

Nitrogen balance studies with the milk-fed lamb

3.* Effect of different nitrogen intakes on growth and nitrogen balance

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The estimation of the protein requirement for the maintenance and growth of young lambs by the factorial method (Mitchell, 1929; Blaxter & Mitchell, 1948) necessitates the determination of the endogenous urinary nitrogen, the metabolic faecal N and the N requirement per unit live-weight gain. In a previous paper (Walker & Faichney, 1964*a*) values were given for the endogenous urinary N and the metabolic faecal N of young lambs. The present paper describes an experiment in which the live-weight gain and N balance of the milk-fed lamb were measured when different amounts of protein were given. The protein content of the diets varied from 18 to 33% of the dry matter: in comparison, ewe's milk contains about 30% protein on a dry-matter basis (Perrin, 1958).

EXPERIMENTAL

Animals and their management

Twenty-four male Merino lambs were used. The lambs were born at pasture, their birth weights ranged from 2.25 to 5.50 kg and they remained with the ewe for about 1 week after birth. During the experiment the lambs were housed in metabolism cages with wire mesh floors which allowed the urine to be collected quantitatively (Walker & Faichney, 1964*a*). The urine was collected in a beaker which contained sufficient 2.5% (v/v) HCl to keep the urine at an acid pH and was stored at 5°. Faeces were collected in Polythene bags (Walker & Faichney, 1964*a*). After the determination of total N on the fresh faeces at the end of the collection period, the rest of the bulk sample was stored at -15°.

Experimental design

Four dietary treatments were used. The lambs were allotted at random to the diets so that no two lambs received the four diets in the same sequence. Each diet was given for a preliminary period of 1 week followed by a collection period of 1 week, so that each lamb received all four diets over an 8-week period. The lambs were weighed in the morning, before feeding, at the beginning and at the end of the collection period. The mean of these two weights was taken as the mean live weight for the period. For statistical analysis the diet sequences were arranged in replicated Latin squares (Cochran & Cox, 1957) to enable the estimation of the direct effects of the diets, the period effects, the residual (carry-over) effects of the diets and the lamb effects.

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Diets

The composition of the experimental diets is shown in Table 1. The basal diet (no. 3) was spray-dried whole cow's milk prepared commercially. Whole milk was forewarmed at 230 °F for 1 min, and then concentrated to 46% total solids in a double-effect evaporator. The concentrated milk was then spray-dried, with a drying air temperature of 300 °F. The protein concentration was varied by the addition of glucose (diet nos. 1 and 2) or casein (diet no. 4). Sodium citrate (3.6 g/l. milk) was added to prevent the formation of a dense curd in the abomasum (Ling, Kon & Porter, 1961), and to facilitate digestion (Sheehy, 1955). It has been shown (Owen, Jacobson, Allen & Homeyer, 1958) that the reduction of curd tension by sodium citrate does not increase the incidence of nutritional scours. In other experiments (Walker & Faichney, 1964*a, b*) the sodium citrate was omitted without any apparent deleterious effect.

Table 1. *Composition of the diets (values expressed per 100 g dry matter)*

Constituent	'Milk' to contain 15% total solids			
	Diet no. 1	Diet no. 2	Diet no. 3	Diet no. 4
Dried whole milk (g)	61.8	81.0	100	93.6
Glucose (g)	38.2	19.0	—	—
Casein (g)	—	—	—	6.4
Crude protein* (g)	17.6	23.1	28.5	32.8
Ether extractives (g)	17.1	22.3	27.6	25.8
Ash (g)	3.7	4.8	5.9	5.7
Nitrogen-free extractives (by difference) (g)	61.6	49.8	38.0	35.7
Energy (kcal)	483	521	556	556
Lactose (g)	22.6	29.6	36.6	34.2
Protein calories as % of total calories	20.2	24.8	28.7	33.0

* $N \times 6.38$.

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 2 days before the third 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 $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ was added to all diets to increase the concentration of these metals in the dry matter by 50 p.p.m. Fe, 50 p.p.m. Cu and 0.1 p.p.m. Co.

