Studies of protein requirements of ruminants

3.* Nitrogen balance trials on Blackhead Persian sheep given diets of different energy and protein content

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Elliott & Topps (1963*a*) have shown that only one-third of the intake of digestible crude protein advocated by Brody (1945) is necessary to keep steers of two breeds of African cattle in nitrogen equilibrium. In feeding trials of 15 weeks' duration (Elliott & Topps, 1963*b*), these same animals gained approximately ½ lb/head daily when given a diet adequate in energy but containing only 60% of the quantity of protein normally prescribed for maintenance.

The digestive abilities of cattle and sheep are similar and their nutritional requirements are often assumed to be similar when stated on the basis of metabolic size (body-weight$^{0.73}$). Since wide differences have been noted between the requirements of African cattle and accepted standards for maintenance, the same may apply to African sheep. The observations of Topps (1963) would be consistent with this concept. The object of the trials now reported was to assess by means of balance trials the protein requirement for maintenance of Blackhead Persian sheep and to study their utilization of N at various levels of dietary protein. The diets used contained differing proportions of ground roughage to concentrate so that the effect of these proportions on protein use could be ascertained.

EXPERIMENTAL

Sixteen different diets were offered to wether sheep in N balance trials. These diets were designed to give four protein levels each with four levels of energy. It was expected that different energy levels would be obtained by varying the proportions of roughage in the form of ground lucerne and maize cobs to concentrate in the diets. However, the diets supplied very similar amounts of total digestible nutrient (see Table 2). The detailed composition of the diets is given in Table 1. Roughage and concentrate portions were finely milled and thoroughly mixed to preclude selection of the more palatable components.

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Sixteen Blackhead Persian wethers were divided into four groups which were allocated at random to each of the four energy levels (hereafter called ratios). Within each of the ratio groups, a four-unit latin square design was used to examine the four different protein diets in four test periods.

Table 1. *Crude protein and constituents of the diets of different protein and energy content* (%)

Ratio, ground roughage: concentrate	Crude protein content	Constituent				
		Cottonseed cake	Maize	Cassava	Lucerne	Ground maize cobs
1:1	4	1·9	1·4	46·7	6·6	43·4
	9	11·5	4·4	34·1	7·7	42·3
	14	21·1	7·3	21·6	8·8	41·2
	19	30·7	10·2	9·1	9·9	40·1
2:1	4	1·3	1·3	30·7	5·8	60·9
	9	7·7	3·2	22·4	21·3	45·4
	14	14·0	5·1	14·2	36·6	30·1
	19	20·5	7·1	5·7	51·8	14·9
3:1	4	1·0	1·2	22·8	5·5	69·5
	9	5·8	2·6	16·6	28·1	46·9
	14	10·6	4·1	10·3	50·4	24·6
	19	15·4	5·5	4·1	73·1	1·9
4:1	4	0·8	1·1	18·1	5·5	74·5
	9	4·6	2·3	13·1	32·2	47·8
	14	8·4	3·4	8·2	59·0	21·0
	19	13·8	5·0	1·2	78·9	1·1

Each N balance trial consisted of a 7-day preliminary period followed by a 7-day collection period during which the sheep were harnessed for collection of faeces and were housed in metabolism crates. Quantitative measurements were made of food eaten and urinary and faecal losses. The sheep were weighed at the beginning of each preliminary period and immediately before and after each collection period. The animals were each offered a total of 900 g air-dry food daily in equal portions at 7 am and 4 pm. Urine was collected in sufficient sulphuric acid to maintain the liquid at a pH of 2·0–2·5. After every feeding, the volumes of urine were measured, and faeces were weighed. One-tenth of the urine from each sheep was stored at 0°. Similarly two one-tenth subsamples of faeces were taken; one was stored at −15° and the other dried to constant weight at 105°. Samples taken over the whole collection period were pooled for each sheep.

