Nitrogen balance studies with the milk-fed lamb

4.* Effect of different nitrogen and sulphur intakes on live-weight gain and wool growth and on nitrogen and sulphur balances

BY D. M. WALKER AND L. J. COOK

Department of Animal Husbandry, University of Sydney, Australia

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1. Sixteen male cross-bred lambs were given four diets, which differed in the ratio of protein to energy. The protein contents of the diets (on a dry-matter basis) were: 6·1 % (diet A), 11·9 % (diet B), 17·5 % (diet C) and 22·9 % (diet D).

2. The experimental period of 7 weeks was divided into two 2-week periods (periods 1 and 2), and one 3-week period. The diets given to the lambs were changed between period 1 and period 2. In period 3 all the lambs were given the same dietary treatment as in period 2.

3. The daily feed intake of the lambs was regulated according to live weight. Adjustments were made at the beginning of period 1 and of period 2. The level of feeding was 121 kcal/kg live weight 24 h. In period 3 the intake was the same as in period 2.

4. Diet digestibility, live-weight gain, and nitrogen and sulphur balances were calculated for each lamb on each diet in all the periods. Wool growth on sample areas was measured over a 2-week and a 4-week period. These wool growth periods corresponded to treatment period 1, and treatment periods 2 and 3, respectively. At the end of the experiment the lambs were slaughtered and the composition of carcass and organs was determined.

5. The mean digestibilities of energy, N, ether extractives and dry matter increased significantly as the protein content of the diet increased.

6. The live-weight gain increased with increasing protein content of the diets, but only the difference between the means for diet A and all other diets was significant.

7. The N and S balances increased with increasing intake of apparently digested N and S, and all differences between the means for individual diets were highly significant.

8. There was a significant correlation between N balance and live-weight gain for each diet. However, when compared at the same rate of gain, N balance increased as the protein content or the diet increased.

9. Wool growth on the sample areas increased with an increase in the protein content of the diet and all differences between the means for individual diets were highly significant. There were no significant differences between the dietary treatments in their effect on the N and S contents of the wool. The mean values were 15·7 % N and 2·87 % S.

10. The retention of N in the wool grown did not account for the increased N retention on the diets of higher protein content. The lambs given diet A, retained more N and S in wool than was supplied by the diet.

11. The percentage of fat in the carcass decreased, and the percentage of protein increased as the protein content of the diet increased. The percentages of moisture and ash were not significantly affected by the dietary treatments.

12. The percentages of moisture in the liver, pancreas and muscle decreased and the percentages of protein in the liver and muscle increased as the protein content of the diet increased. The ratio of N in the organs (with the exception of the spleen and pancreas) to N in the carcass was highest for lambs given the diet with least protein (diet A).

Nitrogen retention in the milk-fed lamb has been shown to be closely related to N intake within the range of 20·2–33·0 % protein calories (Walker & Faichney, 1964*d*). N balance and live-weight gain were closely related within each dietary treatment, but comparisons between diets showed that the N retention at the same live-weight gain increased as the dietary protein level increased. These results suggested that either

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the composition of the gain varied from diet to diet, or that different amounts of N were retained in the wool grown, or both.

The present paper describes an experiment in which N and sulphur balances and live-weight gain of the milk-fed lamb were measured, when the dietary protein levels were considerably lower than the protein concentration of ewe's milk (26·5%; Ling, Kon & Porter, 1961). The effect of the dietary treatments on wool growth, wool and body composition were also measured.

EXPERIMENTAL

Animals and their management

Sixteen male cross-bred lambs ((Border Leicester ♂ × Merino ♀) × Dorset Horn ♂) were used. The lambs were born at pasture, their birth weights ranged from 2·8 to 5·2 kg and they remained with the ewe until the experimental treatment began at about 1 week after birth. The experimental management, collection and storage of urine and faeces were as described for previous experiments (Walker & Faichney, 1964*a*). The mean daily maximum temperature in the animal house was 21° and the minimum 11°.

Experimental design

Newborn lambs vary considerably in their weight at birth and in their development, as a result of differences in the prenatal nutrition of the ewe (Thomson & Thomson, 1948–9). In the present experiment the effects of differences in the postnatal nutrition of the lamb on live-weight gain and on N and S balances were studied. The diets of the lambs were changed after 2 weeks (period 1) to determine whether their response in the subsequent period (period 2) was affected by the previous dietary treatment. After the initial change in diet at the end of period 1 the dietary treatment remained the same for 5 weeks (periods 2 and 3), so that the prolonged effect of the diet on body composition could be measured.

Four dietary treatments were used. The experimental period of 7 weeks was divided into two 2-week periods (periods 1 and 2), and one 3-week period (period 3). In periods 1 and 2 the diet was given for 1 week before making the separate collection of faeces and urine in the 2nd and 4th weeks. In period 3 collections were made in the final week.

The experimental design is illustrated in Table 1 for four of the sixteen lambs. Three of the lambs which were given diet A in the first period were then given diets B, C or D in the second period. In period 3 the allocation of dietary treatments was the same as in period 2. The other three groups of four lambs were given diet B, C or D in period 1 and, in periods 2 and 3, these lambs were allocated as in Table 1.

The results in the first, second and third periods were analysed separately. Those in the first period were analysed as a completely randomized design; those for the second period were analysed as a randomized complete block, the block effects being the period treatment residual effects. One lamb which was given diet B in period 1 refused to drink when changed to diet A in period 2. A missing value was calculated for it in the second period analysis. Since the block effects were negligible, it was concluded that there were no residual effects from the first period treatments. The results from all periods were then pooled. In this latter analysis the values for a reserve lamb of live weight and age similar to the one which refused to drink were used. This lamb had been fed on a diet containing 28·7% protein calories (dried whole milk) from 1 week of age.

The lambs were weighed daily, 4 h after the morning feed, and live-weight gain was estimated by a regression analysis of the daily weights.