The diets were prepared with distilled water every 2 days and stored at 5°. 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 level of feeding was determined by the live weight of the lamb at the beginning of the preliminary period. It was intended to feed all lambs with milk that contained 15% solids at the rate of 20% of their live weight daily. However, with very young lambs

and with those that weighed more than about 9 kg this quantity was often greater than the appetite of the lamb. For this reason all lambs were fed at 15% of live weight daily in the first period (weeks 1 and 2) and at 20% in the second period (weeks 3 and 4). In the third period (weeks 5 and 6) most lambs were fed at 20%, with some being fed at 15%. In the fourth and final period (weeks 7 and 8) a few lambs were fed at 20%, the majority at 15%, and a few at 12.5% of live weight daily. The energy values of the diets (Table 1) were calculated from the experimentally determined values for the individual dietary constituents as follows: casein 5.575 kcal/g, dried whole cow's milk 5.558 kcal/g, glucose 3.736 kcal/g (all values expressed on a dry-matter basis).

Analytical methods

Dietary constituents. Total N was determined by the Kjeldahl method, with copper and selenium as catalysts, fat by the method of Hopkins, Murray & Campbell (1955) as modified by Walker & Faichney (1964*a*), lactose by the method of Shaffer & Hartmann (1920-1), ash by incineration at 600°, dry-matter content by drying to constant weight at 60° under reduced pressure, and gross energy with the bomb calorimeter (Baird and Tatlock Ltd).

Faeces. Total N was determined in the fresh faeces, and fat, ash, dry matter and gross energy in faeces dried to constant weight at 60° under reduced pressure by the methods used for the dietary constituents.

Urine. Total N was determined by the Kjeldahl method.

Table 2. Mean values with their standard errors and ranges for intakes of protein and energy and mean live weights of the lambs on each diet

	Diet no. 1	Diet no. 2	Diet no. 3	Diet no. 4
Digestible energy intake (kcal/kg per week)	695 ± 21	753 ± 15	825 ± 19	823 ± 22
Range	393-825	601-895	633-943	494-926
Protein* intake (g/kg per week)	27.13 ± 0.87	34.73 ± 0.64	43.62 ± 0.99	49.77 ± 1.31
Range	14.78-32.29	28.49-40.11	35.98-49.93	29.54-56.09
Live weight (kg)	8.20 ± 0.64	8.39 ± 0.68	8.05 ± 0.46	8.27 ± 0.56
Range	2.41-13.18	2.59-14.63	3.23-12.03	3.40-11.86

* N × 6.38.

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 2. The lambs tended to scour when they were given diets nos. 1 and 2, particularly diet no. 1. With some lambs the scouring was so profuse that quantitative separation of faeces and urine was impracticable and the energy content was determined on the mixture. For these lambs, urine N was estimated from the regressions of urine N on N intake and an estimate of the energy content of the urine was made by using a value of 8.5 kcal/g N

(Blaxter, 1961). It was then possible to obtain values for the digestibility of N and of energy. The regression equations, with their residual standard deviations, were:

$$\text{Diet no. 1 } UN = 0.278NI + 6.89 (\pm 4.16), \quad (1a)$$

$$\text{Diet no. 2 } UN = 0.381NI + 2.56 (\pm 3.31), \quad (1b)$$

where UN = urine N (gN) per week and NI = N intake (gN) per week.

Table 3. Mean values with their standard errors for apparent digestibility coefficients of the dietary components

	Diet no. 1	Diet no. 2	Diet no. 3	Diet no. 4
Energy	92.90 ± 0.93	95.75 ± 0.46	97.06 ± 0.33	97.62 ± 0.18
Total N	90.12 ± 0.92	91.81 ± 0.69	94.85 ± 0.44	95.92 ± 0.32
Ether extractives	97.02 ± 0.36	96.65 ± 0.46	97.33 ± 0.50	98.10 ± 0.23
Nitrogen-free extractives	96.49 ± 0.66	97.04 ± 0.57	98.10 ± 0.18	98.15 ± 0.13
Dry matter	94.98 ± 0.61	95.11 ± 0.53	96.24 ± 0.31	96.59 ± 0.26
Digestible protein energy as percentage of digestible energy intake	18.99 ± 0.16	23.76 ± 0.16	28.04 ± 0.06	32.41 ± 0.05

Table 3 gives the mean values with their standard errors for the digestibilities of energy, total N, ether extractives, nitrogen-free extractives (NFE) and dry matter. The mean digestibilities of the energy and the N were higher at the higher levels of protein intake. The differences were highly significant ($P < 0.01$) except for the difference in energy digestibility between diets nos. 2 and 3 ($P < 0.05$), and the differences between diets nos. 3 and 4 in both energy and N digestibility, which were not significant. The mean coefficients of digestibility of dry matter, NFE and ether extractives were similar for all diets.