The N content of all samples (food, frozen faeces and urine) was determined by a macro-Kjeldhal method. Ether extract, ash and crude fibre in food and dried faeces were determined by the methods of the Association of Official Agricultural Chemists (1955).

RESULTS

Intake and digestibility of food. Mean intakes of the diets are shown in Table 2. Consumption of the more acceptable diets was often complete, and mean intakes were probably somewhat less than the voluntary intake of the sheep would have been on *ad lib.* feeding.

The digestibility of all the 4% crude protein (CP) diets was significantly lower than that of the higher-protein diets. Daily amounts of dry matter and total digestible nutrient (TDN) eaten declined with CP content and digestibility of the diet. Although the groups of diets varied widely in their content of ground roughage and concentrate, mean digestibilities and intakes of dry matter and TDN by the sheep did not differ significantly between groups.

Table 2. *Mean values with standard deviations for contents of total digestible nutrient (TDN) of sixteen diets, and for intakes of dry matter and TDN in diets by four sheep*

Ratio, ground roughage: concentrate in diet	Crude protein content of diet (%)	TDN content of diet (%)	Dry-matter* intake (g/24 h)	TDN intake (g/kg $W^{0.73}$)
1:1	4	50·6	799 ± 81	32·9
	9	65·8	825 ± 8	44·6
	14	68·5	817 ± 14	47·5
	19	67·5	829 ± 9	44·5
2:1	4	43·7	667 ± 174	24·2
	9	64·2	801 ± 42	43·4
	14	65·1	801 ± 44	43·3
	19	65·3	823 ± 4	45·2
3:1	4	54·2	633 ± 130	29·4
	9	62·2	718 ± 142	36·2
	14	64·3	798 ± 51	42·6
	19	63·7	802 ± 31	47·9
4:1	4	51·9	678 ± 85	29·5
	9	61·3	804 ± 51	41·7
	14	59·6	799 ± 45	40·9
	19	62·7	794 ± 51	43·0
Pooled SD	—	± 4·1	—	± 5·5

* Each sheep was offered 900 g air-dry diet daily. The low variability of dry-matter intake with many of the diets was due to complete, or nearly complete, consumption of the food offered.

The main object of these trials was to determine the maintenance needs of sheep for digestible protein. Adequate amounts of digestible energy were thus necessary to ensure that protein would not be wastefully used as a source of energy. According to Blaxter (1962), the daily fasting energy katabolism of wethers is 58 kcal/kg $W^{0.73}$, where W = body-weight. This value multiplied by 1·35 gives the metabolizable energy required for maintenance. On the assumption that 1 g TDN in these diets supplied 3·56 kcal metabolizable energy, the maintenance requirements of the sheep in these trials were approximately 22 g TDN/kg $W^{0.73}$. If these criteria apply to Blackhead Persian sheep, all the diets, on average, provided more than adequate digestible energy for maintenance. In only one of the sixty-four balance trials, when a 4% CP diet was given, did TDN intake fall below the calculated maintenance need.

Apparent digestibility of N. Highly significant ($P < 0·01$) linear correlations were obtained between the percentage total N in each of the four ratio groups of diets and the percentage of apparently digestible N. Covariance analysis of these regressions showed no statistically significant differences amongst their regression coefficients and intercept values. The pooled values ($n = 64$) showed a high correlation ($r = 0.995$) between the variates, and the regression equation expressing the relationship was $Y = 0.933X - 0.545$, where X is the percentage of N and Y the percentage of apparently digestible N in the dry food. The N in all diets was apparently equally well digested and the intercept value (0.545 ± 0.011 g N/100 g dry food) provides a common estimate of metabolic faecal N excretion.

Urinary N loss and N balance. Analyses of variance were carried out on each of the following measurements and derived variates: live weights of sheep, daily amounts of total N and digested N eaten, daily N loss in urine and daily N balance. Significant differences were found between the mean weights of sheep in the various ratio groups of diets, and it was necessary to correct the values for both sheep and period effects. The corrected results, mean values for which are given in Table 3, have been used in the regression models quoted below.

