2), the diets with 60 and 120 g crude protein per kg DM supplied insufficient amounts of all EAA (except diets SL for lysine). The diets containing 180 g crude protein per kg DM were deficient in histidine, leucine and glycine + serine. The diets with 240 g crude protein per kg DM provided an excess of all AAs. Overall (Table 3), the supplementation with L-lysine (20 g/kg, diets SL) and with sufficient amount of DL-methionine (2 g/kg, diets SM) resulted in decreases or improvements, respectively, in daily body gain and PER, although the effect was only significant for diets SM ($P < 0{\cdot}05$). Nieto *et al.* (1994 and 1995) had previously observed significant increases in daily body gain and N retention in growing chickens given isonitrogenous (200 g crude protein per kg DM) semipurified diets based on soya-bean meal supplemented with 20 g/kg L-lysine or 2 g/kg DL-methionine as the quality of dietary protein improved (SL < S < SM).

It has been reported that plasma concentrations of lysine increased (in rats, Pawlak and Pion, 1968; in chickens, Hill *et al.*, 1961; May *et al.*, 1972; Mandev and Tomova, 1982) and those of other AAs decreased (arginine and tryptophan, Hill *et al.*, 1961; arginine, May *et al.*, 1972; Mandev and Tomova, 1982) in animals given graded levels of dietary lysine (up to three or four times the requirement of this AA, Edmonds and Baker, 1987). Thus, there is evidence that excess dietary lysine can antagonize arginine. We found that the addition of lysine gave rise to increased plasma concentrations of lysine and that this effect was concomitant with a significant ($P < 0{\cdot}05$) decrease of free arginine and tryptophan, although interactions between factors assayed were significant (Table 4). On the other hand, Pion (1973) found in rats that the supplementation with methionine in diets deficient in this AA (from 1 to 8 g/kg) led to a rise in plasma free methionine concentrations. Our results agree with these previous observations. Addition of methionine to diets S caused a significant fall in plasma free levels of lysine and threonine. The low-protein diets showed the highest differences between treatments. This may indicate improved AA balance with AA supplementation which enhanced net protein synthesis and therefore removal of EAAs from plasma.

In experiments conducted to evaluate the effects of graded amounts of lysine when added to diets deficient in this AA (Pawlak and Pion, 1968; Jacobs and Crandall, 1972; Pion, 1973) it has been reported that the amount of dietary lysine was better correlated with free lysine in muscle than in blood. In the current work changes in free levels of lysine in muscle were quantitatively more important than in plasma and showed a tendency to increase with the amount of protein ingested. Edmonds and Baker (1987) found that in pigs excess lysine tended to raise the concentration of lysine in muscle but that of arginine was unaffected. Our results are in agreement with these observations. The rise in the concentrations of free valine, isoleucine and phenylalanine caused by feeding excess of lysine may reflect a decline in their utilization for protein synthesis, as these EAAs were not limiting in our experimental diets. Concerning the supplementation with methionine, in studies with rats Pion (1973) indicated that although the free methionine concentrations were low when the diets were deficient in this AA, they increased with their dietary level, more quickly in muscle than in blood. In our experiments diets S and SM showed significant differences (Table 4) in concentrations of free methionine in plasma ($P < 0{\cdot}001$) and muscle ($P < 0{\cdot}05$), although differences between experimental treatments (interaction diet × protein level) did not attain statistical significance for muscle ($P < 0{\cdot}05$; Table 5).

