Nitrogen balance studies with the milk-fed lamb

9*. Energy and protein requirements for maintenance, live-weight gain and wool growth

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I. Nineteen male cross-bred lambs were allotted to each of three dietary treatments. The protein contents of the diets (on a dry-matter basis) were $12 \cdot 0\%$ (diet A), $28 \cdot 5\%$ (diet B) and $45 \cdot 5\%$ (diet C). The energy intakes of the lambs within each dietary treatment ranged from below maintenance to *ad lib*.

2. The experimental period of 3 weeks was divided into three 7 d periods (periods 1, 2 and 3). Diet digestibility, live-weight gain and nitrogen balances were calculated for all lambs in all periods. Sulphur balances were calculated in periods 1 and 2. Wool growth on sample areas was measured in periods 2 and 3. The lambs were slaughtered at the end of the experiment and N retention was estimated by the comparative slaughter method.

3. There were significant differences between the dietary treatments in the gross energy requirements for the maintenance of body-weight or N equilibrium. The gross energy requirements for empty body-weight equilibrium (with 95% confidence limits) were 160.4 ± 6.7 kcal/kg^{0.73} per d for diet A, 111.2 ± 10.6 for diet B and 113.7 ± 9.8 for diet C. The gross energy requirements for N equilibrium were 181.3 ± 15.0 kcal/kg^{0.73} per d for diet A, 115.4 ± 10.3 for diet B and 94.5 ± 8.4 for diet C.

4. The mean value for wool growth of lambs given diet A was $4\cdot 2 \pm 0\cdot 2 \text{ mg/cm}^2$ per week. The wool growth of lambs given diet B increased from $5\cdot 5$ to $12\cdot 8 \text{ mg/cm}^2$ per week, and that of lambs given diet C from $4\cdot 0$ to $15\cdot 4 \text{ mg/cm}^2$ per week as the gross energy intake increased.

5. There were significant effects of the dietary treatments on the N and S contents of the wool. The mean values for N content were 15.79 ± 0.05 , 16.10 ± 0.04 and 16.15 ± 0.04 %; and for S content 2.46 ± 0.05 , 2.75 ± 0.04 and 2.90 ± 0.04 %, for diets A, B and C, respectively.

6. N retention, S balance and empty body-weight gain were closely related to gross energy intake with all diets; from these relationships, estimates were made of the energy and protein requirements for live-weight gain and wool growth.

In previous studies on the utilization of the protein of cow's milk by the preruminant lamb, diets were used in which the protein-calorie concentrations were between 5 and 33 %. The energy intake per unit of body-weight was kept constant in these experiments and, whilst the energy intake was sufficient for some growth, it was considerably below the *ad lib*. intake of normal lambs (Walker & Cook, 1967; Walker & Faichney, 1964*b*). In the present experiment the diets contained protein in concentrations that supplied either 10, 29 or 45% of the total energy. These diets were given in amounts that were insufficient to maintain body-weight at one extreme, to *ad lib*. at the other.

The effects of the dietary treatments on live-weight gain and wool growth, and on

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[†] Present address: Department of Biochemistry and Nutrition, University of New England, Armidale, NSW 2351, Australia. the retention of nitrogen and sulphur in the tissues and wool, were measured. Estimates were made of the requirements of energy and protein for maintenance, and for the growth of the pre-ruminant lamb from birth to 4 weeks of age.

EXPERIMENTAL

Animals and their management

Fifty-seven male cross-bred lambs, (Border Leicester $3 \times \text{Merino } \mathfrak{P}) \times \text{Dorset}$ Horn 3, were used. The lambs were born at pasture and, at the commencement of the experiment at between 2 and 5 d of age, their live weights ranged from $3 \cdot 2$ to $8 \cdot 4$ kg. Each lamb was housed separately in a metabolism cage and faeces and urine were collected as described previously (Walker & Faichney, 1964*a*). The mean daily maximum and minimum temperatures in the animal house were 27 and 14° respectively.