Table 4. Mean values (g/week) with their standard errors for nitrogen balance of the lambs

Diet no.	No. of lambs in group	N intake	Urine N	Faecal N	N balance
1	17*	30.84 ± 2.78	15.46 ± 1.25	3.18 ± 0.34	12.20 ± 2.09
	24	33.96 ± 2.28	—	—	14.48 ± 1.69
2	23*	45.15 ± 3.46	19.76 ± 1.48	3.59 ± 0.39	21.80 ± 2.07
	24	44.98 ± 3.32	—	—	21.71 ± 1.99
3	23†	54.46 ± 3.04	23.50 ± 1.47	2.77 ± 0.29	28.19 ± 1.77
	24	54.53 ± 2.91	23.64 ± 1.42	3.34 ± 0.64	27.55 ± 1.81
4	24	64.20 ± 4.67	30.46 ± 2.36	2.66 ± 0.35	31.08 ± 2.41

* Omitted lambs with diarrhoea whose urine and faeces collected together.

† Omitted one lamb with high faecal N value.

Nitrogen balance

The mean values for N balance, with their standard errors, are given for each diet in Table 4. For analysis of variance the results were expressed as mg N/kg mean live weight per week and as mg N/100 digestible kcal ingested and were arranged in

replicated Latin squares (Cochran & Cox, 1957). There was no significant residual (carry-over) effect of the diets and neither the lamb nor the period effects were significant. The effect of diet was very highly significant ($P < 0.001$) when the results were expressed in either way. Comparisons between the means for individual diets showed that all differences were significant, with the exception of that between diets nos. 3 and 4.

An analysis of covariance of the relation between N balance and the intake of digestible energy showed that there were highly significant correlations for diets nos. 3 and 4 ($P < 0.001$) and for diet no. 2 ($P < 0.01$). The greater variability between lambs and the inclusion of calculated values reduced the significance of the correlation obtained for diet no. 1 ($P < 0.05$). There were no statistically significant differences between the slopes of the individual regressions relating N balance to the intake of digestible energy. The mean regression was very highly significant ($P < 0.001$). The differences between N balances, when compared at the same intake of digestible energy for each of the diets, were highly significant ($P < 0.01$) with the exception of the difference between diets nos. 3 and 4. These results confirmed those obtained from the Latin square analysis of variance. The equations relating N balance and digestible energy intake, with their residual standard deviations, were:

$$NB_1 = 0.00577DC_1 - 2.34 (\pm 1.01), \quad (2a)$$

$$NB_2 = 0.00577DC_2 - 1.76 (\pm 0.49), \quad (2b)$$

$$NB_3 = 0.00577DC_3 - 1.28 (\pm 0.43), \quad (2c)$$

$$NB_4 = 0.00577DC_4 - 0.93 (\pm 0.48), \quad (2d)$$

where NB = N balance (gN)/kg mean live weight per week, DC = intake of digestible energy (kcal)/kg mean live weight per week and the subscripts refer to diets nos. 1, 2, 3 and 4.

Live-weight gain

The mean values for the live-weight gains of the lambs, with their standard errors, were 65.8 ± 9.7 g/kg mean live weight per week for diet no. 1 (range from -31.2 to 127.0), 90.8 ± 6.7 for diet no. 2 (range $41.1-161.1$), 114.6 ± 7.1 for diet no. 3 (range $13.6-153.3$), and 125.9 ± 6.5 for diet no. 4 (range $45.7-187.1$). For the analysis of variance the live-weight gains were expressed as g/kg mean live weight per week and as g/100 digestible kcal ingested and were arranged in replicated Latin squares (Cochran & Cox, 1957). There was no significant residual (carry-over) effect of the diets and neither the lamb nor the period effects were significant. When live-weight gains were expressed in terms of the mean live weight for the period, comparisons between the means for individual diets showed that all differences were significant with the exception of that between diets nos. 3 and 4.

