

## Protein requirements of growing lambs

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1. The protein requirements of lambs were established by measuring nitrogen balance in seventy-four animals given liquid diets which passed direct to the abomasum. Four groups of lambs weighing approximately 8 kg (group 1), 13 kg (group 2), 21 kg (group 3) and 30 kg (group 4) received diets in which 0.10, 0.15, 0.20, 0.25, 0.30, 0.35 or 0.40 of the digestible energy was provided as protein (DPE:DE ratio) and a gross energy intake of from 1.30 to 1.42 MJ/kg<sup>0.73</sup> per d.

2. When the protein requirements were taken to correspond to the protein intake at the point of intersection of the line describing the increase in N balance with increase in protein intake and the line representing the maximum N balance, values of 0.25, 0.23, 0.17 and 0.12 DPE:DE ratio were obtained for groups 1-4 respectively. The requirements expressed in these terms can be applied only to lambs fed on liquid diets which contain milk proteins and escape fermentation in the rumen. To enable the results to be applied to lambs given other diets, the requirements were expressed as g reference-protein (defined as a theoretical protein with the ideal pattern of amino acids) per MJ net energy and were 11.6, 10.4, 8.0 and 6.2 for groups 1-4 respectively. The relationship between protein requirement ( $Y$ , g reference protein/MJ net energy) and live weight ( $X$ , kg) was:  $Y = 13.4 - 0.242X$ .

3. The influence of energy intake on protein requirements in lambs is discussed and it is concluded that the results obtained are applicable to lambs given a metabolizable energy intake of more than about 1.75 times their maintenance requirement.

4. Application of the estimated requirements to ruminant lambs and methods of formulating diets to supply the required quantity of reference protein/MJ net energy are discussed.

A large variation exists in the estimated protein requirements of growing and fattening lambs (Table 1). There are many possible reasons for this variation, but differences in feedstuffs used for the various determinations, and particularly differences in the extent of degradation of dietary protein within the rumen and subsequent amino acid absorption, are likely to be principal causes.

Nutrient requirements of animals are assessed to facilitate the formulation of their rations, but to do this effectively it is essential to know (*a*) the total needs of the animal's tissues for each nutrient, and (*b*) the capacity of the diet to provide the tissues with these nutrients. On the one hand, the needs of the tissues for nutrients in similar animals in a particular physiological state should theoretically be fairly constant. On the other hand, the availability of dietary nutrients to the tissues is highly variable and depends upon many factors, including the physical and chemical properties of the feedstuffs, their form of preparation and the proportion digested by micro-organisms. Most estimations of protein requirements in lambs combine these two factors, since the requirements are assumed to coincide with the protein intake at which the response in some measured criterion such as live weight or nitrogen retention ceases to increase with increasing protein intake. These estimations are therefore applicable

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Table 1. *Selected estimates obtained from feeding trials of protein requirements in growing and fattening lambs*

Authority	Live weight (kg)	Estimated protein requirement (crude protein as g/kg dry matter)
Andrews & Ørskov (1970)	20	175
	25	150
	30	125
	35	125
Hinds, Mansfield & Lewis (1964)	25 +	177
Hudson, Glimp, Little & Woolfolk (1969)	16-40	140
Miller (1968)	14-20	180
	20-27	150
	27-34	120
Preston & Burroughs (1958)	43	140
Ranhotra & Jordan (1966)	15-20	140
Robinson & Forbes (1970)	39 +	100-130
Walker & Faichney (1964 <i>b</i> )	8	285*

\* Milk-fed lamb.

only to the very limited conditions of the particular experiments and could vary widely between experiments. If, however, the total needs of the tissues for amino acids are known, it is possible to accurately formulate diets by using values for the availability of nutrients in sheep given many different feedstuffs.

It is proposed that the requirement of the tissues for protein in growing lambs can be estimated from the results of a feeding trial by measuring the response in N retention to changes in protein intake if (a) the absorption of nutrients from the digestive tract is accurately assessed, and (b) the efficiency of utilization of the absorbed nutrients is taken into account. A method of assessing nutrient absorption in lambs is to infuse a readily digestible diet direct into the abomasum and measure the disappearance of nutrients between there and the faeces. The efficiency of utilization of absorbed nutrients depends upon several factors including: (1) how closely the amount and pattern of the absorbed amino acids resembles the needs of the animal's tissues, (2) the availability of energy in a biologically suitable form (ATP) in relation to the amino acid absorption and (3) the availability of certain micro-nutrients which are required either directly in the formation of peptide bonds or in other reactions involved in amino acid metabolism or ATP production.

Because most practical diets contain sufficient micro-nutrients, it is the quantity and pattern of absorbed amino acids and the amount of ATP produced which usually determine the capacity of a diet to promote protein synthesis. A suitable method of expressing the total requirements of an animal's tissue for protein should therefore make allowance for these two factors. With our present state of knowledge, it is not possible to accurately define the pattern of amino acids required by the tissues of young lambs. Nevertheless, it is possible to determine the total N requirement and by expressing this in terms of a quantity of reference protein (defined as a theoretical

protein with the correct pattern of amino acids and which is therefore used only for protein synthesis and supplying the amino acids lost during inevitable catabolism (FAO, 1965)), the requirement of a perfectly balanced protein can be predicted. A close approximation of the reference-protein requirement can be obtained from the expression (maximum N retention + total endogenous N losses)  $\times$  6.25. The amino acid pattern of a reference protein is unknown and will differ for animals in different physiological states. However, until the precise amino acid pattern has been defined for growing lambs the reference-protein concept can serve a useful purpose.