In liver, some studies (Jacobs and Crandall, 1972; Edmonds and Baker, 1987) have shown that free lysine concentrations increase on increasing the amount of dietary lysine, although this effect was, however, more marked in muscle than in liver. Similar observations have been made in the present work. Edmonds and Baker (1987) found that excess dietary lysine in pigs increased lysine and methionine in liver and tended to lower free arginine. In our experiments the addition of lysine resulted in a significant ($P < 0{\cdot}001$) decrease of free arginine and phenylalanine and a significant ($P < 0{\cdot}001$) increase of tryptophan. On the other hand, on increasing the level of dietary methionine the free methionine in liver was reduced. This fall was concomitant with increases in methionine in plasma and muscle, which may indicate that this AA is either rapidly metabolized by liver or readily released into the peripheral blood stream. Also, the supplementation with methionine had a lowering effect on concentration of free glycine in muscle and increased the concentration of this AA in the liver. In the above mentioned experiment of Fernández-Fígares *et al.* (1996), we found that the supplementation with methionine gave rise to significant decreases in the excretion of total nitrogen, uric acid, ammonia and urea and also that the excretion of these catabolites increased with the increase in protein intake. Two of the four N atoms of excreted uric acid arise from amide-N of glutamine and the others from aspartate and glycine, so that excretion of uric acid parallels excretion of glycine. Consequently, the lower excretion of uric acid found for the chickens given diets SM in comparison with those receiving diets S seems to be in agreement with the higher concentrations of free glycine in liver encountered in the present experiments. We have observed also, within each of the protein levels studied, a negative relationship between concentrations of free glycine in liver and muscle, suggesting the existence of an efficient exchange between free glycine pools in plasma and muscle and also an efficient uptake of free glycine by the liver to remove this AA and keep plasma glycine concentrations fairly constant.

Concerning the effect of dietary protein content on free AA concentrations, the available data indicate that the concentrations of most EAAs in plasma increase (Tasaki and Ohno, 1971; Tews and Harper, 1985; Peters and Harper, 1985; Soemitro *et al.*, 1989; Jansen *et al.*, 1986 and 1991), mostly those of branched-chain AAs (Tews and Harper, 1985; Peters and Harper, 1985; Soemitro *et al.*, 1989), proportionally to the dietary protein level, whereas the non-essential AA concentrations decrease (Peters and Harper, 1985) or remain unaffected (Soemitro *et al.*, 1989; Jansen *et al.*, 1986 and 1991). Tasaki and Ohno (1971), in experiments with 5-month-old chickens, and Soemitro *et al.* (1989), in adult rats, found different response curves of the plasma AA concentrations to the dietary protein level, which suggests that these trends are a reflexion of the metabolic activities of the individual AAs and also that plasma AA concentrations are accurately regulated, for example by antagonisms between AAs or the deamination rate of AAs. In this respect, it has been shown (Tasaki and Ohno, 1971; Jansen *et al.*, 1986 and 1991) that the plasma concentrations of lysine and threonine, which are relatively resistant to deamination, are frequently higher than those of the rest of AAs. Our results agree with these observations, particularly in case of lysine. Body pools of most AAs increase in animals immediately after they have consumed a meal, and the increases are proportional to the protein content of the diet (Dixon and Harper, 1984). Since most AA-degrading enzymes have high K_m values, rates of oxidation of amino acids increase in response to increased substrate concentration until body pools are restored to the previous state; the time required for this depends upon the size of the meal and its protein content (Soemitro *et al.*, 1989). If protein intake is in excess of the animal's needs, the time

required for restoration of body pools to normal is greatly prolonged and there is a transitory depression in food intake in direct proportion to the degree of AA surplus, and most of AA-degrading enzymes are induced. This reduction is associated with elevated concentrations of EAA in plasma. Then as the animal adapts to a high protein diet, the capacity of the liver to degrade most AA increases and the concentrations of AAs other than the branched-chain AAs in the peripheral blood do not exceed those of animals consuming less protein (Peters and Harper, 1985). Conversely, if protein intake does not match requirements, most AA-degrading enzymes fall to low activities, which should contribute to conservation of AAs for protein synthesis and maintenance of body free AA pools (Krebs, 1972; Harper, 1974). In our studies we have found, as a general trend, that when the low-protein diets (60 and 120 g crude protein per kg DM) were given, plasma concentrations of most EAAs increased with the highest protein intake and that the same pattern was noticed when the adequate-protein (180 g crude protein per kg DM) and the high-protein (240 g crude protein per kg DM) diets were given. In comparison with values observed in muscle, plasma fluctuations of some EAAs (lysine, arginine, threonine) were rather moderate. These findings and the observations described above on relationships found between plasma AA concentrations and AA profile of the protein ingested suggest that the measurement of plasma AA concentrations does not offer a practical tool for the assessment of the nutritional value of dietary protein.