Experimental design

Nineteen lambs were allotted to each of three dietary treatments. The diets were of low (diet A), medium (diet B), or high (diet C) protein content. The experimental period of 21 d was divided into three separate 7 d periods (periods 1, 2 and 3). Two lambs, one that was being fed on diet B and the other on diet C, both at the lowest energy intakes, lost weight continuously and died after 16 and 13 d on experiment respectively. The lambs were kept in metabolism cages and the faeces and urine were collected daily 4 h after the morning feed and were bulked for each 7 d period. 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. Wool growth was measured over a 2-week period (periods 2 and 3) by the method described by Walker & Cook (1967). All wool was sheared from the lambs at slaughter and total wool grown during the 2-week experimental period was estimated by the method of Ferguson, Carter & Hardy (1949). The N retention during the experimental period of 3 weeks was determined by the comparative slaughter method. The initial body composition was calculated from live weight using the regression equations established by Jagusch, Norton & Walker (1970) for lambs of this breed and age.

Diets

The compositions of the experimental diets are shown in Table 1. The diets were prepared by methods previously outlined (Walker & Cook, 1967; Walker & Faichney, 1964*a*, *b*). A mineral and vitamin mixture was added to diet A so that the final composition was similar to that of ewe's milk (Walker & Faichney, 1964*a*). A solution which contained FeSO₄, CuSO₄ and CoCl₂ was added to diets B and C to increase the concentration of these metals in the dry matter by 50 ppm Fe, 5 ppm Cu and 0.1 ppm Co. All lambs were dosed with a groundnut-oil solution of 100000 i.u. retinyl acetate and 10000 i.u. ergocalciferol on the 1st day of the experiment. Aureomycin soluble (0.45 g) (Cyanamid of Great Britain Ltd), which contained 25 mg chlortetracycline hydrochloride, was given daily, dissolved in the milk, to each lamb.

Groups of three lambs within each dietary treatment were given sufficient milk to

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provide energy at five different levels (90–110, 120–140, 150–200, 201–250, 251–300; all values expressed as kcal gross energy/kg^{0.73} d). The intake of milk by each lamb was adjusted three times weekly to allow for an increase, but not for a decrease, in live weight. The lambs were bottle-fed twice daily at 07.00 and 18.00 hours after the diets had been warmed to about 37° by immersion in a constant-temperature bath. A group of four lambs within each dietary treatment was fed to appetite by the method described by Walker, Cook & Jagusch (1967).

Table 1. Constituents and composition of the diets (values expressed per 100 g dry matter); as fed to the lambs, each diet contained 15 % dry matter

Constituent	Diet A	Diet B	Diet C
Dried whole milk (g)	42.1	100.0	74.0
Calcium caseinate* (g)			26·0
Butter oil (g)	39.9		
Lactose (g)	15.2		
Minerals (g)	2.5		
Sulphur† (mg)	113	269	388
Crude protein [†] (g)	12.0	28.5	45.5
Ether extractives (g)	51.2	27.6	20.4
Ash (g)	5.0	5.9	5.2
N-free extractives (by difference) (g)	31.2	38.0	28.9
Energy (kcal)	663	556	556
Protein calories as %	10.1	28.7	45.3

* Casinal (Glaxo Laboratories Ltd).

† S content of dried whole milk and calcium caseinate: 2.69 and 7.29 mg/g dry matter respectively. 1×6.38 .

Analytical methods

The methods used for the analysis of the dietary constituents, faeces, urine, wool and body components, were those described previously (Walker & Cook, 1967; Walker & Faichney, 1964a, b).

Statistical methods

In this experiment the relationships between intake (X) and retention (Y) were frequently curvilinear, with a sharp inflexion at intakes corresponding to zero retention. The line of best fit was obtained by an equation of the type $Y = a+b \log X$. Where the growth of the lamb was of primary interest, negative values of Y were excluded from the relationship; where the intake (Y) corresponding to zero retention (X) was of primary interest, only those values below or around zero retention were used. These latter relationships were usually linear or could be described by quadratic equations.

RESULTS

The regression equations given in this paper are applicable only within the range of values given in Table 2.

Digestibility of the diets

The mean values for the apparent digestibilities of the dietary components in period 2 are given in Table 3. An analysis of variance within each diet showed that there was no significant effect of the level of energy intake on apparent digestibility, with two exceptions. The apparent digestibilities of N and S by the two lambs given diet A at the lowest energy intake were much lower than those for all other lambs in this group; they were excluded from the means in Table 3. The analysis of variance between diets showed that the apparent digestibilities of dry matter, energy, N, S and ether extractives increased significantly with an increase in the protein concentration of the diet. The mean digestibilities of the N-free extractives were similar for all diets.