Table 1. *Experimental design for lambs given diet A in period 1 and diets A, B, C or D in periods 2 and 3*

Lamb no. ...	1	2	3	4
Period 1	A	A	A	A
Period 2	A	B	C	D
Period 3	A	B	C	D

Table 2. *Composition of the diets (per 100 g dry matter)*

Constituent	'Milk' to contain 15% total solids			
	Diet A	Diet B	Diet C	Diet D
Dried whole milk (g)	21·1	41·3	60·7	79·5
Glucose (g)	27·1	18·9	10·8	3·0
Butter oil (g)	46·8	36·5	26·8	17·5
Minerals (g)	5·0	3·3	1·7	—
Crude protein* (g)	6·1	11·9	17·5	22·9
Ether extractives (g)	52·7	48·1	43·8	39·8
Ash (g)	6·1	5·5	4·9	4·2
Nitrogen-free extractives (by difference) (g)	35·1	34·5	33·8	33·1
Energy (kcal)	651	637	625	615
Protein calories as % of total calories	5·2	10·4	15·6	20·8

* N × 6·38.

Diets

The composition of the experimental diets is shown in Table 2. The protein source in each diet was spray-dried whole cow's milk (Walker & Faichney, 1964*d*). Glucose and butter oil were added to supply additional energy, and the total intake of 'hexose equivalent' for each lamb did not exceed 6·2 g/kg mean live weight 24 h (Walker & Faichney, 1964*c*). A concentrated mixture which contained butter oil, dried whole milk and water in the proportions (w/w) of 2·2 parts butter oil, 1 part dried whole milk and 10 parts water was homogenized once weekly in a Weir Junior Homogenizer at a pressure of 850 lb/in^2 and at a temperature of 65–70°. The individual diets were prepared with distilled water every 2 or 3 days and stored at 5°. A mineral and vitamin mixture was added to each diet so that the final composition was similar to that of ewe's milk (Walker & Faichney, 1964*a*). All lambs were dosed with 1 ml of a groundnut-oil solution of 100000 i.u. vitamin A acetate and 10000 i.u. ergocalciferol 2 days before the first collection period and again 1 day after the end of the second collection period. Aureomycin soluble (0·45 g; Cyanamid of Great Britain Ltd), which contained chlortetracycline hydrochloride (25 mg), was given daily, dissolved in the milk, to each lamb. A solution which contained $FeSO_4$, $CuSO_4$ and $CoCl_2$ was added to all diets to increase the concentration of these metals in the dry matter by 50 ppm Fe, 5 ppm Cu and 0·1 ppm Co.

The lambs were bottle-fed after the diets had been warmed to about 37° by immersion in a constant-temperature bath. Feeding was three times daily at 07·00, 14.00 and 22.00 h. The lambs were given sufficient milk to provide 121 kcal/kg live-weight 24 h, based upon their live weight at the beginning of period 1 and period 2. This energy intake was sufficient to permit growth if an adequate diet was given, and was within the range of appetite of all lambs. In the third period feed intake was the same as in the second period and was not adjusted for any change in live weight.

The energy values of the diets (cf. Table 2) were calculated from the experimentally determined values of the individual dietary constituents as follows: dried whole cow's milk 5·558 kcal/g, glucose 3·736 kcal/g, butter oil 9·235 kcal/g (all values expressed on a dry-matter basis).

Analytical methods

Dietary constituents, faeces and urine. Total N, fat, ash, dry matter, energy, urea, ammonia, creatinine and total S were determined by the methods of Walker & Faichney (1964*a*, *b*). Total S in the dried whole milk and dried faeces was determined by bomb calorimetry and subsequent precipitation of the S as $BaSO_4$.

Wool. Total N was determined by the Kjeldahl method and total S by the method of Macdonald (1959) as applied to wool by Earland (1961).

Tissues. Dry-matter content was determined by drying to constant weight at 100°, total N by the Kjeldahl method, and liver fat by successive 4 h Soxhlet extractions with ethanol and diethyl ether on the dried liver.

Measurement of wool growth

Growth of wool was measured on sample areas over a 2-week and a 4-week period. These periods corresponded to period 1, and periods 2 and 3, respectively. The 2-week growth period began at the end of the 1st week of the experiment, and the 4-week period at the end of the 3rd week. It was assumed that on the average the growth of wool in any one week reflected the dietary treatment in the previous week.

Two sample areas, approximately 4 × 4 cm, were defined by tattoo lines on the shoulders of each lamb at the beginning of the experiment. The area within the tattoo lines was measured at the end of the first wool collection period and again at the end of the experiment. The wool samples were cleaned by the method of Reis & Schinckel (1961) to remove wool wax and suint, and the oven-dry weights were obtained.

Body composition

At slaughter the lambs were bled and the skin and viscera removed. The carcass (including the head and feet) was frozen, sectioned and minced finely in a Jeffco Cutter Grinder (Jeffress Bros. Ltd, Brisbane), with precautions to avoid loss of body moisture. Dry-matter content was obtained by drying to constant weight at 100°, total N by the Kjeldahl method, ash by incineration at 600°, and fat by Soxhlet extraction with anhydrous diethyl ether for 16 h.

RESULTS

Digestibility of the diets

Mean values, with their standard errors, for the intake of protein and of digestible energy and for the mean live weight of the lambs are shown in Table 3. Although the lambs were allocated to their initial dietary treatments at random, those lambs which were given diet D had a heavier mean live weight than those given diet A. At the end of period 1 after the dietary treatments had been changed, this effect did not disappear. The mean live weights of each group became more widely separated owing to the greater live-weight gain of lambs given the diets of higher protein content. The differences in mean digestible energy intake over the experimental period are also explained by the differences in live-weight gain between dietary treatments.

The mean values, with their standard errors, for the digestibilities of energy, total N, ether extractives, nitrogen-free extractives (NFE) and dry matter are given in Table 4. There was no diarrhoea amongst any of the lambs given the different dietary treatments. The mean digestibilities of energy, N, ether extractives and dry matter were higher at the higher levels of protein intake. The differences were highly significant ($P < 0.01$) between diet A and all other diets, and significant between diets B and D for the digestibility of N and dry matter. The mean digestibilities of NFE were similar for all diets.