There are also difficulties in expressing the energy needs of lambs in terms of ATP, but because nutrients are used with the same efficiency in supplying energy for maintenance as they are for the theoretical conversion into energy in ATP (Chudy & Schiemann, 1969), it is proposed that energy requirements could be appropriately expressed in terms of net energy (NE). By expressing the tissue protein requirements of lambs in terms of g reference protein/MJ NE, and with a knowledge of the efficiency with which dietary protein and energy are used to supply the tissue needs, it is possible to formulate diets for either weaned or liquid-fed lambs given protein from different sources.

The present paper describes an experiment designed to determine the tissue requirements for protein of lambs ranging in weight from 8 to 30 kg. A preliminary report of this work has been presented elsewhere (Black, 1971).

#### EXPERIMENTAL

##### *Outline of experiment*

Cross-bred lambs at 2 d of age were placed in individual metabolism cages heated to 23° and given entirely liquid diets which passed direct to the abomasum. The lambs were randomly divided into four groups and reared on cow's milk until the mean live weights of the animals in the groups were 6, 10, 18 or 28 kg. Experimental diets were then given to the lambs of each group for 14–16 d and change in live weight was measured throughout the period, whereas N balance was measured during the last 8 d. The experimental diets, which were equienergetic and which contained from 0.10 to 0.40 of the digestible energy as protein (DPE:DE ratio), provided each lamb with 1.32–1.40 MJ/kg<sup>0.73</sup> gross energy daily. The number of lambs allocated to each dietary treatment and the mean live weight during the period when N balance was measured are given for the four groups of lambs in Table 2.

##### *Lambs and management*

Seventy-four entire male cross-bred [(Merino ♀  $\times$  Border Leicester ♂) ♀  $\times$  Dorset Horn ♂] lambs weighing between 3.5 and 5.7 kg at birth were given 150 mg retinol, 1875  $\mu$ g cholecalciferol and 34 mg D- $\alpha$ -tocopherol by intramuscular injection when 2 d old and again 42 d later.

The management procedures adopted for the lambs of groups 1 and 2 were different from those used with groups 3 and 4.

Because the lambs of groups 1 and 2 were less than 24 d old, it was assumed that

Table 2. Mean live weight of lambs in each group and number of lambs allocated to each dietary treatment

Group .. .. .	1	2	3	4
Mean live wt (kg) .. .. .	7.8	12.6	20.8	30.4
	No. of lambs			
Treatment*	— — — —			
0.10	—	—	4	4
0.15	4	3	4	3
0.20	4	4	4	4
0.25	4	3	4	3
0.30	4	4	4	—
0.35	4	3	—	—
0.40	3	—	—	—

\* Digestible protein energy: digestible energy ratio of diet.

when they drank eagerly from a bottle and teat the reticular groove functioned effectively and the diets escaped fermentation in the rumen (Watson, 1944). The diets were therefore given in this way in four daily feeds at 07.00, 12.00, 17.00 and 22.00 hours. During the rearing period when the lambs were growing to the weight required for the introduction of the experimental diets (6 kg group 1, 10 kg group 2) they were given reconstituted, spray-dried cow's milk to appetite, but the experimental diets were offered in four equal amounts to provide a total energy intake of 1.40 MJ/kg<sup>0.73</sup> daily. Intakes were adjusted daily in accordance with the live weight on the previous day, and live weight was recorded daily at 06.30 hours.

Lambs in groups 3 and 4 were given the experimental diets by abomasal infusion. These lambs were reared to the weights required for the introduction of the experimental diets with cow's milk, which the lambs could drink *ad lib.* from a metal tank fitted with teats. A catheter was inserted into the abomasum of each lamb when those of group 3 weighed 14 kg and when those of group 4 weighed 20 kg. Infusion of the experimental diets was begun when the lambs of groups 3 and 4 weighed 18 and 28 kg respectively. The daily infusions began at 15.00 hours and continued for 20–22 h. Each lamb was given between 1.32 and 1.38 MJ gross energy/kg<sup>0.73</sup> daily, and live weight was recorded daily at 14.00 hours.

#### *Diets*

The constituents and chemical compositions of the diets given to groups 1 and 2 and groups 3 and 4 are given respectively in Tables 3 and 4. To each 30 l mixture of reconstituted diet, 1.5 g dimethylpolysiloxane was added as an anti-foaming agent and 0.3 ml H<sub>2</sub>O<sub>2</sub>/l was used as a preservative. Butterfat was incorporated into the mixture with an homogenizer (model 15M-8BA, APV Co. Ltd, Crawley, Sussex, England).