Table 2.	Intake and	l retention	of nutrients	by lamb	os given d	liets of	different	protein	content
	(range	of values	expressed in	units of	f weight o	or ener	gy/kg ^{0·73} (d)	

	Diet A	Diet B	Diet C
Experimental period (d)	21	21	21
No. of lambs	19	19	19
Initial live wt (kg)	3.2 to 6.7	3.5 to 8.4	3.5 to 7.3
Mean live wt (kg ⁰ ⁷³)	2.7 to 4.7	2.9 to 5.9	2.4 to 5.4
Gross energy intake (kcal)	95 to 422	83 to 426	91 to 354
Live-wt gain (g)	-14 to $+47$	-15 to $+83$	-25 to $+68$
Empty body-wt gain (g)	-17 to $+37$	-21 to $+79$	-37 to $+65$
N intake (g)	0.27 to 1.20	0.68 to 3.49	1.16 to 4.54
N retention (g)	-0.51 to $+0.61$	-0.62 to $+2.19$	-0.67 to $+1.87$
S intake (mg)	17 to 81	40 to 206	53 to 265
S balance (mg)	-10 to $+58$	-2 to $+132$	-19 to $+165$
Wool N retention (mg)	49 to 111	88 to 240	91 to 264
Wool growth (mg/cm ² week)	2.0 to 6.1	5.5 to 12.8	4.0 to 12.4

Table 3. Mean values with their standard errors for the apparent digestibility coefficients of the dietary components in the nineteen lambs on each diet

	Diet A	Diet B	Diet C	se of group mean
Energy	95.0	98.2	98·4	г·í
Total N	89.5*	96.3	97.6	1.2
Total S	79·6 *	91.0	93.9	3.3
Ether extractives	94.9	97.3	99.2	1.8
N-free extractives	90 .1	-98 ·o	99 .0	0.4
Dry matter	95.3	98·o	98.1	1.0

* Seventeen lambs.

N retention

There was a highly significant correlation (P < 0.001) between N balance (measured by total collection of urine and faeces) and N retention (measured by the comparative slaughter method) for all dietary treatments.

An analysis of covariance was carried out to compare the between-levels and withinlevels regression coefficients. For diet A the within-levels coefficient was significantly less than the between-levels coefficient. For diets B and C the within-levels coefficient

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did not differ significantly from the between-levels coefficient. The slopes of the individual regressions (diets B and C) did not differ significantly from the common regression coefficient, but the differences between diets were significant. The regression equations (n = 19), with their correlation coefficients (r), residual standard deviations (RSD), and the RSD expressed as a percentage of the mean of the dependent variable, were:

$$NB_A = 0.350 NR_A + 2.151 GE_A - 267 [RSD = \pm 81 (37.1\%) (r = 0.97)], (1a)$$

$$NB_{B} = 1.064 NR_{B} + 103 [RSD = \pm 99 (12.9\%) (r = 0.99)], \qquad (1b)$$

$$NB_{C} = 1.037 NR_{C} - 7 [RSD = \pm 67 (7.0\%) (r = 0.99)],$$
 (1c)

where NB = N balance (mg/kg^{0.73} d), NR = N retention (mg/kg^{0.73} d), GE = gross energy intake (kcal/kg^{0.73} d) and the subscripts refer to diets A, B and C.

There was a highly significant curvilinear relationship between N retention and gross energy intake for each diet (P < 0.001). An analysis of covariance showed that there were significant differences between the slopes of the individual regressions. The regression equations, omitting values for lambs in negative N balance, were:

$$NR_{A} = 1.434 \log GE_{A} - 3.207 (n = 12) [RSD = \pm 0.084 (32.3\%) (r = 0.92)],$$
(2a)

$$NR_{\rm B} = 3.261 \log \text{GE}_{\rm B} - 6.632 (n = 15) \quad [RSD = \pm 0.131 (12.9\%) (r = 0.97)],$$
(2b)

$$NR_{\rm C} = 3.086 \log \text{GE}_{\rm C} - 6.033 (n = 17) \quad [RSD = \pm 0.079 (6.8\%) (r = 0.99)],$$
(2c)

where NR = N retention (g/kg^{0.73} d) and GE =gross energy intake (kcal/kg^{0.73} d).