The mean values for the live-weight gains, with their standard errors, expressed as g/100 digestible kcal ingested were 9.4 ± 1.3 for diet no. 1 (range from -4.1 to 20.1), 12.1 ± 0.9 for diet no. 2 (range $5.9-21.9$), 13.7 ± 0.8 for diet no. 3 (range $2.2-18.8$), and 15.4 ± 0.9 for diet no. 4 (range $6.0-26.5$). Comparisons between the means for individual diets showed that only the difference between diets nos. 1 and 3 and that between diets nos. 1 and 4 were significant.

An analysis of covariance of the relation between the live-weight gain (g/kg mean live weight per week) and the intake of digestible energy (kcal/kg mean live weight per week) showed that the effect of diet, compared at a particular energy intake, was highly significant ($P < 0.01$). However, the absence of significant correlations for diets nos. 1, 2 and 4 makes it questionable whether valid relations can be obtained directly from the data.

A very highly significant correlation ($P < 0.001$) was obtained between N balance (gN/week) and live-weight gain (g/week). An analysis of covariance showed that there were highly significant differences between diets ($P < 0.01$) but not between the slopes of the individual regressions. An analysis of covariance of the relation between N balance and live-weight gain, when each was expressed in terms of the mean live weight for the collection period, also showed very highly significant differences between diets ($P < 0.001$). All individual differences between diets were highly significant, with the exception of that between diets nos. 3 and 4. There were no significant differences between the slopes of the individual regressions. The equations, with their residual standard deviations, were:

$$NB_1 = 0.0147G_1 + 0.70 (\pm 0.68), \quad (3a)$$

$$NB_2 = 0.0147G_2 + 1.25 (\pm 0.51), \quad (3b)$$

$$NB_3 = 0.0147G_3 + 1.80 (\pm 0.45), \quad (3c)$$

$$NB_4 = 0.0147G_4 + 1.97 (\pm 0.60), \quad (3d)$$

where NB = N balance (gN)/kg mean live weight per week and G = live-weight gain (g)/kg mean live weight per week.

Faecal nitrogen

The regression equations relating faecal N and faecal dry matter, with their residual standard deviations, were:

$$FN_1 = 0.041FDM_1 + 1.02 (\pm 0.706), \quad (4a)$$

$$FN_2 = 0.049FDM_2 + 0.73 (\pm 1.170), \quad (4b)$$

$$FN_3 = 0.060FDM_3 + 0.01 (\pm 0.407), \quad (4c)$$

$$FN_4 = 0.060FDM_4 + 0.06 (\pm 0.471), \quad (4d)$$

where FN = faecal N (gN) per week and FDM = faecal dry matter (g) per week.

The regression equations relating faecal N and dry-matter intake, with their residual standard deviations, were:

$$FN_1 = 0.00190DMI_1 + 1.06 (\pm 1.22), \quad (5a)$$

$$FN_2 = 0.00229DMI_2 + 0.73 (\pm 1.63), \quad (5b)$$

$$FN_3 = 0.00212DMI_3 + 0.18 (\pm 1.26), \quad (5c)$$

$$FN_4 = 0.00223DMI_4 - 0.13 (\pm 1.08), \quad (5d)$$

where FN = faecal N (gN) per week and DMI = dry-matter intake (g) per week.

The values for lambs that scoured profusely were omitted in calculating the regression coefficients. The value for one lamb was omitted from the values for diet

no. 3 because of an abnormally high faecal N excretion. 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 highly significant ($P < 0.01$). The mean percentage of N in the dry faeces was 6.0% for diet no. 1 (seventeen lambs), 6.2% for diet no. 2 (twenty-three lambs), 6.0% for diet no. 3 (twenty-three lambs) and 6.1% for diet no. 4 (twenty-four lambs).