Table 3. *Mean values with their standard errors for intakes of protein and energy and mean live weights of the four lambs on each diet in each period*

Period	Diet A	Diet B	Diet C	Diet D	SE of the diet mean
		Digestible energy intake (kcal/kg per week)			
1	760	744	756	738	±16
2	737	739	728	719	±12
3	650	630	599	584	±14
		Protein* intake (g/kg per week)			
1	7.8	14.7	21.9	28.3	±0.3
2	7.7	14.3	21.0	27.5	±0.2
3	6.8	12.1	17.2	22.3	±0.3
		Live weight (kg)			
1	5.6	5.6	6.0	6.3	±0.7
2	5.3	6.5	7.4	7.6	±0.7
3	5.8	7.7	9.0	9.4	±1.0

* N × 6.38.

Nitrogen balance

The mean values for N balance, with their standard errors, are given for each diet in Table 5. An analysis of multiple covariance showed that there were highly significant

Table 4. *Mean values with their standard errors for apparent digestibility coefficients of the dietary components in the four lambs on each diet in each period*

Period	Diet A	Diet B	Diet C	Diet D	SE of the diet mean
			Energy		
1	92·1	95·4	97·4	98·1	±1·4
2	90·3	97·2	97·9	98·7	±1·5
3	90·9	98·1	98·5	99·0	±1·2
			Total N		
1	81·4	91·2	94·2	97·2	±2·0
2	75·7	95·2	95·7	97·8	±2·3
3	76·0	94·9	96·5	97·9	±2·9
			Ether extractives		
1	91·0	95·1	98·0	98·3	±1·8
2	89·3	97·3	98·3	98·8	±1·7
3	90·0	98·5	99·0	99·3	±1·8
			Nitrogen-free extractives		
1	98·9	99·0	98·7	99·2	±0·3
2	97·6	98·3	98·6	98·9	±0·8
3	98·5	98·1	98·5	99·0	±0·3
			Dry matter		
1	92·6	95·5	97·3	98·2	±1·2
2	89·8	96·6	97·4	98·3	±1·3
3	90·2	96·5	97·7	98·4	±1·3
			Digestible protein energy as percentage of digestible energy intake		
1	4·6	10·0	15·2	20·8	±0·1
2	4·4	10·3	15·4	20·8	±0·1
3	4·3	10·2	15·4	20·7	±0·1

($P < 0·01$) differences in N balance, adjusted to equal live weight and digestible energy intake, between all diets. The N balances of the lambs in the first period of the experiment were significantly lower ($P < 0·05$) than those of lambs in periods 2 and 3.

Live-weight gain

The mean values for the live-weight gains of the lambs, with their standard errors, are given in Table 6. The individual values ranged from 16 to 670 for diet A, from 19 to 645 for diet B, from 200 to 1057 for diet C, and from 276 to 905 g/week for diet D. One lamb which was given diet A in the second and third periods became oedematous during the last collection period and had an apparent gain of 670 g during that week. An analysis of multiple covariance showed that there was a highly significant difference ($P < 0·01$) in live-weight gain (transformed to a logarithmic scale to reduce error variance in periods 2 and 3), adjusted to equal live weight and digestible energy intake, between diet A and all the other diets. There were no other significant differences. The live-weight gains of the lambs in period 1 were significantly lower ($P < 0·05$) than those of lambs in periods 2 and 3.

A very highly significant correlation ($P < 0·001$) was obtained between N balance (g N/week) and live-weight gain (g/week). An analysis of covariance showed that

Table 5. *Mean values (g/week) with their standard errors for the nitrogen balances of the four lambs on each diet in each period*

Diet	N intake	Urinary N	Faecal N	N balance
		Period 1		
A	6·9	5·7	1·3	−0·1
B	12·3	6·7	1·0	4·6
C	20·7	8·9	1·2	10·6
D	27·9	11·4	0·8	15·7
			SE of diet mean	±1·1
		Period 2		
A	6·4	4·6	1·5	0·3
B	14·6	6·5	0·7	7·4
C	24·3	9·0	1·1	14·2
D	32·7	11·5	0·8	20·4
			SE of diet mean	±1·6
		Period 3		
A	6·4	4·5	1·4	0·5
B	14·6	6·2	0·7	7·7
C	24·3	9·8	0·9	13·6
D	32·7	13·5	0·7	18·5
			SE of diet mean	±1·5

Table 6. *Mean values (g/week) with their standard errors for the live-weight gain of the four lambs on each diet in each period*

Period	Diet A	Diet B	Diet C	Diet D	SE of the diet mean
1	114	271	281	407	± 69
2	204	492	709	719	±111
3	303	496	512	572	±105

there were highly significant differences between diets ($P < 0·01$) in the N balance corresponding to any given live-weight gain, but not between the slopes of the individual regressions. The equations ($n = 12$), with their correlation coefficients (r), residual standard deviations (RSD), and the RSD expressed as a percentage of the mean of the dependent variable, were:

$$NB_A = 0·0053G_A - 0·70 \quad (1a)$$
$$RSD = \pm 0·4\ (343·7\%)\ (r = +0·80),$$

$$NB_B = 0·0093G_B + 2·45 \quad (1b)$$
$$RSD = \pm 1·1\ (17·6\%)\ (r = +0·86),$$

$$NB_C = 0·0101G_C + 7·77 \quad (1c)$$
$$RSD = \pm 1·4\ (11·2\%)\ (r = +0·85),$$

$$NB_D = 0·0186G_D + 7·77 \quad (1d)$$
$$RSD = \pm 2·4\ (14·0\%)\ (r = +0·89),$$

where NB = N balance (g N) per week, G = live-weight gain (g) per week and the subscripts refer to diets A, B, C and D.