In muscle, the general trend reported, with few exceptions, is an increase in the concentrations of EAAs (Kunachowicz *et al.*, 1983; Tews and Harper, 1985; Jansen *et al.*, 1986; Soemitro *et al.*, 1989), mostly in those of branched-chain AAs (Tews and Harper, 1985; Soemitro *et al.*, 1989), with increasing dietary protein level, whereas the concentration of non-essential AAs remains practically unaltered (Tews and Harper, 1985; Jansen *et al.*, 1986; Soemitro *et al.*, 1989). In the present work we have found different response curves for individual AAs. In particular, there was an increase in histidine, valine and threonine with increases in dietary protein level. The increase in the concentration of threonine agrees with previous results obtained in rats (Pion, 1973; Kunachowicz *et al.*, 1983) and is concomitant with the high concentrations of these AAs found in plasma.

Earlier observations (Tews and Harper, 1985; Jansen *et al.*, 1986; Soemitro *et al.*, 1989) indicated that free EAA concentrations in liver, particularly branched-chain AAs, increased on increasing dietary protein intake, while changes in the concentrations of non-essential AAs were inconsistent. In our experiments we have found an increase in the concentrations of free isoleucine, glycine and threonine proportional to the level of dietary protein.

It is now well documented that protein metabolism is regulated, and profoundly influenced, by changes in the supply of free AAs to the various tissues and organs. It has been postulated (Munro, 1970) that while there is often a close association between changes in the free AA composition in plasma and muscle, the liver may behave differently since dietary AAs first cross the liver, which can be buffered against AA depletion. On the other hand, the liver proteins undergo large changes in turn-over rate (although balanced by dietary uptake) and the endogenous AAs thus released provide a major part of the liver AA pool. In general, with minor exceptions, there was no clear direct relationship between the AA content of the protein ingested and the free AA concentrations in plasma, muscle and liver. This might stem from the fact that the concentrations of free AAs in tissues account for only a minor fraction of the AA intake. In this respect, it has been calculated from values obtained with diet S at different protein levels that the amount of free AAs in plasma, muscle and liver represented 1, 23 and 8 g/kg of the absorbed AAs. It has been stated that it is difficult to interpret changes in free AA pools without additional information about AA flux (Munro, 1970; Waterlow and Fern, 1981). Moreover, considerable attention should be paid to protein degradation in tissues, as it has been reported (Riis, 1983) that it can represent more than half of free AAs released into the extracellular pool.

In summary, we undertook the present study to test the hypothesis that skeletal muscle and liver would provide more suitable pools than plasma to establish close relationships between free AAs profile in tissues and AA intake. Some significant relationships have been observed. Particularly, the changes in the ratios lysine : phenylalanine, lysine : valine and methionine : glycine in muscle together with that of methionine : glycine in liver seem to be appropriate indexes of the adequacy of the AA profile of the diet. Moreover, the measurement of the changes in tissue concentrations of free methionine is a sensitive test to evaluate this AA in the diet. However, as the present study related to the balance or imbalance effect of supplementing with a single AA (methionine or lysine, the amino acids most likely to be limiting in practice) and a unique protein source was used, extrapolation of data to the range of protein qualities encountered in typical practical diets would need further validation.