Utilization of digested nitrogen

A very highly significant relation ($P < 0.001$) was obtained between N balance and the intake of apparently digested N (ADN). The individual regression coefficients were 0.71 for diet no. 1, 0.60 for diet no. 2, 0.58 for diet no. 3 and 0.51 for diet no. 4. Analysis of covariance showed that these coefficients were not significantly different from the mean coefficient of 0.57 and that there was no significant difference in N balance between the four diets when compared at the same intake of digestible N. Analysis of variance showed that there were no significant differences between the diets in the proportion of the digested N that was retained, confirming the results of the analysis of covariance. The values for the proportion of the digested N retained were 49.3% for diet no. 1, 51.1% for diet no. 2, 53.4% for diet no. 3 and 50.7% for diet no. 4.

DISCUSSION

Digestibility of the diets

Many workers have shown that the digestibilities of dietary dry matter, N, carbohydrate, fat and energy increase as the intake of protein increases. This effect is most often reported for the digestibility of dietary N. Whiting, Slen & Bezeau (1959) found that, when the level of protein in the diet of ewe lambs about 6 months old and on a roughage-type diet was increased, an increase in protein digestibility resulted; there was no significant effect on the digestibilities of dry matter and energy. Cunningham & Brisson (1957) and Brisson, Cunningham & Haskell (1957) showed that when milk-fed dairy calves were given low-protein diets the digestibilities of dietary dry matter, N, carbohydrate, fat and energy were depressed when compared with values obtained with medium- and high-protein diets. The lowered digestibilities were associated with a high incidence of diarrhoea. Blaxter & Wood (1951*a*) observed a marked decrease in dry-matter digestibility and in fat digestibility when Ayrshire calves were given a N-free liquid diet; all calves had diarrhoea on this diet. In an experiment using diets of different protein content, Blaxter & Wood (1951*b*) reported that the digestibilities of the dry matter, fat and protein tended to be higher when the diet high in protein was given. However, the differences were not significant. There was considerable variability in the results and this was attributed to diarrhoea, which varied from mild to acute. In a subsequent experiment, Blaxter & Wood (1952) suggested that the low digestibility of all the dietary components was due to the low level of dietary protein used, and that part of the decline 'was possibly due to the presence of a metabolic component in the faecal excretion of both fat and nitrogen'.

The values for the digestibility of the dietary components obtained in our experiment were a little higher than those of Blaxter & Wood (1951*b*) for calves given liquid diets. Brisson *et al.* (1957) obtained lower results in one trial, but in a second trial their results were in substantial agreement with ours. Adams, Gander, Gullickson & Sautter (1959) gave 'filled milks' (skim milk homogenized with various fats) to dairy calves and also obtained dry-matter digestibilities which were a little lower and fat digestibilities which, with the exception of the butter-oil 'filled milk', were markedly lower than those obtained in our studies with lambs.

There are a number of known factors that could contribute to the decline of digestibility as the protein intake declined, though in our experiment there seemed little doubt that the lowered digestibility was associated with an increase in diarrhoea. It seems likely that this diarrhoea was caused by the high levels of sugar in diets nos. 1 and 2 (Walker & Faichney, 1964*b*).

Faecal nitrogen

Blaxter & Wood (1951*a*) found that the faecal N excretion of calves given a liquid diet based on dried skim cow's milk was not significantly different from the faecal N excretion of the same calves when given a N-free diet. It seems, then, that with diets that contain a highly digestible protein, such as milk protein, faecal N excretion may be a reasonable estimate of metabolic faecal N. This assumption may not, however, be valid when the dry-matter digestibility is lowered by diarrhoea. The value of about 0.2 gN/100 g dry matter ingested (Table 4) is considerably less than the value 0.41–0.45 gN/100 g dry matter ingested obtained by Blaxter & Wood (1951*a*) with calves and is more in line with values quoted for adult non-ruminants (Schneider 1934, 1935). It is in close agreement with the value for lambs of 0.29 gN/100 g dry matter ingested, reported in an earlier paper (Walker & Faichney, 1964*a*). In the study described here a much closer relation was found between faecal N and dry-matter excretion than between faecal N and dry-matter intake, which supports the suggestion of Blaxter & Wood (1951*a*) that the digestibility of the dry matter of the diet determines the metabolic faecal N excretion.