Faecal N

The regression equations relating faecal N and faecal dry matter ($n = 12$), were:

$$FN_A = 0.0168FDM_A + 0.423 \quad (2a)$$
$$RSD = \pm 0.2\ (13.1\%)\ (r = +0.96),$$

$$FN_B = 0.0342FDM_B - 0.087 \quad (2b)$$
$$RSD = \pm 0.2\ (20.2\%)\ (r = +0.94),$$

$$FN_C = 0.0383FDM_C + 0.244 \quad (2c)$$
$$RSD = \pm 0.3\ (31.0\%)\ (r = +0.78),$$

$$FN_D = 0.0445FDM_D + 0.117 \quad (2d)$$
$$RSD = \pm 0.2\ (27.1\%)\ (r = +0.91),$$

where FN = faecal N (g N) per week and FDM = faecal dry matter (g) per week. It can be seen from these regressions that the faecal N excretion per 100 g faecal dry matter increased from diet A to diet D, and that the value of 1.68 g N/100 g dry matter excreted for diet A was lower than the mean value of 3.86, and below the range 2.61–4.46, for the metabolic faecal N loss determined with a N-free diet (Walker & Faichney, 1964*a*). Roy, Gaston, Shillam, Thompson, Stobo & Greatorex (1964) have shown that the faecal N loss of calves given a diet of cow's milk is lower than that of calves given N-free diets, and Hodge (1965) also found that when lambs were given ewe's milk they excreted smaller amounts of N in the faeces per 100 g dry matter consumed than had been observed by Walker & Faichney (1964*a*) with a N-free diet.

The regression equations relating faecal N and dry-matter intake ($n = 12$), were:

$$FN_A = 0.00223DMI_A - 0.019 \quad (3a)$$
$$RSD = \pm 0.5\ (35.2\%)\ (r = +0.63),$$

$$FN_B = 0.00128DMI_B - 0.064 \quad (3b)$$
$$RSD = \pm 0.5\ (58.9\%)\ (r = +0.25),$$

$$FN_C = 0.00239DMI_C - 0.907 \quad (3c)$$
$$RSD = \pm 0.4\ (37.2\%)\ (r = +0.65),$$

$$FN_D = 0.00165DMI_D - 0.623 \quad (3d)$$
$$RSD = \pm 0.4\ (46.4\%)\ (r = +0.72),$$

where FN = faecal N (g N) per week and DMI = dry matter intake (g) per week. The values for the faecal N excretion per 100 g dry matter consumed were all within the range observed when lambs were given a N-free diet.

The relation between faecal N and faecal dry matter was very highly significant ($P < 0.001$) and the relation between faecal N and dry-matter intake was significant ($P < 0.05$). The mean percentage of N in the dry faeces was 2.7 for diet A, 3.2 for diet B, 5.4 for diet C and 5.5 for diet D. The differences between diets A and B, and diets C and D were highly significant ($P < 0.01$).

Urinary N

The mean values for the excretion of urea N, ammonia N and creatinine N by lambs in period 3 are given in Table 7. The values have also been expressed per kg 'lean body mass' (fat-free carcass weight). Although the total weights of urea N and creatinine N excreted increased with an increase in dietary protein intake, only the urea N excretion showed a relation to dietary treatment when expressed as a proportion of 'lean body mass'. These results support the findings in an earlier experiment (Walker & Faichney, 1964*b*) that the quantity of ammonia and creatinine excreted by the lamb is independent of the dietary protein intake.

Table 7. *Mean values with their standard errors for urinary constituents excreted by the four lambs on each diet during period 3*

	Urea N		Ammonia N		Creatinine N	
Diet	g/week	g/kg lean body mass*	mg/week	mg/kg lean body mass	mg/week	mg/kg lean body mass
A	2·3	0·8	556	194	324	106
B	4·0	0·9	502	115	504	115
C	7·1	1·4	580	118	687	137
D	9·7	1·8	579	111	707	126
SE of the diet mean	±0·7	±0·1	±86	±24	±83	±10

* Fat-free carcass weight.

Utilization of digested N

A highly significant relation ($P < 0·01$) was obtained between N balance (or urinary N excretion) and the intake of apparently digested N (ADN). An analysis of covariance showed that there were no significant differences between diets or between the slopes of the individual regressions. The regression equations, omitting four values for lambs in negative N balance ($n = 44$), were:

$$\mathrm{NB} = 0{\cdot}66\mathrm{ADN} - 2{\cdot}07 \qquad (4a)$$
$$\mathrm{RSD} = \pm 1{\cdot}0\ (9{\cdot}5\%)\ (r = +0{\cdot}99),$$

$$\mathrm{UN} = 0{\cdot}34\mathrm{ADN} + 2{\cdot}07 \qquad (4b)$$
$$\mathrm{RSD} = \pm 1{\cdot}0\ (11{\cdot}5\%)\ (r = +0{\cdot}99),$$

where NB = N balance (g N) per week, UN = urinary N (g N) per week and ADN = apparently digested N (g N) per week.

In a previous experiment (Walker & Faichney, 1964*d*), the comparable equations ($n = 88$) were:

$$\mathrm{NB} = 0{\cdot}56\mathrm{ADN} - 2{\cdot}21 \qquad (5a)$$
$$\mathrm{RSD} = \pm 3{\cdot}9\ (16{\cdot}2\%)\ (r = +0{\cdot}92),$$

$$\mathrm{UN} = 0{\cdot}44\mathrm{ADN} + 2{\cdot}21 \qquad (5b)$$
$$\mathrm{RSD} = \pm 3{\cdot}9\ (17{\cdot}0\%)\ (r = +0{\cdot}92).$$

The intercepts of equations 4*b* and 5*b* represent the urinary N excretions when N intake is equal to faecal N output. These values of 2·07 and 2·21 g N, respectively, whilst they may be considered as estimates of the endogenous N excretion, were much lower than those obtained in a previous experiment when lambs were given N-free diets. Assuming mean live weights of 6·8 and 8·2 kg respectively, for the two experiments, the estimates of endogenous N were 43·2 and 38·4 mg N/kg mean live weight 24 h, compared with a value of 111·8 mg N for lambs with a mean live weight of 5·3 kg given a N-free diet (Walker & Faichney, 1964*a*).