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Effects of isolation on the behaviour, live-weight gain, adrenal capacity and immune responses of weaned red deer hind calves

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Abstract

At weaning at 3 months of age (week 1), 30 red deer hind calves were housed in six groups of five animals at a stocking density of 1·5 m^2 per head and maintained in these groups for 4 weeks. At the start of week 5, all calves were immunized with ovalbumin (OVA). Fifteen calves, from three groups, selected at random, were transferred to individual pens which restricted visual and tactile contact with others (ISO) while the remaining animals were kept in their groups (GP). The behaviour, food intake, live-weight gain, antibody and lymphocyte responses in vitro *to OVA and lymphocyte responses* in vitro *to the non-specific mitogen, concanavalin A (ConA), of all calves were assessed in each of weeks 5 to 9. Isolated calves had a lower mean live-weight gain than GP calves ($P < 0·001$), although there were no differences in food intake. Significantly more time was spent lying ($P < 0·001$) but less time feeding ($P < 0·05$) and self-grooming ($P < 0·001$) by ISO than by GP calves. There was no significant difference between ISO and GP calves in the cortisol response to an ACTH challenge test (10 i.u.) at week 11. Lymphocyte responses and antibody titres to OVA were lower in GP than in ISO calves at weeks 7 ($P < 0·05$) and 8 ($P < 0·05$), respectively. In contrast, GP calves had greater lymphocyte responses to the non-specific mitogen, ConA, in weeks 7 ($P < 0·05$) and 10 ($P < 0·001$) but not in week 9 compared with ISO calves. Differences in lymphocyte stimulation were attributed to the non-specific mitogenic nature of ConA. Factors such as agonistic interactions evident in group housing may have compromised the antibody and lymphocyte responses to OVA by GP calves but conversely the lack of social contact may have also suppressed behavioural activity in ISO calves.*

Keywords: *behaviour, immune response, isolation, red deer, stress.*

Introduction

In Britain, farmed red deer (*Cervus elaphus*) are normally maintained in groups at pasture throughout the year, except calves post weaning, which are housed over-winter because they have a lower proportion of body fat than adults and so are less able to cope with adverse weather conditions (Blaxter *et al.*, 1988). Fighting amongst housed calves is a common problem, often countered by removing bullies or bullied individuals and housing them in isolation.

Red deer are highly gregarious animals (Clutton-Brock *et al.*, 1982). The short-term social isolation of farmed red deer has been shown to increase heart rate and elicit escape behaviour (Pollard *et al.*, 1993). Similar studies on the short or repeated short-term isolation (<1 h to 24 h) of traditional livestock have reported increases in fearfulness (Veissier *et al.*, 1994) and motivation to move and interact (Bowers *et al.*, 1993), greater adrenocortical activity (Parrott, 1990) and increases in leucocyte numbers (Cockram *et al.*, 1994) compared with group-housed conspecifics.

The aims of this study were to assess the effects of long-term social isolation on the behaviour, productivity, adrenal activation and immunity of farmed red deer calves and to investigate further the value of immune responses as potential indices of chronic stress. This was achieved by comparing the antibody and cell-mediated immune responses, behaviour, cortisol response to an

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adrenocorticotrophic hormone (ACTH) challenge test, food intake and live-weight gain of weaned red deer calves housed in isolation or in groups for 5 weeks.

Material and methods

Animals and treatments

In early September, 30 red deer hind calves were abruptly weaned at approximately 3 months of age and immediately transferred from pasture to indoor pens. Calves were allocated by live weight to one of six pre-treatment groups each containing five calves (at a stocking density of approximately 1·5 m^2 per head) and maintained in these groups for 4 weeks, to allow adaptation to weaning (Hanlon *et al.*, 1994) and new groupings. At the start of week 5, three groups of calves selected at random, were transferred into fifteen single pens each of 1·5 m^2 with solid walls 1·5 m high (isolated treatment, ISO), which restricted visual and tactile contact with neighbouring animals. Isolated calves and the remaining group-housed calves (group treatment, GP) were maintained in their respective pens for a further 6 weeks i.e. weeks 5 to 10.

All calves were offered *ad libitum* a complete diet, 'Complete Deer Mix' (North Eastern Farmers Ltd, Aberdeen) at approximately 11.00 h each day, throughout the study. Refusals were weighed daily from weeks 5 to 10.