Energy intake and live-weight gain

It is now generally accepted that the basal metabolism of animals is a function of the metabolically effective body-weight rather than of body-weight and that the maintenance requirement must meet the cost of basal metabolism and of normal activity above the basal level. The metabolically effective body-weight of an animal is defined as its live weight raised to a power, which is less than unity (Brody, 1945).

The results of our experiment have been expressed in terms of measured live weight since the values when plotted did not cover a range great enough to show any evidence of curvilinearity. For this reason the procedure of extrapolating the regression lines beyond the limits of the values found may be subject to error and any predictions made can only apply to lambs in the age and weight range used in this experiment, when given similar diets under conditions of restricted activity. Although an analysis of

covariance of the relation between live-weight gain (g/kg mean live weight per week) and the intake of digestible energy (kcal/kg mean live weight per week) showed that there was a highly significant ($P < 0.01$) effect of diet when compared at a particular energy intake, the absence of significant correlations for diets nos. 1, 2 and 4 did not allow the prediction of live-weight gain from digestible energy intake for each diet. Live-weight gain in our experiment was calculated from the two live weights taken at the beginning and at the end of the collection period. The error attached to this method of estimating live-weight gain, as against that based upon the regression analysis of daily weights, would contribute largely to the failure to obtain significant relationships between live-weight gain and digestible energy intake.

Nitrogen intake and nitrogen balance

Allison & Anderson (1945) found that in adult dogs there was a rectilinear relation between the intake of digestible N and N balance in the region of negative N balance and low positive N balance. The relation became curvilinear in the region of positive N balance. However, in our studies with lambs the rectilinear relation applied above maintenance. This finding reflects the intensity of the N metabolism of the young animal and is in agreement with the results obtained with young calves by Blaxter & Wood (1951*b*).

It can be seen from equations (3*a-d*) that, for a particular rate of gain, the values for N balance increased from the 'low-protein' diet no. 1 to the 'high-protein' diet no. 4. These results suggest that the composition of the live-weight gain may have varied from diet to diet. In the absence of carcass-analysis measurements, such an explanation must remain speculative. However, the regression between N balance and live-weight gain can be used to give an indication of the composition of the gain so far as its N content is concerned. The equations for this relation (3*a-d*) show that each unit of gain contained the same amount of N irrespective of diet though, on substituting $G = 0$, which involves extrapolation for diets nos. 2, 3 and 4, it is seen that the N retention at live-weight maintenance increased from the 'low-protein' diet no. 1 to the 'high-protein' diet no. 4. This retention of N not accounted for in the body tissue has been called 'protein reserve' (Maynard & Loosli, 1962). Rubner (1911) was one of the first to put forward the idea of a 'protein reserve' (cited by Kosterlitz & Campbell, 1945-6). However, the capacity of an animal to store protein as 'protein reserve' is limited (Maynard & Loosli, 1962) and, since the analysis of covariance showed that the difference between diet no. 3 and diet no. 4 in the relation between N balance and live-weight gain was not significant, these results would suggest that lambs which were fed on diet no. 4 had almost reached this limit of 'protein reserve'.

The existence of a 'protein reserve' as suggested above can only be confirmed by experiments in which animals can be shown to retain N when live weight is unchanged. In our experiments, only a few of those lambs that were fed on diet no. 1 merely maintained weight, whereas the majority increased in weight. Extrapolation of equations (3*a-d*) was necessary to obtain values for the N retention at live-weight maintenance for all other lambs, a procedure that may be subject to error and that cannot be used to prove the existence of a 'protein reserve'. The extent of the extra-

polation necessary may be judged from the range of live-weight gains for each diet (see p. 299).