Analysis of variance showed that there were significant differences between the diets in the proportion of the digested N that was retained. The differences were highly significant ($P < 0.01$) between diet A and all other diets, and between diet B and all other diets. The mean values for the percentage of the digested N retained were 5·4 for diet A, 48·8 for diet B, 58·0 for diet C and 59·7 for diet D. The mean value for diet D was higher than the value of 49·3% digested N retained observed for a diet of similar protein content (diet no. 1) in a previous experiment (Walker & Faichney, 1964*d*). In this earlier experiment most of the lambs given diet no. 1 had diarrhoea, thus reducing the amounts of N absorbed and lowering the efficiency with which the absorbed N was utilized.

Table 8. *Mean values (mg/week) with their standard errors for the sulphur balances of the four lambs on each diet in each period*

Diet	S intake*	Urinary S	Faecal S	S balance
		Period 1		
A	405	83	199	123
B	757	152	156	449
C	1219	204	160	855
D	1649	291	109	1249
			SE of the diet mean	±69
		Period 2		
A	373	83	182	108
B	858	140	168	550
C	1433	241	193	999
D	1928	347	157	1424
			SE of the diet mean	±108
		Period 3		
A	360	125	175	60
B	858	130	158	570
C	1433	253	202	978
D	1928	282	176	1470
			SE of diet the mean	±121

* S content of dried whole milk: 2·69 mg/g dry matter.

Sulphur balance

The mean values for S balance, with their standard errors, are given for each diet in Table 8. An analysis of multiple covariance showed that there were highly significant ($P < 0.01$) differences in S balance, adjusted to equal live weight and digestible energy intake between all the diets. There were no significant differences between periods in the S balances of the lambs.

Utilization of digested sulphur

A highly significant relation ($P < 0.01$) was obtained between S balance and the intake of apparently digested S (ADS). An analysis of covariance showed that there were no significant differences between the diets or between the slopes of the individual regressions. The regression equation ($n = 48$) was:

$$\text{SB} = 0.867\text{ADS} - 70 \qquad (6)$$
$$\text{RSD} = \pm 45\ (6.1\%)\ (r = +0.97),$$

where SB = S balance (mg S) per week and ADS = apparently digested S (mg S) per week.

The values for the percentage of the ADS retained were 49.9% for diet A, 78.8% for diet B, 80.2% for diet C and 81.8% for diet D. A comparison of the values for faecal S in this experiment with those in a previous experiment (Walker & Faichney, 1964*b*), where lambs were fed on a S-free diet, suggest that all the S excreted in the faeces was metabolic in origin in this experiment.

Table 9. *Mean values (mg/cm² per week) with their standard errors for the growth of wool of the four lambs on each diet measured on sample patches*

Period	Diet A	Diet B	Diet C	Diet D	SE of the diet mean
1	3.1	4.2	5.6	7.4	±0.3
2 and 3	1.7	2.7	4.4	5.6	±0.4

Wool growth and composition

Mean values for wool growth, with their standard errors, are given in Table 9. These values were based on the growth of wool on the sample patches, whose areas were measured at each wool collection. Analysis of variance showed that there were highly significant differences ($P < 0.01$) between all diets. The wool growth in the first period was significantly higher ($P < 0.01$) than that in the second wool-collection period. This significant period effect may be explained by the method adopted for expressing wool growth, which does not take into account the change in patch size. When total wool growth per animal was used as a basis for comparison, this period difference was evident only for lambs given diet A in periods 2 and 3 (cf. Table 13).

Mean values for the S content of the wool, with their standard errors, were: 2.95 ± 0.05% for diet A, 2.86 ± 0.05% for diet B, 2.77 ± 0.03% for diet C and 2.92 ± 0.06% for diet D.

Mean values for the N content of the wool, with their standard errors, were: 15.82 ± 0.10% for diet A, 15.74 ± 0.12% for diet B, 15.81 ± 0.14% for diet C and 15.64 ± 0.05% for diet D. Analysis of variance showed no significant effect of dietary protein level or periods on the S or N content of wool.

Skin areas were measured when the wool was clipped at the end of period 1 and again at the end of the experiment, 4 weeks later. The mean increases in skin area on the measured patches, with their standard errors, were: 2.0 ± 1.0% for diet A, 14.4 ± 1.2%

for diet B, 17·2 ± 2·2% for diet C and 22·7 ± 4·5% for diet D. The difference between diet A and diet B was significant ($P < 0·05$), and between diet A and diets C and D was highly significant ($P < 0·01$).

Carcass and tissue composition

The mean values for the chemical composition of the lamb carcasses are given in Table 10. An analysis of variance showed that there were significant dietary effects on the percentages of fat ($P < 0·05$) and protein (N × 6·25; $P < 0·01$) in the carcass, but not on the percentages of moisture and ash. Analysis of covariance between the weight of individual constituents and carcass weight showed that the weight of fat decreased and the weight of moisture increased as the protein content of the diet increased. All differences between diets were significant with the exception of that between diets B and C for carcass moisture. There were no significant effects of the diets on the weights of ash or protein (N × 6·25).

Table 10. *Mean values with their standard errors for the carcass composition of the four lambs on each diet at the end of period 3*

	Diet A	Diet B	Diet C	Diet D	SE of the diet mean
Carcass weight (kg)	3·7	5·2	5·9	6·4	±0·6
Water (%)	61·0	62·0	62·6	65·4	±1·1
Fat (%)	17·9	16·5	15·0	11·6	±1·4
Ash (%)	5·0	5·1	4·9	4·8	±0·2
Protein (N × 6·25) (%)	15·7	16·5	17·4	17·9	±0·4
Protein (by difference) (%)	16·0	16·3	17·4	18·1	±0·4
Mean weights (g) adjusted for differences in carcass weights					
Water	3156	3287	3348	3502	—
Fat	1063	881	759	559	—
Ash	243	270	267	260	—
Protein (N × 6·25)	831	875	932	960	—
Protein (by difference)	838	864	930	978	—

The mean values for the composition of the fat-free carcasses are given in Table 11. An analysis of variance showed that diet had no significant effect on the concentration of the components. The weights of protein (N × 6·25), ash and moisture, when adjusted by covariance analysis for differences in fat-free carcass weights, were similar in all dietary treatments. The mean weights of the carcass constituents, adjusted for differences in carcass weights, are given in Tables 10 and 11.