Table 3. *Mean values for intake, amount digested, urinary loss, and retention of nitrogen for groups of four sheep given sixteen different diets*

Ratio, ground roughage: concentrate in diet	Crude protein content of the diet (%)	Nitrogen metabolism (g/24 h)			
		Intake	Digested	Urinary loss	Retention
1:1	4	5·02	0·80	1·30	−0·50
	9	11·73	5·86	4·10	1·76
	14	19·96	13·79	10·37	3·42
	19	24·45	18·06	13·93	4·13
2:1	4	3·81	−0·18	1·49	−1·67
	9	10·09	6·97	3·91	3·06
	14	19·67	14·18	10·21	3·97
	19	25·10	19·15	15·38	3·77
3:1	4	3·58	0·02	1·36	−1·34
	9	11·44	7·01	4·52	2·49
	14	19·30	14·16	9·71	4·45
	19	25·95	19·95	14·55	5·40
4:1	4	5·07	1·19	1·89	−0·70
	9	13·33	8·09	5·73	2·36
	14	20·65	14·71	11·65	3·06
	19	26·29	20·31	15·95	4·36

Table 4. *Relationship between urinary nitrogen (Y) and N intake of sheep (X) (g/24 h)*

Ratio, ground roughage: concentrate in diet	Quadratic model of regression	Variability accounted for by regression (%)	Standard error of coefficient		Confidence limits on intercept†
			b_1	b_2	
1:1	$Y = -0.552 + 0.272X + 0.013X^2$	98·4	0·178	0·006	±2·59
2:1	$Y = 1.199 + 0.064X + 0.020X^2$	95·8*	0·236	0·008	±3·23
3:1	$Y = 0.241 + 9.251X + 0.012X^2$	99·1*	0·099	0·003	±1·44
4:1	$Y = 0.181 + 0.323X + 0.011X^2$	99·1*	0·121	0·004	±1·90
All ratios	$Y = 0.305 + 0.211X + 0.014X^2$	97·6*	0·079	0·003	±1·00

* An asterisk shows that the quadratic term explains a significant portion of the variability remaining after the linear model had been fitted. In all instances the fit of the linear model was highly significant.

† The confidence limits on the intercepts (a_1) are for $P = 0.95$.

Daily urinary N losses by the sheep were found to be highly correlated with the amounts of N consumed. Moreover, a quadratic model significantly reduced deviations from the linear form for three of the four ratio groups (Table 4). Regression coefficients and intercepts did not differ amongst themselves when a quadratic model was used for all ratio diets. Further, the intercept value (a_1 = 0·305) on these pooled values did not differ from zero when no N was consumed. The 95 % confidence limits about the intercept (approximately ± 1 g N) indicate that the level of endogenous N excretion in these trials was significantly lower than the value deduced by Brody (1945). For sheep of the same weight (30·7 kg) the endogenous N katabolism calculated from Brody's equation, endogenous N (g/24 h) = 0·146 $W^{0.73}$, is 1·78 g.

Table 5. *Relationship between nitrogen digested by sheep (Y) and N retention (X) (g/24 h)*

Ratio, ground roughage: concentrate in diet	Quadratic model of regression	Variability accounted for by regression (%)	Standard error of coefficient b_1	b_2	Confidence limits on intercept*
1:1	$Y = 1.737+2.354X+0.329X^2$	94·1	0·923	0·234	±2·30
2:1	$Y = 3.099+2.103X+0.191X^2$	71·1	0·938	0·329	±6·82
3:1	$Y = 2.121+1.948X+0.194X^2$	91·5	0·711	0·160	±3·14
4:1	$Y = 2.424+2.128X+0.433X^2$	93·5	0·871	0·234	±2·63
All ratios	$Y = 2.843+2.407X+0.158X^2$	83·2	0·411	0·104	±1·55

* Confidence limits on the intercept (a_1) are for P = 0·95.