Utilization of digested N

The relationship between N retention and the intake of apparently digested N (ADN) was curvilinear for all diets. An analysis of covariance showed that there were significant differences between the slopes of the individual regressions. The regression equations, omitting values for lambs in negative N balance, were:

$$NR_{A} = 1.403 \log ADN_{A} + 0.503 (n = 12) [RSD = \pm 0.082 (31.5\%) (r = 0.92)],$$
(3a)

$$NR_{B} = 3.098 \log ADN_{B} + 0.283 (n = 15) [RSD = \pm 0.129 (12.7\% (r = 0.97)],$$
(3b)

$$NR_{C} = 2.992 \log ADN_{C} - 0.113 (n = 17) [RSD = \pm 0.083 (7.5\%) (r = 0.99)],$$
(3c)

where NR = N retention $(g/kg^{0.73} d)$ and ADN = apparently digested N $(g/kg^{0.73} d)$.

In Table 4 values are given for the N retained at given intakes of gross energy (equations (2a)-(2c)), and for the percentage of the ADN retained for each dietary treatment.

Table 4. Relation between the gross energy intake, retention and utilization of nitrogen and sulphur, and the empty body-weight gain of lambs given diets of different protein content (values expressed per $kg^{0.73}$ d)

Gross	Empty		Apparently	G	Apparently
energy	body-wt	N	digested N	S	digested S
intake	gain*	retention†	retained	balance‡	retained
(kcal)	(g)	(mg)	(%)	(mg)	(%)
		Di	et A		
150	0	ş	ş	0	0
200	11	93	18.3	21	77.4
250	20	232	36.2	29	85.5
300	27	346	45.4	35	86·o
350	33	442	49.8	41	86.3
		Di	et B		
150	15	465	40.1	48	72.7
200	30	872	56.4	72	81.8
250	42	1189	61.2	91	82.7
300	51	1447	62.3	107	81.0
350	60	1665	61.2	120	77.9
		Di	et C		
150	15	682	36.3	64	65.1
200	30	1068	42.7	91	69.4
250	42	1367	43.7	III	67.8
300	51	1611	42.9	128	65-1
350	60	1818	41.2	142	61.9

* Calculated from equations (5a)-(5c).
‡ Calculated from equations (7a)-(7c).

† Calculated from equations (2a)-(2c). § Negative retention.

Live-weight gain

The live-weight gain (including gut contents) was closely related to empty bodyweight gain. An analysis of covariance showed that there were significant differences between the slopes of the individual regressions. The regression equations (n = 19)were:

$$LWG_A = 1.124 EBWG_A + 4.8 [RSD = \pm 1.5 (9.2\%) (r = 0.99)],$$
 (4*a*)

$$LWG_B = 1.028 EBWG_B + 3.2 [RSD = \pm 2.0 (5.7\%) (r = 0.99)],$$
 (4b)

$$LWG_{C} = 0.981 EBWG_{C} + 4.0 [RSD = \pm 3.1 (10.9\%) (r = 0.99)],$$
 (4c)

where LWG = live-weight gain $(g/kg^{0.73} d)$ and EBWG = empty body-weight gain $(g/kg^{0.73} d)$.

There was a highly significant curvilinear relationship (P < 0.001) between empty body-weight gain and gross energy intake. An analysis of covariance showed that there were significant differences between the slopes of the individual regressions. The regression equations, omitting values for lambs that lost weight, were:

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where EBWG = empty body-weight gain $(g/kg^{0.73} d)$ and GE = gross energy intake $(kcal/kg^{0.73} d)$.

In Table 4 values are given for the empty body-weight gains at given intakes of gross energy (equations (5a)-(5c)) for each dietary treatment.