The mean results for the tissue analyses are given in Table 12. As the protein intake of the lambs increased, the percentages of dry matter and protein in the liver increased significantly, but the N concentration in the dry matter decreased. There was an increase in the fat content of the liver with increasing protein intake, and though the differences between diets were not significant the linear trend was significant ($P < 0·05$). The mean values were: 3·15% for diet A, 4·01% for diet B, 5·11% for diet C and 5·70% for diet D. No estimations of glycogen were made in the liver, and the sum of protein (N × 6·25) plus fat accounted for only about 70% of the dry matter. The changes which occurred in the pancreas were similar to those observed in the liver, but the mean values for the protein contents of the pancreas were not significantly different between diets. The increase in the percentage of dry matter in the liver and pancreas did not continue above 15% protein calories in the diet. These changes in the dry-matter contents of the liver and pancreas were the reverse of those which occurred in the carcass.

Table 11. *Mean values with their standard errors for the carcass composition (expressed on a fat-free basis) of the four lambs on each diet at the end of period 3*

	Diet A	Diet B	Diet C	Diet D	SE of the diet mean
Fat-free carcass weight (kg)	3·0	4·4	5·0	5·5	±0·5
Water (%)	74·4	74·3	73·7	74·1	±0·5
Ash (%)	6·1	6·1	5·8	5·4	±0·2
Protein (N×6·25) (%)	19·1	19·8	20·5	20·3	±0·4
Protein (by difference) (%)	19·5	19·6	20·5	20·5	±0·4
Mean weights (g) adjusted for differences in fat-free carcass weights					
Water	3338	3327	3302	3307	—
Ash	256	273	264	247	—
Protein (N×6·25)	883	886	919	905	—
Protein (by difference)	890	875	916	923	—

Table 12. *Mean values for the composition of organs and muscle of the four lambs on each diet at the end of period 3*

Diet	Liver	Pancreas	Heart	Kidney	Spleen	Lung	Muscle
Weight of organ (g)							
A	131	9	44	25	11	80	—
B	140	10	57	27	13	107	—
C	150	12	59	34	19	119	—
D	152	13	59	39	19	127	—
Dry matter (%)							
A	24·3	18·8	21·5	18·5	22·2	19·2	20·3
B	27·5	19·8	22·6	19·8	22·4	19·5	20·8
C	30·0	22·0	21·3	19·5	22·4	19·5	21·7
D	29·7	21·7	21·5	19·0	22·3	19·5	21·8
Nitrogen (mg/g dry matter)							
A	98	135	120	129	135	138	144
B	87	132	104	127	137	137	141
C	84	119	121	127	138	131	141
D	92	125	117	125	133	135	139
Nitrogen (g/kg carcass protein)							
A	5·67	0·39	1·98	1·08	0·56	3·78	—
B	3·84	0·30	1·55	0·77	0·47	3·31	—
C	3·60	0·31	1·49	0·81	0·58	2·97	—
D	3·64	0·34	1·32	0·83	0·48	2·95	—

There was a significant effect ($P < 0·05$) of diet on the N concentration of muscle (when expressed on a wet weight basis). The mean values for the N concentration (mgN/ g fresh muscle) of the vastus lateralis muscle were: 28·8 for diet A, 29·3 for diet B, 30·7 for diet C and 30·3 for diet D. All other organs examined showed no consistent effect of the dietary treatments. When the N content of individual organs was related to the total N in the carcass, the ratio for all organs, with the exception of the pancreas and spleen, was highest for lambs given the diet of lowest protein content (diet A).

DISCUSSION

Digestibility of the diets

In an earlier experiment with lambs given liquid diets containing from 20·2 to 33·0% protein calories (Walker & Faichney, 1964*d*), the digestibilities of the dietary components increased as the intake of protein increased. In the present experiment there were similar increases in digestibility as the protein calorie percentage increased from 5·2 to 20·8%. No diarrhoea occurred with any of the dietary treatments, and the digestibilities of all constituents were higher for diet D than was observed previously for a diet of similar protein content (diet no. 1; Walker & Faichney, 1964*d*), when diarrhoea occurred in seventeen of twenty-four lambs.

Nitrogen intake and nitrogen balance

There was a proportionate increase in N retained as the percentage of calories derived from protein in the diet increased. This was reflected in a rectilinear relation between the intake of ADN and N balance up to the highest level of protein intake (cf. equation (4)).

A rectilinear relation between the intake of truly absorbed N and N balance has been observed with growing pigs (initial weight 20–25 kg) by Armstrong & Mitchell (1955) up to concentrations of 10% protein in the diet. At concentrations higher than 10% the relation became curvilinear. Jones, Hepburn & Boyne (1961), working with young pigs between 25 and 50 lb live weight, found that the rectilinear relation extended up to concentrations of 18% protein in their control diet. Fomon (1961) showed with infants that the rectilinear relation persisted up to concentrations of 20% protein calories in the diet, and Blaxter & Wood (1951) observed a similar relation between protein calories (%) and N balance in the range of 13–20% for liquid diets given to young calves. The observations with young lambs in our experiments are in agreement with the general finding that young animals have a very high capacity for N retention.

Nitrogen balance and live-weight gain

Although the retention of N increased rectilinearly with an increase in protein intake, there was not a corresponding increase in live-weight gain. There were small, but not significant, differences in live-weight gain as the dietary protein calories increased from 10·4 (diet B) to 20·8% (diet D).