Procedures and measurements

Food intake and live-weight gain. Mean food intake for individual ISO and groups of GP calves was estimated from the difference between food offered and refusals. Live weights of calves were recorded on day 1 in each of weeks 4 to 10.

Behaviour. The activities of all calves were recorded every 5 min during a 2·5 h observation period (09.00 to 11.30 h) on days 6 and 7 of weeks 5 to 10. The classes of activities recorded were standing, lying, feeding (feeding on concentrate or sawdust bedding), self-grooming, pen-directed activities (licking walls, water pipes and troughs), exploring the surrounding environment (standing on hind legs or with forelegs in the food trough to look outside the pen) and other activities (moving, drinking and 'star-gazing' defined as neck outstretched with head and neck turned in a stiffened circular motion).

ACTH stimulation test. An ACTH stimulation test was conducted between 09.00 and 13.30 h on day 2 in week 11. All calves were herded into a handling area behind a drop-floor crate. Individuals were manoeuvred by a stockperson up a ramp into the crate, where the floor was released, securing the animal for blood sample collection. Single 10 ml blood samples were collected from each calf by jugular venipuncture into an evacuated tube containing 10 i.u. heparin per ml. Fifteen calves were randomly selected from ISO (no. = 7) and GP (no. = 8) groups and injected (i.v.) with 2 mg dexamethasone (DXM) sodium phosphate (Dexadreson, Intervet UK Ltd), to suppress endogenous ACTH secretion and then returned to their pens. After 3 h a second 10-ml blood sample was collected from all calves and followed by an injection (i.v.) of 10 i.u. synthetic ACTH (Synacthen, CIBA, Horsham, UK). A final 10 ml blood sample was collected from all calves 1 h later.

The plasma was assayed for total cortisol concentration using a radioimmunoassay kit (Coat-a Count Cortisol, Euro/DPC Ltd, Caernarfon, UK). The assay sensitivity was 2·68 nmol/l and the intra- and inter-assay coefficients were 0·11 and 0·18, respectively.

Immune responses to ovalbumin. On day 1 of week 5, prior to treatment allocation, all calves were immunized with a 1-ml subcutaneous injection containing 5 mg aqueous ovalbumin (OVA) (Sigma Chemical Co. Ltd, Poole, UK) emulsified in an equal volume of incomplete Freund's adjuvant (Difco Laboratories, East Molesey, UK). Single 10-ml blood samples were collected at the time of immunization and then on day 1 of weeks 6 to 10. The primary antibody and lymphocyte responses to OVA were assessed from blood samples using an enzyme-linked immunosorbent assay (ELISA) and *in vitro* lymphocyte stimulation assays (LSA), respectively, as described by Hanlon *et al.* (1994). In addition, lymphocyte responses to stimulation with the non-specific mitogen concanavalinA (ConA) were measured.

Statistical methods

Repeated measures analysis of variance was used to compare the effects of treatment on antibody response to OVA and the proportion of time spent feeding, ingesting sawdust bedding, and grooming. Residual maximum likelihood (REML) was used to analyse live-weight gain, food intake, cortisol response to ACTH, lymphocyte responses to OVA and ConA and the proportion of time spent lying, standing, exploring and in 'other' activities, due to non-orthogonality caused by missing values or variance instability.

A logarithmic transformation (log (1 + value)) was used to normalize skewness in the distribution of antibody and lymphocyte response to OVA, the proportion of time spent ingesting sawdust bedding, grooming, exploring, pen-directed and 'other' activities and food intake. A square root

Table 1 *Mean percentage of time spent in different activities by isolated (ISO) and group-housed† (GP) weaned red deer calves*

	Isolated	Group	s.e.
Lying	60·29	42·61***	5·41
Standing	26·47	26·60	5·983
Feeding	4·85	11·18***	0·847
Licking walls	3·01	4·38*	0·96
Grooming	1·33	2·78***	0·30
Exploring	1·92	2·12	1·26
Eating bedding	1·55	2·39*	0·44
Other	1·56	2·55*	0·51

† Social interactions such as fighting and allogrooming have been excluded because they only applied to GP and account for approximately 5% of the total activity in GP calves.

transformation was used to correct for the variance instability of lymphocyte response to ConA.