#### *Experimental procedures*

The abomasal infusions were made through Foley catheters which were inserted into the lambs of group 3 through Mann–Bellman fistulas (Markowitz, Archibald & Downie, 1964) and into the lambs of group 4 by the Witzel's gastrostomy technique (Markowitz *et al.* 1964). A peristaltic pump was used for the infusions. The pre-weighed

Table 3. *Composition (per kg dry matter) of diets given to lambs in groups 1 and 2*

Constituent	Treatment*						
	RP†	0·15	0·20	0·25	0·30	0·35	0·40
Spray-dried whole milk (g)	1000	475	645	817	820	780	700
Spray-dried whey (g)	—	100	100	100	100	100	100
Sodium caseinate (g)	—	—	—	—	55	120	200
Glucose (g)	—	280	172	63	25	—	—
Butter oil (g)	—	145	83	20	—	—	—
Chemical composition							
Crude protein (N × 6·38) (g)	290	151	200	250	301	350	400
Diethyl ether extractives (g)	268	274	258	238	220	211	190
Ash (g)	58	46	56	66	68	69	67
Gross energy (MJ)	23·8	23·0	23·0	23·0	23·0	23·2	23·1
Digestible protein energy: digestible energy ratio	0·28	0·15	0·20	0·25	0·30	0·35	0·40

Diet given during treatment RP was reconstituted to contain 200 g dry matter/l, whereas all other diets contained 218 g dry matter/l. A mineral mixture was added, providing (mg/kg diet dry matter) Mg, 812; Fe, 240; Cu, 5·2 and Co, 0·12.

\* Digestible protein energy: digestible energy ratio of diet.

† Rearing period.

Table 4. *Composition (per kg dry matter) of diets given to lambs in groups 3 and 4*

Constituent	Treatment*					
	RP†	0·10	0·15	0·20	0·25	0·30
Spray-dried whole milk (g)	988	253	478	650	765	680
Spray-dried whey (g)	—	200	100	100	100	100
Sodium caseinate (g)	—	—	—	—	20	102
Glucose (g)	—	227	186	85	—	—
Butter oil (g)	—	288	207	140	90	93
Minerals and vitamins ‡	12·2	12·2	12·2	12·2	12·2	12·2
CaCO <sub>3</sub> (g)	—	—	4·5	6·3	6·3	6·3
Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> (g)	—	20·0	13·3	6·7	6·7	6·7
Chemical composition						
Crude protein (N × 6·38) (g)	283	100	150	200	251	302
Diethyl ether extractives (g)	260	358	334	312	293	273
Ash (g)	54	65	63	67	74	73
Gross energy (MJ)	23·6	23·8	23·8	23·9	23·8	23·8
Digestible protein energy: digestible energy ratio	0·28	0·10	0·15	0·20	0·25	0·30

Diet given during treatment RP was reconstituted to contain 200 g dry matter/l and all other diets contained 210 g dry matter/l.

\* Digestible protein energy: digestible energy ratio of diet.

† Rearing period.

‡ The mineral and vitamin mixtures are given by Black (1970) and the following amounts were added (mg/kg diet dry matter): Mg, 812; Fe, 240; Zn, 90; Mn, 36; Cu, 5·2; Co, 0·12; I, 0·16; Mo, 0·11; choline chloride, 240; *myo*-inositol, 300; *p*-amino benzoic acid, 30; D- $\alpha$ -tocopherol, 20; thiamin, 10; riboflavin, 5; pyridoxine, 3; nicotinic acid, 35; sodium pantothenate, 25; cyanocobalamin, 0·02; retinol, 4·5; cholecalciferol, 0·188.

daily ration for each lamb was continually stirred. To establish whether any of the diet infused into the abomasum was flowing back into the rumen, 15 g polyethylene glycol (PEG), molecular weight 4000, were dissolved in 100 ml water and added to the diet on the day after completion of the N-balance period. A sample of rumen fluid was obtained by stomach-tube after the infusion and analysed for PEG.

For the determination of N balance, faeces were collected in bags held to the lambs by light harnesses and samples representing half of the daily excretion were bulked during the collection period. Urine was collected in 100 ml 10 M-HCl and samples representing 50 ml/l of the daily excretion were bulked during the collection period. The bulked urine and faeces samples were stored at  $-15^{\circ}$  before analysis. Because feed intake was continually changing, an arbitrary time lag of 1 d was allowed before the collection of urine and faeces corresponding to a particular intake.

Ether extract, N, ash and dry-matter contents of feed, faeces and urine were determined by the methods of the Association of Official Agricultural Chemists (1965). Gross energy was determined in an adiabatic bomb calorimeter. PEG was measured by the method of Smith (1958). N in urine and faeces was determined in fresh samples which had been thoroughly mixed. Other analyses made on faeces were with material dried at  $60^{\circ}$ . Gross energy in urine was determined on freeze-dried samples burnt in gelatin capsules of predetermined energy content.

Analysis of variance was used for within-group comparisons. Significant differences between treatment means were established by the method of Tukey as modified by Snedecor (1959).

## RESULTS

### *PEG recovery*

PEG was not detected in the rumen fluid of lambs after its infusion into the abomasum, and it was concluded that there was no significant flow of abomasal contents to the rumen.

### *Live-weight gain, N balance and energy intake*

Mean values for live-weight gain are given in Table 5, and the N balance results are presented in Table 6.

The responses of live-weight gain and N balance to protein intake were similar for all groups of lambs. Within any group, an increase in protein intake initially produced a significant increase ( $P < 0.05$ ) in both live-weight gain and N balance until apparently maximum levels were reached. After that, further increases in protein intake did not substantially alter the responses.

Faecal N excretion was not significantly affected by N intake in any group. A common regression equation established between N intake (NI, g/kg<sup>0.73</sup> per d) and apparently digestible N (ADN, g/kg<sup>0.73</sup> per d) for all four groups of lambs was

$$\text{ADN} = 1.007 (\pm 0.008) \text{NI} - 0.145 (\pm 0.018); r^2 = 0.995.$$

The fact that the regression coefficient was not significantly different from 1.00 suggests that this value was the true digestibility coefficient of N in the diets. The intercept of this equation ( $-0.145$  g/kg<sup>0.73</sup> per d) represents a negative estimate of the inevitable loss of N in the faeces of these lambs.