Table 6. *Relationship between nitrogen digested by sheep (Y) and urinary N (X) (g/24 h)*

Ratio, ground roughage: concentrate in diet	Quadratic model of regression	Variability accounted for by regression (%)	Standard error of coefficient b_1	b_2	Digestible N required for N equilibrium†
1:1	$Y = 1.167+1.731X-0.036X^2$	99·3	0·201	0·013	1·70±0·80
2:1	$Y = 2.356+2.264X-0.057X^2$	96·6*	0·428	0·035	2·05±1·85
3:1	$Y = 2.444+2.197X-0.046X^2$	98·8*	0·253	0·015	2·23±1·10
4:1	$Y = 1.500+1.665X-0.019X^2$	99·0	0·213	0·012	2·43±1·08
All ratios	$Y = 1.860+1.966X-0.037X^2$	97·8*	0·142	0·008	2·10±0·55

* An asterisk indicates that the quadratic term accounts for a significant amount of variability after the linear model has been fitted at a probability of P = 0·06.

† Confidence limits are calculated at P = 0·95 for digestible N required for N equilibrium. This is the point of intercept of the regression with the straight line $Y = X$.

The amount of digestible N required for N equilibrium is usually considered to be synonymous with the maintenance requirement for digestible N, and was estimated from the experimental values in two ways, the more obvious being from the regression equations of digestible N on N retention which are given in Table 5. The estimate of digestible N for zero N retention, from all the values, is 2·84 ± 1·55 g daily, which is only 40% of the figure of 7·12 g advocated by Brody (1945) as necessary for sheep of similar live weight. The choice of N retention as the independent variable in the regression is a bad one, because it is subject to considerable experimental error which leads to a high value (±1·55 g) for the standard error of the estimate.

The second method of estimating the maintenance requirement for digestible N was to calculate regression equations of digestible N intake on urinary N and from these to estimate the point at which digestible N intake was equal to urinary N. The results are given in Table 6 and yield much more precise estimates than the first method. The pooled estimate, from all the values, was 2·10±0·55 g digestible N daily, which is only 29% of Brody's (1945) estimate.

Effect of ratio of roughage to concentrate on digestible N required for N equilibrium. The hypothesis that the ratio of ground roughage to concentrate in the diets is related to the amount of digestible N required for N equilibrium was tested, and the results are given in Table 7.

Owing to the heterogeneity of the variance (column 3 in Table 7) a weighted least squares regression was applied and reciprocals of the variances were used as weights. A highly significant ($P < 0·01$) positive linear trend was found between the digestible N required for N equilibrium and the ratio of ground roughage to concentrate in these diets. The relationship found was $Y_E = 1·467 + 0·247R$, where Y_E is the N digested at N equilibrium and R is the ratio of ground roughage to concentrate in the diets. The best estimate of this increase in digestible N required daily for N equilibrium was 0·25±0·06 g/unit increase in the ratio. The existence of this linear trend over the four ratios implies that only limited emphasis may be placed on the results obtained from the pooled values (Tables 5 and 6).

Table 7. *Effects of relative amounts of ground roughage and concentrate in diets on the digestible nitrogen required for N equilibrium in sheep*

Ratio, ground roughage: concentrate in diet	Estimated digestible N required for N equilibrium (g/24 h)	Variance of estimate	% of Brody (1945) standard
1:1	1·7	0·11	23·8
2:1	2·1	0·61	28·9
3:1	2·2	0·22	31·4
4:1	2·4	0·21	34·1

DISCUSSION

The results of N balance trials with Blackhead Persian wethers appear to be quantitatively similar to those previously reported for African cattle (Elliott & Topps, 1963*a*). The generally accepted standards for digestible N for maintenance appear to be excessive by a factor of 3 when applied to African cattle and sheep given diets adequate in available energy. This high efficiency of N utilization is apparently associated with low endogenous losses and presumably with high biological values of the protein at N equilibrium. It is possible that genetic factors associated with the animals studied are involved, but the present evidence suggests that the efficiency of N utilization is more probably connected with the high content of available energy in the diets. With such diets losses of protein, caused by bacterial deamination, are minimal.