Untransformed means and standard error terms have been cited in the text and figures. All data were analysed using GENSTAT 5 release 3 (Lawes Agricultural Trust, 1993).

Results

Live-weight gain and food intake

Isolated calves had a lower rate of live-weight gain (138 *v.* 202 (s.e. 14·8) g/day; $P < 0·001$) than GP calves, but there were no significant treatment effects on food intake (1676 *v.* 1715 (s.e. 178·3) g per head per day; $P > 0·05$).

Activity

Significantly less time was spent feeding ($P < 0·001$), ingesting sawdust bedding ($P < 0·05$) and grooming ($P < 0·001$) by ISO than by GP calves and ISO calves spent more time lying than GP calves ($P < 0·001$; Table 1). The incidence of pen-directed activities such as licking walls and 'other' activities were also lower in ISO than in GP calves ($P = 0·05$; Table 1). Treatment had no significant effect on the proportion of time spent standing or exploring, although both ISO and GP calves spent increasingly more time exploring as the study progressed ($P < 0·001$).

ACTH stimulation test

Isolated calves had significantly lower mean plasma cortisol concentrations before DXM administration than GP calves ($P < 0·001$; Table 2). DXM injection resulted in a reduction in plasma cortisol concentrations in both ISO and GP calves compared with no DXM calves ($P < 0·001$; Table 2). Neither treatment (ISO *v.* GP) nor DXM administration had any significant effect on mean increase in plasma cortisol concentration following ACTH injection (Table 2) .

Immune responses

Antibody responses. Antibody response to OVA was consistently higher in ISO than in GP calves and significantly so (12·1 *v.* 8·10 (s.e. 1·02) %; $P < 0·05$) at week 8 (Figure 1). Treatment had no effect on the time taken to initiate an antibody response to OVA (7 to 14 days post immunization) (Figure 1).

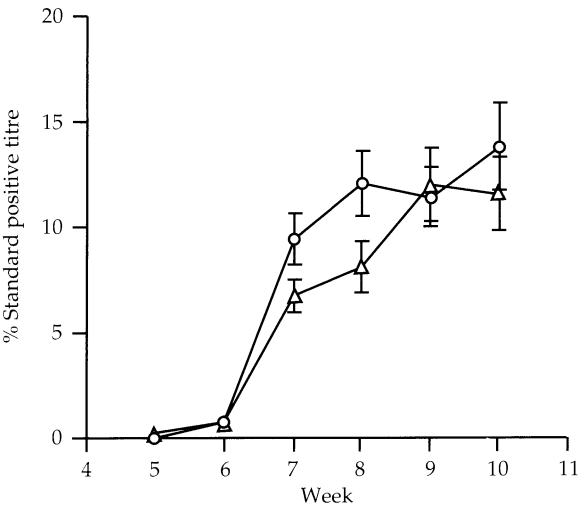

Figure 1 Mean antibody titre (% standard positive titre) to ovalbumin by isolated (—○—) and group-housed (— —) farmed red deer calves.

Table 2 *Effects of 2 mg dexamethasone (DXM) administration on the mean total plasma cortisol concentrations (nmol/l) in isolated (ISO) and group-housed (GP) weaned red deer calves before and after an i.v. injection of 10 i.u. synthetic ACTH*

	Isolated		Group-housed		Pooled	Significance of effect	
	DXM	No DXM	DXM	No DXM	s.e.	Housing	Dexamethasone
Basal	24·14	27·94	47·89	58·67	22·52	***	
Before ACTH	2·69	12·60	4·06	29·28	9·27	*	***
After ACTH	133·1	120·7	125·9	129·9	35·25		
Increase	129·5	107·9	121·0	90·92	38·77		