Table 5. Mean values for live-weight gain of lambs in each group

Treatment*	Live-wt gain (g/d)			
	Group 1	Group 2	Group 3	Group 4
0.10	—	—	209 <sup>a</sup>	274 <sup>a</sup>
0.15	201 <sup>a</sup>	288 <sup>a</sup>	327 <sup>b</sup>	395 <sup>b</sup>
0.20	277 <sup>ab</sup>	370 <sup>b</sup>	394 <sup>bc</sup>	392 <sup>b</sup>
0.25	319 <sup>b</sup>	400 <sup>b</sup>	406 <sup>c</sup>	373 <sup>b</sup>
0.30	316 <sup>b</sup>	392 <sup>b</sup>	381 <sup>bc</sup>	—
0.35	308 <sup>b</sup>	338 <sup>ab</sup>	—	—
0.40	238 <sup>ab</sup>	—	—	—
Approximate standard error	20	15	17	16

<sup>abc</sup> Values for treatments within the one group with different superscripts were significantly different ( $P < 0.05$ ).

\* Digestible protein energy: digestible energy ratio of diet.

Table 6. Mean values (g/d) with their standard errors for the nitrogen balance of lambs in each group

Treatment*	N intake	Faecal N	Urinary N	N retention
Group 1				
0.15	6.17 <sup>a</sup>	0.61 <sup>a</sup>	1.83 <sup>a</sup>	3.73 <sup>b</sup>
0.20	8.68 <sup>b</sup>	0.69 <sup>a</sup>	2.57 <sup>a</sup>	5.42 <sup>b</sup>
0.25	10.88 <sup>c</sup>	0.65 <sup>a</sup>	2.96 <sup>a</sup>	7.27 <sup>c</sup>
0.30	13.44 <sup>d</sup>	0.72 <sup>a</sup>	5.48 <sup>b</sup>	7.24 <sup>c</sup>
0.35	16.11 <sup>e</sup>	0.86 <sup>a</sup>	7.63 <sup>c</sup>	7.62 <sup>c</sup>
0.40	16.32 <sup>e</sup>	0.64 <sup>a</sup>	8.32 <sup>c</sup>	7.37 <sup>c</sup>
Approximate standard error	0.44	0.13	0.32	0.31
Group 2				
0.15	8.97 <sup>a</sup>	0.80 <sup>a</sup>	2.69 <sup>a</sup>	5.47 <sup>a</sup>
0.20	12.32 <sup>b</sup>	0.98 <sup>a</sup>	3.82 <sup>a</sup>	7.60 <sup>b</sup>
0.25	15.55 <sup>c</sup>	0.94 <sup>a</sup>	6.12 <sup>b</sup>	8.49 <sup>bc</sup>
0.30	18.88 <sup>d</sup>	0.76 <sup>a</sup>	8.51 <sup>c</sup>	9.61 <sup>c</sup>
0.35	22.13 <sup>e</sup>	0.64 <sup>a</sup>	12.39 <sup>d</sup>	9.36 <sup>c</sup>
Approximate standard error	0.26	0.16	0.30	0.28
Group 3				
0.10	8.11 <sup>a</sup>	1.02 <sup>a</sup>	2.67 <sup>a</sup>	4.42 <sup>a</sup>
0.15	12.35 <sup>b</sup>	1.08 <sup>a</sup>	4.00 <sup>b</sup>	7.27 <sup>b</sup>
0.20	16.70 <sup>c</sup>	1.03 <sup>a</sup>	6.39 <sup>c</sup>	9.28 <sup>c</sup>
0.25	21.28 <sup>d</sup>	0.82 <sup>a</sup>	10.97 <sup>d</sup>	9.49 <sup>c</sup>
0.30	25.36 <sup>e</sup>	0.84 <sup>a</sup>	14.17 <sup>e</sup>	10.35 <sup>c</sup>
Approximate standard error	0.51	0.14	0.26	0.44
Group 4				
0.10	10.08 <sup>a</sup>	1.41 <sup>a</sup>	3.03 <sup>a</sup>	5.76 <sup>a</sup>
0.15	15.53 <sup>b</sup>	1.83 <sup>a</sup>	5.90 <sup>b</sup>	7.80 <sup>b</sup>
0.20	20.71 <sup>c</sup>	1.97 <sup>a</sup>	10.34 <sup>c</sup>	8.40 <sup>b</sup>
0.25	26.17 <sup>d</sup>	1.43 <sup>a</sup>	15.86 <sup>d</sup>	8.88 <sup>b</sup>
Approximate standard error	0.20	0.27	0.40	0.30

<sup>abcdc</sup> Values for treatments within the one group with different superscripts were significantly different ( $P < 0.05$ ).

\* Digestible protein energy: digestible energy ratio of diet.