There was a highly significant correlation (P < 0.001) between N retention and empty body-weight gain for all dietary treatments. An analysis of covariance showed that there were significant differences between the slopes of the individual regressions. The regression equations (n = 19) were:

$$NR_{A} = 0.0172 EBWG_{A} - 0.11 [RSD = \pm 0.07 (108.2\%) (r = 0.98)], \quad (6a)$$

$$NR_{\rm B} = 0.0266 \, EBWG_{\rm B} + 0.08 \quad [RSD = \pm 0.14 \, (15.4 \,\%) \, (r = 0.97)], \qquad (6b)$$

$$NR_{C} = 0.0259 EBWG_{C} + 0.28 \quad [RSD = \pm 0.10 (11.3\%) (r = 0.99)], \quad (6c)$$

where NR = N retention $(g/kg^{0.73} d)$ and EBWG = empty body-weight gain $(g/kg^{0.73} d)$.

S balance

There was a highly significant curvilinear relationship between S balance and gross energy intake for each diet (P < 0.001). An analysis of covariance showed that there were significant differences between the slopes of the individual regressions. The regression equations (n = 19) were:

$$SB_A = 82.84 \log GE_A - 170 [RSD = \pm 4 (20.0\%) (r = 0.97)],$$
 (7*a*)

$$SB_B = 194.84 \log GE_B - 376 [RSD = \pm 8 (13.8\%) (r = 0.98)],$$
 (7b)

$$SB_{C} = 212.68 \log GE_{C} - 399 [RSD = \pm 11 (12.6\%) (r = 0.98)],$$
 (7c)

where SB = S balance (mg/kg^{0.73} d) and GE = gross energy intake (kcal/kg^{0.73} d).

There was a highly significant correlation (P < 0.001) between S balance and the intake of apparently digested S (ADS) for all dietary treatments in periods 1 and 2 (corresponding to wool growth in periods 2 and 3).

The relationship was curvilinear for all three diets. An analysis of covariance was carried out to compare the between-levels and within-levels regression coefficients. For diet A the within-levels coefficient was significantly less than the between-levels coefficient. For diets B and C the within-levels coefficient did not differ significantly from the between-levels coefficient. There were no significant differences between the diets or between the slopes of the individual regressions for diets B and C. The regression equations (n = 19) were:

$$SB_A = 19.69 \log ADS_A + 0.12 GE_A - 33 [RSD = \pm 4 (17.4\%) (r = 0.98)], (8a)$$

$$SB_B = 181.58 \log ADS_B - 279 [RSD = \pm 6 (10.9\%) (r = 0.99)],$$
 (8b)

$$SB_{C} = 207 \cdot 16 \log ADS_{C} - 348 \quad [RSD = \pm 12 (13 \cdot 7 \, \%) (r = 0.97)],$$
 (8c)

where SB = S balance (mg/kg^{0.73} d), ADS = apparently digested S (mg/kg^{0.73} d) and GE = gross energy intake (kcal/kg^{0.73} d). In Table 4, values are given for the S balance at given intakes of gross energy (equations (7a)-(7c)) and for the percentage of the ADS retained for each dietary treatment.

Wool growth and composition

In Fig. 1 wool growth (mg/cm² per week) is plotted against ADN intake (mg/kg⁰⁻⁷³ d), to illustrate the close relationship that exists when all dietary treatments are combined. There were significant differences between diets in the N and S contents of the wool. The mean values, with their standard errors, are given in Table 5.



Fig. 1. Relation between the intake of apparently digested nitrogen and wool growth of lambs given diets of different protein content. \bigcirc — \bigcirc , diet A; \bigcirc — \bigcirc , diet B; \triangle — \triangle , diet C.

In Table 6 values are given for the total N and S retained in the wool and body tissues. The values for the N retained in wool by lambs given diets B and C were calculated from the relationship between wool N retained and ADN intake. The intake of ADN was calculated from the gross energy intake, knowing the energy and N concentrations in the diets (cf. Table 1), and the apparent digestibilities of the dietary N (cf. Table 3). There were significant curvilinear relationships between wool N retained and ADN intake for lambs given diets B and C, but not for lambs given diet A. The mean value and standard error for the N retained in wool by lambs given diet A (n = 19) was 0.074 ± 0.004 g/kg^{0.73} d. The regression equations for diets B and C (n = 19) were:

 $WN_{\rm B} = 0.2036 \log \text{ADN}_{\rm B} + 0.157 \quad [\text{RSD} = \pm 0.027 (14.6\%) (r = 0.82)], \quad (9a) \\ WN_{\rm C} = 0.0976 \log \text{ADN}_{\rm C} + 0.143 \quad [\text{RSD} = \pm 0.038 (20.9\%) (r = 0.52)], \quad (9b) \\ \text{where WN} = \text{wool N retained } (g/\text{kg}^{0.73} \text{ d}) \text{ and ADN} = \text{apparently digested N intake} \\ (g/\text{kg}^{0.73} \text{ d}).$

Table 5. Mean values with their standard errors for the nitrogen and sulphur contents of wool and for the S:N ratios (all values expressed as g/100 g)

Com- ponent	Diet A*	Diet B*	Diet C*	SE of diet mean
Ν	15.79 ^a	16.10 _p	16.12 ^p	0.12
S	2.46ª	2.75 ^b	2.90°	0.10
S:N	15.6ª	17.16	18.0p	o∙8

Values within a line with different superscripts differ significantly at the 5 % level of probability. * n = 19.

Table 6. Relation between the gross energy intake and the retention of nitrogen and sulphur in the wool and body tissues of lambs given diets of different protein content (values expressed per $kg^{0.73}$ d)

Gross energy intake (kcal)	Wool N* (mg)	Wool S† (mg)	N retention minus wool N (mg)	S balance minus wool S (mg)
		Diet A		
150	74	12	- 160	- 12
200	74	12	19	9
250	74	12	158	17
300	74	12	272	23
350	74	12	368	29
		Diet B		
150	171	29	294	19
200	196	33	676	39
250	216	37	973	54
300	232	40	1215	67
350	245	42	1420	78
		Diet C		
150	169	30	513	34
200	182	33	886	58
250	191	34	1176	77
300	199	36	1412	92
350	205	37	1613	105

* See p. 22.

† Ratio of wool S: wool N for diet A, 0.156: 1.000; for diet B, 0.171: 1.000; and for diet C, 0.180: 1.000.

DISCUSSION

Maintenance requirement

In Table 7 values are given for the intakes of gross energy corresponding to zero N retention and to zero empty body-weight gain. In Table 8 values are given for the N retention in the wool and body tissues when empty body-weight gain was zero. The values in Tables 7 and 8 were calculated from the equations whose constants are given in Table 9. The calculated values in Table 7 show that there were significant differences between diets in the gross energy requirements for the maintenance of N or empty body-weight equilibrium. The values in Table 8 demonstrate the continued retention of N in wool coincident with negative N balance in the tissues of lambs given diets A and B, and the continued retention of N in wool and tissues, in the absence of a change in empty body-weight, by lambs given diet C.

Table 7. Calculated values for the gross energy intakes (kcal/kg⁰⁷³ d), with 95% confidence limits, corresponding to zero nitrogen retention and zero empty body-weight gain of lambs given diets of different protein content

Diet	When N retention is zero	When empty body-weight gain is zero
Α	181·3 [°] ± 15·0	160·4 ^a ±6·7
В	$115.4^{b} \pm 10.3$	$111.2^{b} \pm 10.6$
С	$94.5^{\circ} \pm 8.4$	$113.7^{\rm b} \pm 9.8$

Values within a column with different superscripts differ significantly at the 5 % level of probability.

Table 8. Calculated values for the nitrogen retention in wool and body tissues of the lambs (mg $N/kg^{0.73} d$), with 95% confidence limits, corresponding to zero empty body-weight gain

Diet	N retained*	Wool N†	Tissue N†
A	$-109^{a} \pm 36$	$74^{a}_{b} \pm 8$	$-183^{a} \pm 40$
В	77°±7°	$151^{\circ} \pm 19$	−74°±84
С	284° ± 67	160 ^b ± 23	+ 124° ± 56

Values within a column with different superscripts differ significantly at the 5 % level of probability.

* Calculated from equations (6a)-(6c) (p. 21).

† Calculated from equations in Table 9.