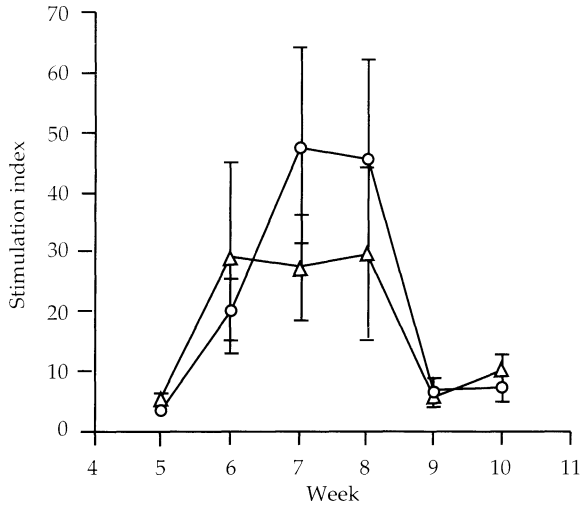

Figure 2 Lymphocyte response (stimulation index) to ovalbumin by isolated (—○—) and group-housed (— —) farmed red deer calves.

Lymphocyte responses. At week 7, ISO calves had a significantly greater lymphocyte response to OVA than GP calves (47·9 *v.* 27·4 (s.e. 9·36); $P < 0·05$) (Figure 2). In contrast, lymphocyte response to ConA was significantly lower in ISO than GP calves at week 7 (255 *v.* 347 (s.e. 29·7); $P < 0·05$) and week 10 (86·2 *v.* 170 (s.e. 21·0); $P < 0·001$), although ISO calves had a higher lymphocyte response to ConA than GP calves at week 9 (155 *v.* 62 (s.e. 25·2); $P < 0·01$) (Figure 3).

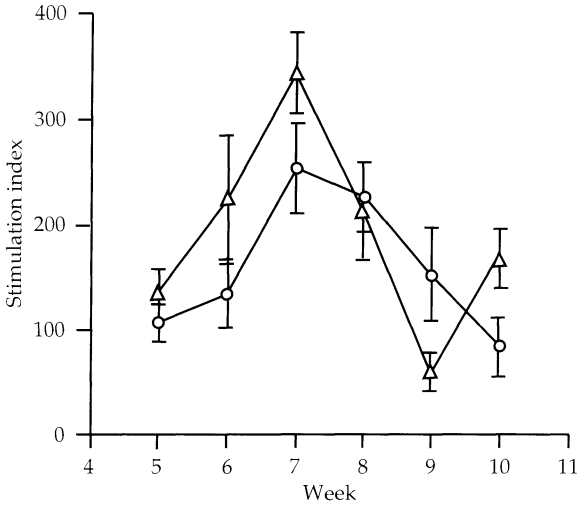

Figure 3 Lymphocyte response (stimulation index) to concanavalin A by isolated (—○—) and group-housed (— —) farmed red deer calves.

Discussion

Livestock such as cattle and pigs are sometimes maintained in social isolation to increase their live-weight gain and reproductive success (Gonyou *et al.*, 1992; McGlone *et al.*, 1994). In this study, GP calves had a greater live-weight gain than ISO calves, and although reproductive success was not measured in this experiment, lowered live-weight gain has been shown to delay the onset of puberty and reduce reproductive rate in farmed red deer hinds (Blaxter *et al.*, 1988). Isolated calves spent less time feeding during the observation periods than GP calves, although there were no differences in food intake. Calves were given food mid-way through the behavioural observations and less time spent feeding in ISO calves may reflect the lack of social facilitation to feed as observed by Hansen *et al.* (1982) in pigs or lack of competition for feeding space. Both treatment groups were observed ingesting sawdust bedding during the study and, although GP calves spent more time eating sawdust, its nutritional content is negligible and so it is unlikely to have contributed to the differences in live-weight gain.