Table 7. Mean and range for values of energy intake (Mj/d) in each group of lambs

Group	Gross energy	Apparently digestible energy	Metabolizable energy
1	6.36 (4.92-6.91)	6.13 (4.56-6.65)	5.83 (4.35-6.29)
2	9.08 (7.93-9.49)	8.75 (7.53-9.29)	8.41 (7.30-8.77)
3	12.68 (12.20-13.09)	12.28 (11.63-12.92)	11.90 (11.38-12.37)
4	15.68 (15.02-16.29)	14.99 (14.00-15.89)	14.48 (13.69-15.28)

Urinary N excretion increased in a biphasic way in all groups of lambs as protein intake was raised. The rate of increase changed sharply when the protein intake reached the level at which N balance ceased to increase, and after that virtually all the additional N provided in the diets was excreted in the urine. The relationship between urinary N excretion (UN, g/kg<sup>0.73</sup> per d) and NI (g/kg<sup>0.73</sup> per d) for the lambs during the initial phase did not depart significantly from linearity and was similar for all groups. An equation describing this relationship was established from the pooled results for lambs from the treatments selected on the same basis as that described below for the relationship between N balance and N intake (treatments 0.15, 0.20, 0.25, group 1; 0.15, 0.20, group 2; 0.10, 0.15, group 3; 0.10, group 4).

$$UN = 0.267 (\pm 0.021) NI + 0.056 (\pm 0.034); r^2 = 0.850.$$

The intercept of this equation (0.056 g/kg<sup>0.73</sup> per d) represents the inevitable loss of N in the urine of these animals. The total endogenous N loss in urine and faeces of the lambs was therefore  $0.20 \pm 0.04$  g/kg<sup>0.73</sup> per d.

Mean intakes and range of gross energy, digestible energy and metabolizable energy are given in Table 7. These were not significantly influenced by protein intake, although energy loss in the urine increased within each group ( $P < 0.05$ ) as protein intake was raised. For example, in group 1, the lambs receiving treatment 0.15 lost 3.9% of digestible energy in urine compared with 6.7% for the lambs receiving treatment 0.40.

#### *Estimation of protein requirements*

Initially, in accordance with customary methods of estimating protein requirements, the best estimate of the minimum requirement of each group of lambs was taken to be at the intersection of the line describing the increase in N balance as protein intake was raised with the horizontal line representing the maximum response in N balance (Hegsted, 1964). The line describing the increase in N balance is essentially rectilinear when plotted against protein intake (Hegsted & Neff, 1970; Velu, Baker & Scott, 1971), but may be curvilinear when plotted against protein concentration in the diet (Miller & Payne, 1963) since geometric increases in protein concentration rarely correspond to geometric increases in protein intake in practical diets. Because maximum N balance of animals is dependent on energy intake, it is important to specify energy intake as well as protein intake when defining protein requirements.



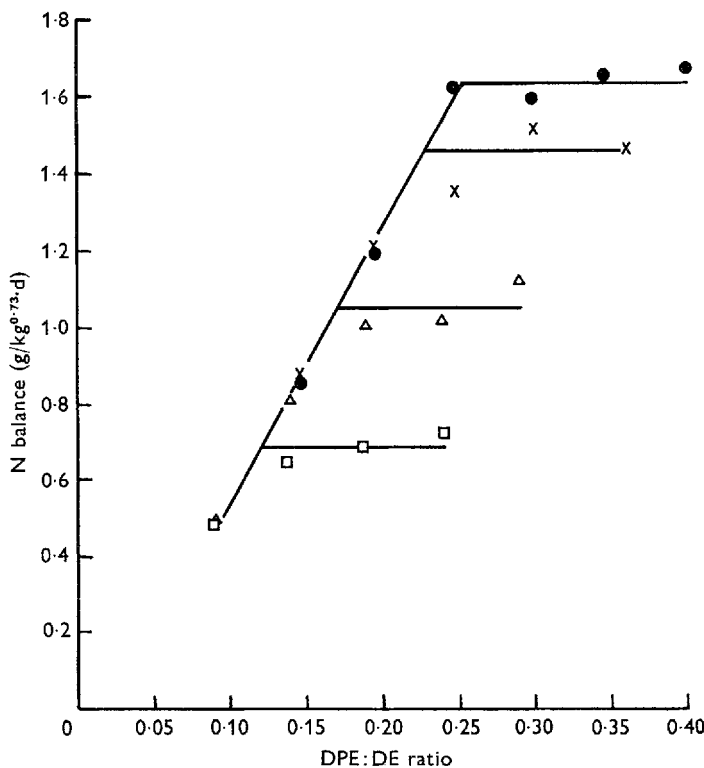


Fig. 1. Relationship between nitrogen balance and the digestible protein energy: digestible energy ratio (DPE:DE ratio) of the dietary intake for the groups of lambs weighing 8 (●), 12.5 (×), 21 (△) and 30 (□) kg. Each point represents the mean for either three or four lambs.

Therefore, with the present results, N balance was plotted, in Fig. 1, against the contribution protein made to the digestible energy intake (DPE:DE ratio). Because the diets were equienergetic and given to all lambs at a similar level of digestible energy intake relative to metabolic live weight, the relationship between N balance and DPE:DE ratio was linear over the initial phase.

A piece-wise linear regression model was adopted to describe the over-all relationship between N balance and protein intake. The optimal split of treatments into an initial phase common to all four groups of lambs in which N balance was less than maximum, and a second horizontal phase representing the maximum N balance for each group was chosen to be that split which minimized the total residual sum of squares over both phases of the regression. The fitting of an asymptotic regression model, either Mischelich or logistic, did not significantly improve the fit or alter the estimates of maximum N balance compared with the piece-wise linear approach adopted.