The utilization of digested nitrogen

Swanson (1951) stated that N balance is affected by the energy intake and showed with rats that the efficiency of N utilization increased as the energy intake increased, while the intake of dietary N was held constant. Munro (1951) also stated that N balance on a diet was improved by adding extra energy as carbohydrate or fat. Blaxter & Wood (1951*b*) reported that 'for equal amounts of N apparently digested. . . the storage of N increases with decreasing protein content of the diet'. Allison & Anderson (1945) found a linear relation between N balance and the intake of apparently digestible N with dogs in the region of negative and low positive N balance. They termed the regression coefficient the 'N balance index'. Allison (1948) stated that the 'N balance index' was a function of, but not necessarily equal to, the biological value of the protein being given. The regression coefficients for the relation between N balance and the intake of apparently digested N obtained in our experiment may not be equivalent to the 'N balance index' of Allison & Anderson (1945), since our results were obtained in the region of positive N balance. However, the regression coefficients showed a tendency to decrease as the protein content of the diet increased, which suggests that at lower intakes of N relative to energy intake the utilization of the absorbed N was more efficient. This would be in agreement with the findings of other workers, previously mentioned.

Although, as shown earlier, there was no difference between treatments in the relation between ADN and N balance the percentage of digested N that was retained tended to increase as the protein content of the diet increased from diet no. 1 to diet no. 3 and then decreased for diet no. 4. This effect can be accounted for by the greater proportion of digested N that is excreted as endogenous N as the N intake is reduced. The fall in percentage retention between diets nos. 3 and 4 suggests that protein in excess of requirements was being provided in diet no. 4, a finding in keeping with the suggestion that the upper limit of 'protein reserve' had been reached.

SUMMARY

1. Twenty-four male Merino lambs were given a sequence of four diets, which differed in protein:energy ratio, in a replicated Latin square experiment. The protein contents of the diets (on a dry-matter basis) were: 17.6% (diet no. 1), 23.1% (diet no. 2), 28.5% (diet no. 3) and 32.8% (diet no. 4).
2. The daily feed intake of the lambs was regulated according to live weight. Adjustments were made for appetite for very young and for large lambs. Feeding times were 07.00, 14.00 and 22.00 h.
3. The lambs were weighed once a week before feeding. Total collections of faeces and urine were made over 7-day periods for each diet. The collection was made after a 7-day preliminary period.
4. Live-weight gain, nitrogen balance and diet digestibility were calculated for each lamb on each diet.

5. The live-weight gain (g/100 digestible kcal ingested) increased with increasing protein content of the diets, but only the differences between the means of diets nos. 1 and 3 and between diets nos. 1 and 4 were significant.

6. The N balance increased with increasing intake of apparently digested N, and all differences between the means for individual diets were significant, with the exception of that between diets nos. 3 and 4.

7. There was a statistically significant ($P < 0.01$) decrease in the digestibility of dietary protein and energy as the protein content of the diet fell. This decrease in digestibility was attributed to the high incidence of nutritional diarrhoea on the diets of lower protein content.

8. Faecal N was more closely related to dry-matter excretion ($P < 0.001$) than to dry-matter intake ($P < 0.01$). An approximate estimate of 0.2 gN/100 g dry-matter intake was made for the metabolic faecal N.

9. There was a very close relation between N balance and the intake of apparently digested N ($P < 0.001$). There were no significant differences between the diets, although there was a tendency for the regression coefficients ('N balance index') to decrease as the protein content of the diet increased. The mean values for the proportion of the apparently digested N retained from the diets were not significantly different.

10. There was a very close relation between N balance and live-weight gain ($P < 0.001$). The composition of the gain with respect to N, taken from the regression, was the same on all diets. Compared at the same rate of gain, however, N balance increased as the protein content of the diet increased. The increases were statistically significant except for the difference between the N balances at the two highest protein levels. This was explained in terms of a 'protein reserve' of limited capacity, the limit being reached when diets with a protein content similar to the highest level used in this study were given.

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Note added in proof. In paper 1 of this series (Walker & Faichney, 1964*a*) we referred to the classic work of Terroine and his school indirectly by quoting a review article by Smuts (1935). Of course it was Terroine & Sorg-Matter (1927*a, b*) who showed that the excretion of nitrogen in the urine and faeces of a number of animals fed on N-free diets was related to the basal heat production. The relation, which is now termed the Terroine-Sorg-Matter law, expressed as a ratio, varied from 2.3 to 2.9 mg N/basal kcal.