Growth requirements

In Table 10 estimates are given of the gross energy and available protein requirements for particular gains in weight of a 'reference' lamb with an initial empty bodyweight of 5 kg. The gross energy requirements were calculated from the following equations, for lambs that gained weight:

$\log \text{GE}_{A} = 0.0107 \text{ EBWG}_{A} + 2.1905 (n = 12)$	$[RSD = \pm 0.025 (r = 0.98)], (14a)$
$\log \text{GE}_{\text{B}} = 0.0078 \text{ EBWG}_{\text{B}} + 2.0699 (n = 15)$	$[RSD = \pm 0.250 (r = 0.97)], (14b)$
$\log \text{GE}_{\text{C}} = 0.0080 \text{ EBWG}_{\text{C}} + 2.0620 (n = 16)$	$[RSD = \pm 0.130 (r = 0.98)], (14c)$
where $GE = gross energy intake (kcal/kg^{0.73} d)$	and $EBWG = empty body-weight$

gain (g/kg^{0.73} d).

Lambs given diet A that weighed 5 kg initially could not achieve gains in weight greater than about 130 g/d (corresponding to 40 g/kg^{0.73} d) owing to the limitations of appetite (cf. Table 2).

The requirements for available protein were calculated from the equation

$$AP = (EUN + G) \times 6.25 \times 100/BV, \tag{15}$$

where AP = available protein (g/d), EUN = endogenous urinary N (g/d), G = N content of gain (g/d) and BV = biological value. The endogenous urinary N excretion was taken as 111.8 mg/d per kg (Walker & Faichney, 1964*a*) and the N content of the gain was predicted for each diet from equations (6*a*)-(6*c*).

 $(EBWG) (g/kg^{0.73} d)$

(see p. 23) Equation no. воі qoi 120 100 IIa q_{II} IΙC 12b120 13*a* 13*b* 13*c* -0.51 to +0.26 -0.62 to +0.80 -0.67 to +1.13-21 to +79-37 to +65-21 to +19-17 to +17-37 to +29 -17 to +37-21 to +79 -37 to +65-17 to +37 $\underset{X}{\operatorname{Range}}$ Mean o.749 0.074 0.175 0.180 0.381 - 0.014 Þ 123 127 154 123 127 154 0.072 0.129 0.036 0200 10.3 10.3 9.5 RSD 1.51 0.1 l (Models: $Y = a + b_1 X$ and $Y = a + b_1 X + b_2 X^2$) Correlation coefficient 0.93 0.85 0.98 86.0 86.0 66.o 0.75 0.55 0.94 26.0 66.0 1 Intercept +181.3 +0.151 +111.2 +113.7 - 0.074 +0.160 +115.4+ 160.4 -0.183 + 94.5 +0.124 a I Independent variable EBWG EBWG EBWG EBWG EBWG EBWG EBWG EBWG × ZR RR 010.0 0.035 8.65 ļ l ļ l ļ \tilde{b}_2^2 ł coefficient Regression 0.0245 0.0251 0.0174 0.0013 213.8 1.850 1.818 4.327 53.9 1.04 NC \tilde{p}_1 Dependent variable NW GEGE GE GE **N** lambs of No. 12 ទួ ទួ 12 12 5 61 61 61 61 Diet ABC CBA CBA CBA

NC, no correlation.

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The BV of the milk proteins was dependent upon the dietary protein concentration and upon the level of energy intake. The metabolic faecal N (MFN) was taken as 0.29 g N/100 g dry-matter intake (Walker & Faichney, 1964*a*), though in many instances this factor led to the situation where the sum of ADN plus MFN was greater than N intake. It seemed likely that with diets of high digestibility the factor 0.29, as determined with a N-free diet of significantly lower digestibility (Walker &

Table 10. Requirements of available protein (AP), truly digestible protein (TP), digestible crude protein (DCP) and gross energy for the maintenance and growth of lambs with an empty body-weight of 5 kg

	Empty	Empty body-weight gain (g/d)			
Diet	50	100	200		
	A	vailable protein	(g/d)		
Α	9	17			
В	18	30	53		
С	35	51	83		
	G	Gross energy (kcal/d)			
Α	736	1076	_		
В	504	665	1155		
С	497	661	1160		
	Convert AP ir	nto TP Conv	vert AP into DCP		
А	Add 25.2	* D†	Add 7.1‡ D		
В	Add 25.1	D	Add 6 9 D		
С	Add 36.2	D	Add 18.1 D		

* 6.25 (MFN × 100/BV), where MFN is metabolic faecal N (g N/kg dry-matter intake per d) and BV is biological value of the protein.