The equation describing the initial phase was

$$NB = 7.19 (\pm 0.28) P - 0.175 (\pm 0.046); \quad r^2 = 0.957,$$

where NB = N balance ( $\text{g}/\text{kg}^{0.73}$  per d) and P is the DPE:DE ratio of the diet. The maximum N balances for each group were  $1.64 (\pm 0.04)$ ,  $1.46 (\pm 0.04)$ ,  $1.05 (\pm 0.02)$

Table 8. *Estimated ratio of reference protein to net energy (NE) intake of the lambs of varying weights*

Live wt (kg)	Mean maximum N balance (g/kg <sup>0.73</sup> per d)	Endogenous N losses (g/kg <sup>0.73</sup> per d)	Reference-protein intake* (g/kg <sup>0.73</sup> per d)	Mean ME intake† (MJ/kg <sup>0.73</sup> per d)	Estimated NE intake‡ (MJ/kg <sup>0.73</sup> per d)	Ratio, reference-protein: NE (g/MJ)
7.8	1.64	0.20	11.50	1.33	0.99	11.6
12.6	1.46	0.20	10.38	1.34	1.00	10.4
20.8	1.05	0.20	7.81	1.31	0.97	8.0
30.4	0.69	0.20	5.56	1.20	0.90	6.2

\* (Mean maximum N balance + endogenous N losses) × 6.25.

† Mean metabolizable energy (ME) intake of lambs from which maximum N balance was calculated.

‡ Net energy intake estimated by assuming that 0.44 MJ/kg<sup>0.73</sup> per d of ME required for maintenance, and the coefficient of efficiency of utilization of ME for maintenance is 0.813, and for production is 0.710 (Walker & Jagusch, 1969); e.g. for 7.8 kg lamb, ME = 1.33 MJ; maintenance NE = 0.44 × 0.813 = 0.36; ME production = 1.33 - 0.44 = 0.89; production NE = 0.89 × 0.710 = 0.63; total NE = 0.36 + 0.63 = 0.99.

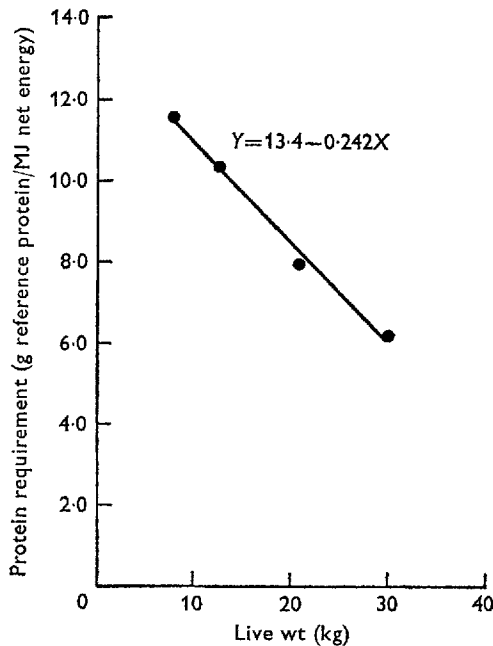


Fig. 2. Effect of live weight on the estimated protein requirements of liquid-fed lambs.

and 0.69 ( $\pm$  0.02) g/kg<sup>0.73</sup> per d respectively, for groups 1-4. The requirements of DPE:DE ratio estimated from the intersection of the lines representing the two phases were 0.25, 0.23, 0.17 and 0.12 respectively for groups 1-4.

However, when protein requirements are expressed in terms of DPE:DE ratio, they provide a combined estimate of the needs of the tissues and of the capacity of the diets to provide these needs, and therefore apply only to the particular conditions of this experiment. It is suggested on p. 46 that the results would be more useful if they gave an estimate of the tissue requirements of the lambs so that they could be used to

formulate rations for lambs given alternative diets. It was proposed to achieve this by expressing the requirements as g reference protein/MJ NE.

The protein requirements of the four groups of lambs expressed in these terms and the method by which they were derived are given in Table 8. The requirement of reference protein was calculated for each group by multiplying the sum of the respective maximum N balance and the total endogenous N loss in urine and faeces by 6.25. The NE intake of each group was calculated from the average metabolizable energy intakes of the lambs used to establish the maximum level of N balance. The proportions of this energy used for maintenance and for production were estimated and then, using published values for the efficiencies of utilization of metabolizable energy for both maintenance and production (Walker & Jagusch, 1969), the total NE intake was derived. The requirements of the lambs weighing 7.8, 12.6, 20.8 and 30.4 kg were 11.6, 10.4, 8.0 and 6.2 g reference protein/MJ NE respectively. The relationship between protein requirements and live weight of the lambs, which does not depart significantly from linearity, is given in Fig. 2.

#### DISCUSSION

The tissue requirements for protein in lambs weighing from 8 to 30 kg have been established by giving liquid diets which passed direct to the abomasum. In order that these results can be used to assess the needs of similar animals given alternative diets, the requirements were expressed in g reference protein/MJ NE. However, in doing so the following assumptions were made: (a) that measurement of N balance gives an accurate estimation of the N retained in the lambs; (b) that the inevitable losses of N in urine and faeces can be determined by an extrapolation to zero protein intake of the results from animals given a wide range of protein intakes; and that (c) the inevitable N losses per  $\text{kg}^{0.73}$ , (d) the energy requirements for maintenance per  $\text{kg}^{0.73}$ , and (e) the efficiencies of utilization of metabolizable energy for maintenance and for production are constant over the range in live weight and diets covered by the experiment. The validity of these assumptions is considered in turn below.