The overall relationship between live-weight gain and N balance was highly significant, and within each diet there was a significant correlation. At the same live-weight gain the N retention increased from diet A to diet D. This increase in N retention at a particular live-weight gain, with an increase in the protein content of the diet, was also reported in a previous experiment with milk-fed lambs within the range of 20·2 to 33·0% protein calories (Walker & Faichney, 1964*d*). In this earlier experiment there was a very highly significant correlation between N balance and live-weight gain. An analysis of covariance showed that the individual slopes of the regressions did not differ significantly from the common regression coefficient of 0·0215, but all differences between diets were significant, with the exception of that between diets nos. 3 and 4. The regression equations ($n = 24$), were:

$$NB_1 = 0{\cdot}0157G_1+5{\cdot}82 \quad (7a)$$
$$\text{RSD} = \pm 6{\cdot}0\ (41{\cdot}4\%)\ (r = +0{\cdot}71),$$

$$NB_2 = 0{\cdot}0211G_2+6{\cdot}15 \quad (7b)$$
$$\text{RSD} = \pm 6{\cdot}8\ (31{\cdot}2\%)\ (r = +0{\cdot}73),$$

$$NB_3 = 0{\cdot}0217G_3+7{\cdot}71 \quad (7c)$$
$$\text{RSD} = \pm 3{\cdot}8\ (13{\cdot}7\%)\ (r = +0{\cdot}91),$$

$$NB_4 = 0{\cdot}0272G_4+3{\cdot}24 \quad (7d)$$
$$\text{RSD} = \pm 5{\cdot}6\ (17{\cdot}9\%)\ (r = +0{\cdot}89),$$

where NB = N balance (g N) per week, G = live-weight gain (g) per week, and the subscripts refer to diets nos. 1–4. The individual regression coefficients in the present experiment were 0·0053, 0·0093, 0·0101 and 0·0186 for diets A, B, C and D, respectively. As mentioned above, an analysis of covariance showed that these individual coefficients were not significantly different from the mean coefficient of 0·0132. However, it is apparent that there was an increase in the slope of the line as the protein content of the diet increased, and this would suggest that each increment in live-weight gain made by lambs given high-protein diets contained much more N than that of lambs given low-protein diets. It is clear from an examination of equations (1*a*–*d*) and (7*a*–*d*) that for a particular gain in weight the values for N balance increased linearly from the 'low-protein' diet A to the 'high-protein' diet no. 4. For example, at a live-weight gain of 400 g/week the N balance increased from 1·4 g for diet A (5·2% protein calories) to 14·1 g for diet no. 4 (33·0% protein calories).

A storage of N in the lamb not associated with an increase in live-weight gain could be explained by differences in the weight of N stored in wool, or by an increase in the N concentration of particular organs and tissues.

Table 13 gives the mean values for total wool production and for total wool N. The calculation of total wool growth from the growth on the sample patches is subject to a number of errors. The relation between live weight and surface area may be affected by changes in body composition and body conformation as a result of the dietary treatments. No corrections were made for the specific gravities of the carcasses (cf. Mitchell, 1962). The assumption was also made that wool growth over the whole body surface was the same as that on the sample areas. This assumption is not correct, since the total surface area includes portions on the head and legs not covered by wool.

The amounts of N stored in wool were insufficient to account for the increased N retention with the diets of higher protein content (Fig. 1). Lambs given diets B, C and and D had increasing amounts of N available for tissue growth after correcting the N retained for that stored as wool N. On the other hand, lambs given diet A must have

Table 13. *Mean values for total wool growth, wool nitrogen retention and wool sulphur retention of the four lambs on each diet in each collection period*

Period	Total wool growth (g/week)*	Total wool N (g/week)†	Total wool S (mg/week)‡	N balance minus wool N (g/week)	S balance minus wool S (mg/week)
		Diet A			
1	11·11	1·75	319	−1·90	−196
2 and 3	6·20	0·98	178	−0·58	−94
		Diet B			
1	13·95	2·20	400	2·29	49
2 and 3	12·78	2·01	367	5·51	193
		Diet C			
1	20·73	3·26	595	7·34	260
2 and 3	23·11	3·64	663	10·28	325
		Diet D			
1	27·65	4·35	794	11·33	455
2 and 3	31·29	4·93	898	14·50	549

* Surface area calculated at the end of each wool collection period from the equation of Peirce (1934): (m^2 area) = 0·121 (kg body-weight)$^{0·59}$.
† N content of wool (%) = 15·75.
‡ S content of wool (%) = 2·87.

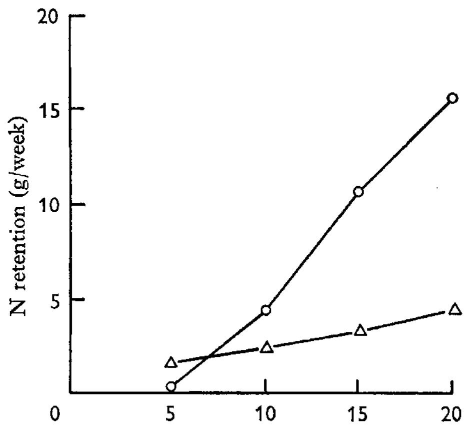

Fig. 1. Relation between protein calories in the diet (%) and the retention of nitrogen by the lambs during period 1. ○—○, N balance; △—△, wool N. Each value is the mean for four lambs.

withdrawn N from the tissues to meet the requirement for N in the wool grown. The composition of the small gain in weight made by lambs fed on diet A must, therefore, have differed considerably from that of lambs given the diets of higher protein content.

The analyses of the carcasses and of individual organs at the end of the experiment do not allow conclusions to be drawn as to the quantitative changes which occurred in the body composition during the period of the experiment. However, there were significant effects of the dietary treatments on the carcass composition. The fat percentage decreased and the protein percentage increased with an increase in the protein content of the diet (cf. Table 10).

Filer & Churella (1963) found that when young pigs were fed on diets which contained either 11 or 39% dietary protein calories, the fat content of the carcass was considerably higher on the low-protein diet up to the age of 6 weeks, but that by 8 weeks this difference had largely disappeared.

Filer, Baur & Rezebak (1960) had already shown that this effect of dietary protein concentration on the fat content of the carcass was influenced by the level of fat (or conversely, by the level of carbohydrate) in the diet. Low-fat diets did not have such a marked effect as high-fat diets. Wallace, Weil & Taylor (1958) did not observe this effect of dietary fat level on carcass fat content in rats fed on diets in which the fat varied from 6·5 to 32·0% by weight. In our experiment fat replaced protein isocalorically in the low-protein diet, since high-carbohydrate diets have been shown to cause diarrhoea in lambs (Walker & Faichney, 1964*c*). The levels of fat in our diets varied from 39·8 to 52·7% of the dry matter.