Conversely, animals given diets composed of poor-quality roughage and a protein-rich concentrate are likely to have low N retention and an apparently high protein requirement. The work of Majumdar (1960), in which goats fed on chaffed wheat straw and rapeseed cake were found to have a high N requirement for maintenance, supports this concept.

No account has been taken of N losses in the form of hair and skin abrasions. We are unaware of any published reports of such losses in hair-coated sheep. These sheep continually shed their hair and accurate short-term estimates are very difficult to obtain. Blaxter (1959) uses an estimate of daily loss for cattle, derived from seventeen experiments, of 5.0 ± 0.5 mg N/kg $W^{0.73}$. Marston (1955) provides similar estimates for Merino sheep on maintenance diets. Neither estimate may be applicable to Blackhead Persian sheep, but they indicate a probable loss of about 60 mg N/day for the size of sheep used and it is unlikely that the loss would exceed 100 mg. If this additional loss is taken into account, the maintenance requirement for digestible N increases only slightly.

Blaxter (1962) has shown that the fasting metabolism of adult sheep expressed per kg of metabolic size is considerably less (about 30%) than that of adult cattle. This difference is reflected in the energy requirements for maintenance of the two species. It is possible that maintenance requirements for N may follow the same trend and that when comparable diets are used differences between values for sheep and for cattle may be of the same magnitude. As yet sufficient data are not available to provide strong support for this concept.

The validity of using balance trials to estimate nutrient requirements of animals has been questioned (Duncan, 1958), mainly because these trials are invariably of short duration and errors of sampling and analysis can be appreciable. The results obtained by the balance method should be confirmed by long-term feeding trials and also by carcass analyses. Despite the fact that balance trials may not provide accurate absolute estimates, it is generally agreed that they do provide valid relative measurements of the efficiency of nutrient utilization. That N requirements for maintenance are dependent upon the composition of the diet has been clearly shown in the trials described here. The inclusion of increasing proportions of ground roughage in the diets resulted in substantial increments in digestible N required for N equilibrium. This means that any statement of N requirements for maintenance of ruminants should be qualified by information on the type and amount of other dietary components, and particularly of the energy-yielding components of the diet.

Finally, it should be recognized that the results, from which estimates of N equilibrium were derived, may have been affected by the level of TDN intake. TDN intake was not equal at all levels of dietary protein. As the energy intake was less with the 4% CP diets the estimates of digestible N intake for N equilibrium may have been slightly overestimated.

SUMMARY

1. Nitrogen balance trials were carried out with sixteen Blackhead Persian wether sheep given four levels of protein in diets with four different proportions of ground roughage and concentrate.

2. Digestibilities of the diets, which contained different proportions of ground roughage and concentrate but had the same protein content, were not significantly different. The digestibilities of the diets containing 4% crude protein, and their intake by sheep, were appreciably lower than the corresponding values obtained for the diets higher in protein.

3. The crude protein in all the diets was equally well digested.

4. The level of endogenous N excretion by the sheep, obtained by extrapolation of the regression relating urinary N loss to dietary N consumed, was low (0·305 g/24 h for a 30·7 kg sheep) compared with 1·78 g/24 h derived from the Brody (1945) equation.

5. The level of digestible N required for N equilibrium was also low: estimates varied from 1·70 to 2·43 g/24 h. These estimates were only 24–34% of the Brody (1945) maintenance standard.

6. The level of ground roughage in the diet was shown to affect the digestible N required for maintenance, and unit change in the ratio of ground roughage to concentrate (in the range 1:1 to 4:1) caused a linear increase of 0·25 ± 0·06 g digestible N required daily for maintenance.

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