Because protein requirements of growing animals change rapidly with live weight, N retention was estimated by N balance rather than by either comparative slaughter (Duncan, 1966) or from an estimation of body water (Reardon, 1969) since the latter techniques can have considerable error over narrow ranges in live weight. Measurements of N balance, in general, systematically overestimate N retention (Duncan, 1966), but any overestimation in protein requirement resulting from its use in the present experiment should have been small. With careful experimentation the common losses of N due to errors in urine collection and failure to consider dermal losses in sheep range from only 1.2 to 2.6% of the faecal and urinary output of N (Martin, 1966), which, at the most, would be equivalent to only 5% of maximum N balance in the present experiment. Moreover, because N loss from the integument is a result of metabolism and is inevitable, it should be included in an estimation of protein requirements.

The endogenous losses of N in urine and faeces of liquid-fed lambs have been

established by giving protein-free diets (Walker & Faichney, 1964*a*), but the values of 170 mg/kg<sup>0.73</sup> per d for urine and 290 mg/100 g dry-matter intake for faeces are considerably higher than those of 56 mg/kg<sup>0.73</sup> per d and 204 mg/100 g dry-matter intake respectively obtained in the present experiment. However, when N-free diets are given to animals their protein metabolism is substantially altered (Waterlow, 1968) and the estimates of endogenous N losses made under these conditions may not apply to animals given protein at higher levels. Since the estimates required for the calculation of reference protein should describe the inevitable N losses which occur in animals given sufficient protein to produce maximum N retention but not those losses associated with the catabolism of excess dietary amino acids, it is felt that satisfactory estimates are obtained by extrapolation from values obtained with lambs given more than 10 g protein/100 g dry matter, as was done in the present experiment. If, however, the estimates obtained by Walker & Faichney (1964*a*) for endogenous N excretion were used in the calculation of reference protein, the requirement would increase by 7% in group 1 and by 14% in group 4.

The total endogenous N losses per kg<sup>0.73</sup> may not be constant under all conditions. The results of Ashworth & Cowgill (1938) suggest that in rats the amount of endogenous urinary N per kg<sup>0.73</sup> rises slightly as live weight increases, but there is insufficient published information to identify a similar trend in milk-fed lambs. Since the endogenous N excretion was calculated by pooling values for the four groups of lambs, inaccuracies in the estimated reference-protein requirement caused by the assumption that the endogenous urinary N loss per kg<sup>0.73</sup> was the same for all groups are not likely to be great. This may not be so with endogenous N excretion in faeces, since it is influenced by dry-matter intake (Schneider, 1935) and the crude fibre content of the diet (Whiting & Bezeau, 1957). Walker & Faichney (1964*b*) found that 100 g change in dry-matter intake in milk-fed lambs resulted in a change of 0.2 g N in faeces. Because dietary N in milk is completely digested, this reflects a change in endogenous faecal N excretion which would result in an increase in the estimated reference-protein requirement of lambs in groups 1 and 4 of only 2 and 4% respectively if dry-matter intake was raised by 100 g in a diet providing the same NE intake. The effects of dietary fibre on endogenous faecal N excretion may be greater. When Whiting & Bezeau (1957) increased the fibre content of the diet of young pigs from 50 to 150 g/kg, endogenous faecal N rose by 35% and an increase of this magnitude would raise the protein requirements of group 4 lambs by about 6%. On the other hand, Mason (1971) has recently found that, although endogenous faecal N excretion is positively related to the intake of indigestible dry-matter in lambs weighing from 19 to 27 kg, the significance of the relationship declined with age and was not apparent in adult sheep.

The estimates given by Walker & Jagusch (1969) for the energy requirements of maintenance and for the efficiency of utilization of metabolizable energy for both maintenance and production were for lambs weighing from 3.5 to 8.5 kg and given cow's milk. They are therefore strictly applicable only to group 1 of the present experiment. Graham & Searle (1970) have found that the fasting heat production per kg<sup>0.73</sup> falls by about 40% as lambs increase in weight from 8 to 30 kg. Nevertheless, if it is assumed that the maintenance requirement of the lambs in group 4 was 60% of

that estimated by Walker & Jagusch, the daily NE intake falls only from 0.90 to 0.87 MJ/kg<sup>0.73</sup> when the same factors for the efficiency of utilization of metabolizable energy are used in both calculations. There is little evidence to suggest that the efficiencies of utilization of the metabolizable energy of a diet for maintenance and for production change substantially as animals increase in live weight (Blaxter, Clapperton & Wainman, 1966; Graham & Searle, 1972), or as the protein content of the diet needed to fulfil the requirements changes over the range observed in the present experiment (Walker & Norton, 1971).

#### *Effect of energy intake on protein requirements of lambs*

Although the lambs had an energy intake equivalent to about three times their maintenance requirement, the protein requirements established in this paper can also be applied to lambs given other levels of energy intake. Walker & Jagusch (1969) measured the retention of protein and energy in the carcasses of lambs fed on re-constituted cow's milk at various levels, providing intakes of metabolizable energy ranging from below maintenance to about 3.4 times maintenance. The influence of energy intake on the estimated requirement of reference protein/MJ NE for these lambs is given in Fig. 3. It can be seen that as energy intake was raised above maintenance there was initially a rapid increase in the requirement, but between the energy intakes of 1.75 and 3.4 times maintenance it rose by only about 10%. The results of the present experiment should therefore be satisfactory for the range of intakes associated with *ad lib.* feeding.