Table 14. *Calculated composition of the live-weight gain of the four lambs on each diet during period* 3

	Diet A	Diet B	Diet C	Diet D
No. of lambs ...	4	4	4	4
N in fat-free carcass (%)	3·06	3·17	3·28	3·25
Factor*	32·7	31·5	30·5	30·8
Live-weight gain (g/week)	303	496	512	572
N balance (g/week)	0·5	7·7	13·6	18·4
Fat-free gain (g/week)	16	243	415	567
Fat gain (g/week)	287	253	97	5
Fat in gain (%)	94·7	51·0	18·9	0·9

* Factor for computing the weight of fat-free material retained/g N.

Although the composition of the live-weight gain could not be estimated directly in this experiment, it was possible to determine the composition indirectly in period 3 from a knowledge of the N retention and the composition of the fat-free empty body, using the method referred to by Blaxter (1962). In our experiment the body composition was determined on the carcass and not on the total empty body. The analyses of the wool, skin and viscera were not included. However, the fat-free carcass composition of lambs given diet D differed only slightly from that of the total fat-free empty body of lambs given a dried whole milk diet (28·7% protein calories) in a subsequent experiment (Walker, Cook & Jagusch, 1967), though the percentage of ash in the carcass was higher than that in the total body. On average, the carcass represented about 65% of the weight of the total empty body. In Table 14 the fat content of the live-weight gain with the different dietary treatments has been estimated. These values are necessarily only approximate and in certain instances the calculated fat-free gain was greater than the measured gain. However, the differences in the calculated fat content of the gain are considerable and are in agreement with the differences observed in the analyses of the carcasses.

It would appear that, in our experiment, the rate of growth of body tissues was limited by the dietary supply of N and not by that of calories. On the diet with least protein, it would seem that the supply of calories was considerably in excess of that required for the utilization of the available N. The excess calories were thus stored as fat. As the dietary protein level increased, more calories were utilized in wool and tissue growth and fewer were stored as fat. Since the fat-free gain in live weight is associated with a greater retention of water than the deposition of fat or the storage of N in wool, it would not be expected, when the proportions of these constituents in the live-weight gain differed so considerably between treatments, that the retention of N would be closely related to live-weight gain. The apparent increase in N retention at the same live-weight gain with an increase in N intake (cf. equations (1 *a–d*)) is thus largely explained by differences in the fat content of the tissue gain and by the variable proportion of the retained N stored in wool.

Waterlow (1959) has suggested, from the results of experiments with growing rats, that the integrity of the internal organs and tissues is maintained during protein deficiency at the expense of skin and skeletal muscle proteins. In the present experiment no quantitative measurements of changes in individual organs or tissues could be made, and all comparisons were based on values obtained at slaughter. Lambs given diet A retained more N in wool than was given in the diet. If this N was withdrawn preferentially from the carcass, it would be expected that the ratio of N in the organs to N in the carcass would be higher for lambs given diet A than for lambs given diets containing N in excess of that required for wool growth. This was in fact observed. Those lambs given diet A had significantly higher amounts of N, relative to that in the carcass, in all organs with the exception of the pancreas and the spleen, than lambs given the higher protein diets (cf. Table 12). This preferential withdrawal of N from the carcass was reflected in differences in the protein contents of the carcass. Although these differences were not significant, the weight of carcass protein increased from diet A to diet D (cf. Table 11) and the linear trend was significant ($P < 0.05$).

Sulphur balance and wool growth

The values for S balance showed a similar increase to those of N balance with an increase in the protein content of the diet. The retentions of S in the wool grown are given in Table 13.

The mean S content of the wool grown in both periods was 2·87%, which is considerably below the average S content of wool grown by adult sheep (3·38%) in the experiments of Reis & Schinckel (1963). There was no significant effect of the dietary treatments on the wool S content.

Lambs given diet A continued to grow wool even though the intake of dietary S was low and, in fact, the lambs were in negative S balance. This effect was more evident in period 1 when the total wool S was considerably in excess of the S balance for lambs given diet A (Fig. 2). These results are in agreement with the observations of Marston (1948) with adult sheep, that wool continues to grow even when an animal is living entirely at the expense of its own tissues. Wool growth is known to be considerably affected by nutrition (for references see Reis & Schinckel, 1963). Variations in the intake of energy, protein and S-containing amino acids have all been suggested as factors affecting both the quantity and quality of wool grown. Since the energy intake was constant for all diets, it would appear that wool growth in our experiment

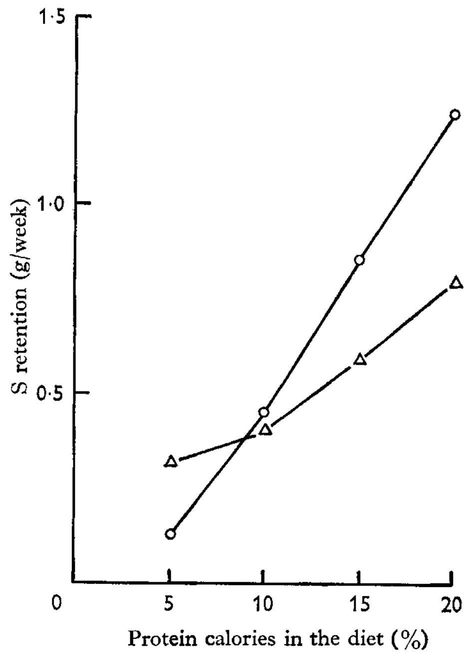

Fig. 2. Relation between protein calories in the diet (%) and the retention of sulphur by the lambs during period 1. ○—○, S balance; △—△, wool S. Each value is the mean for four lambs.

was limited both by the supply of S-containing amino acids and by the dietary supply of N. The design of the experiment did not allow a conclusion to be drawn as to which was of greater importance.

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