Farm animals are sometimes isolated to prevent bullying or during clinical illness, but isolation may reduce behavioural activity caused by the lack of exteroceptive stimulation or the inability to express normal behaviour. In general, behavioural diversity decreases with increasing confinement (Dantzer, 1986), partly due to the physical constraints of the environment restricting, for example, movement and social behaviour (Bowers *et al.*, 1993; Veissier *et al.*, 1994). This is reflected by the greater proportion of time ISO calves spent lying inactive compared with GP calves.

Throughout the study individual GP calves were observed to stand with their forelegs in the food trough, peering into adjacent pens (the pen walls were 1·5 m high) and, as the experiment progressed, similar behaviour was observed in ISO calves. Isolated animals are highly motivated to interact with conspecifics (Price and Thos, 1980; Bowers *et al.*, 1993; Veissier *et al.*, 1994). No physical contact was observed between ISO individuals, although the repeated occurrence of this behaviour may suggest a need to interact with or be within sight of neighbouring animals. The latter suggestion is supported by the relatively small proportion of time (approx. 0·05 of their total activity budget) GP calves spent in social activities such as allogrooming.

GP calves had higher basal and 'before-ACTH' plasma cortisol concentrations than ISO calves, although these differences disappeared following ACTH administration. The elevated basal cortisol concentration and subsequent decrease before ACTH

administration in GP calves, may be indicative of an acute response to handling (Hanlon *et al.*, 1995). Purcell and Arave (1991) showed that heifer calves reared in groups were more fearful, when handled in isolation, than their twins reared in individual pens, because grouped individuals had less direct physical contact with the stockpersons than the isolated calves. The potential stressor associated with handling and restraint may therefore have been perceived as greater by individual GP calves than ISO calves, due to their relative inexperience or lack of direct contact with stockpersons (Hanlon *et al.*, 1995).

Adrenal activation produces differential effects on the immune responses, depending on the degree, timing and duration of glucocorticoid release relative to an immune challenge (Griffin, 1989). T lymphocytes involved in the cellular responses are considered to be more sensitive to cortisol than B lymphocytes, which mediate antibody responses (Siegel, 1987; Coppinger *et al.*, 1991). There were no apparent treatment differences in adrenal activity between ISO and GP calves, although cortisol concentration in plasma was measured on only one occasion. Group-housed calves had significantly lower lymphocyte and antibody responses to OVA than ISO calves, 2 to 3 weeks after treatment allocation, which may have been induced by social stressors such as agonistic interactions associated with living in groups. In a previous study (Hanlon *et al.*, 1995) social stress, caused by repeated hierarchical challenges was shown to impair lymphocyte responses in juvenile red deer. In contrast, in the present experiment, GP calves had greater lymphocyte responses to ConA than ISO calves on two of the occasions that it was measured. Concanavalin A is a non-specific mitogen, which produces a blanket stimulation of blastogenesis in up to 90% of all circulating T lymphocytes (Gershwin *et al.*, 1995). It is used as an index to assess the general responsiveness of T lymphocytes and does not reflect the level of immunocompetence. In contrast, stimulation by an antigen such as OVA is a more specific response and selects both T and B lymphocytes (less than 0·1% of the lymphocyte population) which have been primed following immunization with OVA (Gershwin *et al.*, 1995). Lymphocyte stimulation to OVA is therefore a more accurate indicator of immunological competence. On the basis of the responses to OVA, it can be concluded that the GP calves had a lower immune status than ISO calves at certain times during the experiment.

Isolated calves had greater antibody and lymphocyte responses to OVA than GP calves mid-way through and at the end of the experiment. This may reflect the impact of social stressors, such as fighting, on the immunocompetence of GP calves (Hanlon *et al.*, 1995). In contrast, some of the behaviour data, in particular the higher level of inactivity and repeated attempts to see their neighbours by ISO calves, may be indicative of the lack of exteroceptive stimulation in single pens associated with a sub-optimal environment. These apparently contradictory observations, drawn from the study, suggest that it will not be possible to draw clear conclusions about the welfare implications of social isolation in farmed deer without further research to determine the value of the respective criteria as indices of welfare. This research would include investigation of group structures and the mechanisms through which stress may affect the immune system.

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