#### *Application of estimated protein requirement to ruminant lambs*

Although it is hoped that the estimations of protein requirement established in this paper may also be applicable to ruminant lambs of the same tissue weight, care must be taken when doing so because the effects of absorption of volatile fatty acids instead of carbohydrates and long-chain fatty acids on the amino acid metabolism of these animals have not been established. For example, because ruminant lambs obtain a substantial amount of their glucose from gluconeogenic reactions involving amino acids, their protein requirements per unit of NE may be higher than in non-ruminant lambs. Similarly, the absorption of different nutrients may lead to an alteration in the secretion of hormones which influence amino acid metabolism.

The protein requirements of lambs estimated from the present experiment are less than the recommendations made by the Agricultural Research Council (1965). For a 20 kg lamb, gaining weight at 300 g/d and consuming a diet providing 12.6 MJ metabolizable energy/kg dry-matter, it can be calculated from the report that the suggested requirement is 9.8 g reference protein/MJ NE, compared with a value of approximately 7.9 for a lamb of the similar weight from the present experiment. Because ruminant lambs of this weight may contain as much as 2 kg more contents in their digestive tracts than the milk-fed lambs, an appropriate adjustment would lift the estimated requirement of a 20 kg ruminant lamb made from the present experiment to about 8.4 g reference protein/MJ NE. A further contributing factor for the higher estimate of the Agricultural Research Council is the larger endogenous faecal N losses

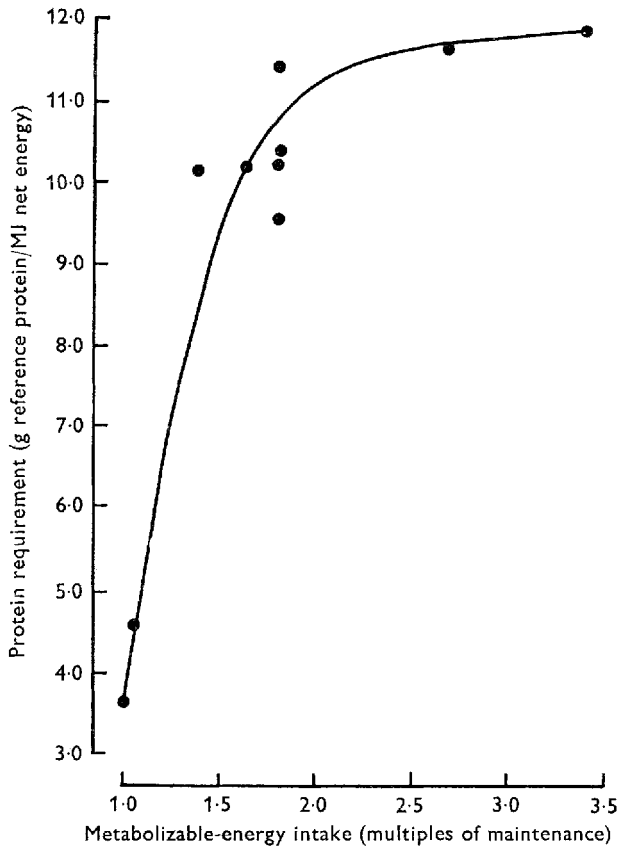


Fig. 3. Influence of metabolizable-energy intake on the estimated protein requirements of liquid-fed lambs. Calculated from the results of Walker & Jagusch (1969).

used in their calculation. The high value used is not entirely justifiable because it includes an amount of N in undigested microbial residues which, in N-containing diets, is not solely derived from endogenous sources (Mason, 1971).

#### *Formulation of rations*

The method of formulating rations to satisfy the requirement of reference protein/MJ NE for lambs given either liquid or solid diets has been given in detail previously (Black, 1970) and a brief outline of the principle only is given here. The amount of dietary crude protein needed is calculated from the reference-protein requirements by making adjustment for (a) the efficiency with which amino acids absorbed from the digestive tract are used for protein synthesis, that is biological value in non-ruminants as a coefficient (BV) and (b) the proportion of dietary crude protein absorbed as amino acids, that is true digestibility in non-ruminants as a coefficient (TD). By the use of intestinal cannulas similar information is also becoming available for ruminants (see Clarke, Ellinger & Phillipson, 1966; Ørskov, Fraser & McDonald, 1971).

Thus, g crude protein = g reference protein  $\times$  (1/BV)  $\times$  (1/TD).

The amount of dietary energy needed to provide 1 MJ NE can be calculated by

making adjustments for: (a) the percentage of NE used for maintenance ( $NE_m$ ) and for production ( $NE_p$ ) as coefficients, (b) the efficiency with which metabolizable energy is used for maintenance ( $k_m$ ) and for production ( $k_p$ ) as coefficients, and (c) the efficiency of conversion of gross energy into metabolizable energy (M) as a coefficient.

Thus, dietary energy needed to provide

$$1 \text{ MJ NE} = \left( \frac{NE_m}{k_m} + \frac{NE_p}{k_p} \right) \frac{1}{M}$$

An example of ration formulation for ruminant lambs is given by Black (1970).

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