 $\dagger D = dry-matter intake in kg/d.$

 $\ddagger 6.25[(MFN \times 100/BV) - MFN].$

Faichney, 1964*a*), overestimated MFN, and a factor of 0.20 would have given a more realistic estimate. By were calculated from the equation

$$BV = \frac{IOO(NR + MFN + EUN)}{ADN + MFN},$$
 (16)

where NR = N retention (g/d), MFN = metabolic faecal N (g/d), EUN = endogenous urinary N (g/d) and ADN = apparently digested N (g/d).

In the present experiment N retention was estimated by the comparative slaughter method (NR) and by the N balance method (NB). The total N retained by lambs given the low-protein diet (diet A) did not exceed $0.61 \text{ g/kg}^{0.73}$ d at the highest intakes of gross energy. The comparable values for diets B and C were 2.19 and $1.87 \text{ g/kg}^{0.73}$ d, respectively. With the majority of lambs NB gave higher estimates of N retained than NR. However, as the protein content of the diets increased there was closer agreement between the values for N retained as estimated by NR and NB (cf. equations (1a)-(1c)). It is apparent that the values for ADN retained, and for BV, will be

affected by these different estimates of N retained. In previous papers in this series and in the majority of published experiments with other domestic animals, N retention has been estimated from NB. It is now agreed that NB usually overestimates the true N retention (cf. Duncan, 1966).



Fig. 2. Relation between the intake of gross energy, the retention of apparently digested N and the biological value, of milk proteins fed to lambs in diets of low, medium or high protein content (diets A, B and C respectively). O, N retention by comparative slaughter method; \bullet , N balance.

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Fig. 2 shows for each diet the individual lamb values for the percentage of ADN retained, and individual BV, calculated from NR and NB, related to the intake of gross energy. It will be noted that with the medium- and high-protein diets (B and C) BV became relatively constant at a lower intake of gross energy than they did with the low-protein diet (A). This difference was a reflection of the significant differences in the requirements of gross energy for N equilibrium (cf. Table 7). When N retention was negative the value of the numerator in equation (16) was low, relative to the value of the denominator, and BV were correspondingly low. BV increased as the intake of gross energy increased with all diets, but were relatively constant when the intakes of gross energy were in excess of 230, 130 and 130 kcal/kg⁰⁻⁷³ d for diets A, B and C respectively. When the intakes of gross energy were below these values the BV decreased to zero. Mean values, with their standard errors, for the BV of the milk proteins, calculated from NR, were 71.8 ± 3.4 for diet A (n = 7), 66.4 ± 2.2 for diet B (n = 13)and 49.5 ± 0.8 for diet C (n = 13). The corresponding BV, calculated from NB, were 89.0 \pm 0.3 for diet A (n = 7), 72.2 \pm 1.4 for diet B (n = 13) and 51.1 \pm 0.8 for diet C (n = 13).

The values in Table 10 are minimum requirements and are applicable for 1 d only to lambs with an initial empty body-weight (EBW) of 5 kg. After 1 d the EBW of the lamb will have increased and different allowances of available protein and gross energy will be necessary simply to achieve the same EBW gain as on the 1st day. An alternative method of expressing requirements, in terms of the metabolic body-weight (kg^{0.73}), would obviate the necessity for multiple tables of requirements. However, regular adjustment of the food intake would still be obligatory if the predetermined daily rate of gain were to be achieved.

The values in Table 10 (diet B) for available protein may be compared directly with those given in the Agricultural Research Council (1965) recommendations of nutrient requirements for ruminants.

Wool composition

The significant differences in wool N and S contents (cf. Table 5) were unexpected and were not in agreement with the results obtained in a previous experiment (Walker & Cook, 1967). Although the N content of wool has been reported to vary widely (Simmonds, 1954; Block & Weiss, 1956), no relationship with dietary protein concentration or N intake has been reported. The N content of the wool was similar to that in adult sheep, but the S content was much lower. The S content of wool from adult sheep varies between 3.0 and 4.0% and is influenced by dietary S intake (Reis, 1965), but the S content of lambs' wool has not exceeded 3.2% in our studies. This low concentration of S may be attributed to the high proportion of medullated fibres (low S content) relative to non-medullated fibres (high S content) in the fleece of the young lamb (Barritt & King, 1926, 1931; Larose & Tweedie, 1937).

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