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Nitrogen balance studies with the milk-fed lamb

8*. Labile protein reserves

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1. Three male cross-bred lambs (aged 3 d) were given a nitrogen-free diet for 7 d and then slaughtered (group A). Three more lambs (aged 3 d) were given a medium-protein diet for 14 d, a N-free diet for 7 d, and were then slaughtered (group B).

2. Labile protein reserves were measured during the N-free periods. The reserves of group A were 827 ± 58 mg N per kg live weight (2·8 % of initial body N), and for group B 178 ± 18 mg N per kg live weight (0·6 % of initial body N).

3. The labile protein reserves plus the endogenous urinary N losses were 5·8 and 3·1 % of the initial body N for groups A and B, respectively. These N losses were derived mainly from the carcass (57 %), but also from the skin (29 %) and to a lesser extent from the liver (4 %). The losses from the skin and liver represented 19 and 20 % respectively of their initial N content. The loss of N from other organs such as the lungs, kidneys, spleen and pancreas varied from 2 to 15 % of their initial N content. All organs and compartments lost N during the N-free periods, with the exception of wool, which gained 13 ± 3 % of its initial N content.

Starvation in all animals is associated with an increased excretion of nitrogen in the urine. A part of this excess N arises from the catabolism of body protein, which supplies energy and aids survival. During a 4 d starvation period the pre-ruminant lamb loses 447 ± 37 mg N/d per kg live weight (Walker, 1967). This urinary N loss contains an endogenous N component of 112 ± 11 mg N/d per kg (Walker & Faichney, 1964*a*). Walker (1967) assumed that the excess N lost during starvation represented N derived from muscle catabolism. However, no account was taken of the labile protein reserves (as defined by Allison, Wannemacher & Banks, 1963), which may contribute significantly to the N loss during starvation, and no attempt was made to determine the extent of the N loss from individual organs and tissues.

The experiment now reported was concerned with measuring the labile protein reserves of the pre-ruminant lamb, and determining the relative contributions of individual organs to this reserve.

EXPERIMENTAL

Experimental design

Six male cross-bred lambs, (Border Leicester ♂ × Merino ♀) × Dorset Horn ♂, were taken from the ewe at pasture when they were 3 d of age. Three of the lambs (group A) were fed on a N-free diet for 7 d (N-free period). The other three lambs (group B) were fed on reconstituted dried whole milk (28·7 % protein calories) for

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2 weeks (preliminary period) and were then given the N-free diet for 7 d (N-free period). The cross-bred lambs in group B of this experiment were given exactly the same treatment as four of the Merino lambs (nos. 101, 106, 107 and 111) in the experiment of Walker & Faichney (1964*a*). The apparent digestibility coefficients of the diets and the N balances of the lambs were determined in each period. The urinary N excretion of each lamb was determined daily during the N-free periods.

The mean daily maximum and minimum temperatures in the animal house during the experimental period were 25 and 13° respectively.

The management and feeding of the lambs were as described in the earlier paper (Walker & Faichney, 1964*a*). The diets were similar in composition, except that lard was used as the source of fat in the N-free diet instead of butter oil. The lambs were given sufficient milk to supply 210 kcal (879 kJ) gross energy/kg$^{0.73}$ per d. This intake of gross energy was frequently in excess of appetite during the N-free period.

Comparative slaughter method

The lambs were slaughtered at the end of the N-free periods and some individual organs, together with the skin and the wool, were removed for the estimation of their N content by the Kjeldahl method. The change in the N content of individual organs and compartments of the body was estimated by the comparative slaughter method. The N content of the body and organs of each lamb was estimated at the start of the N-free period by the use of previously established regression equations (Jagusch, Norton & Walker (1970), for lambs in group A; Norton (1968), for lambs in group B). The minimum loss of N from each organ necessary for significance at the 5% level of probability ($n = 3$) was calculated by Stein's two-stage sample test (Steel & Torrie, 1960). Minimum values of N losses for lambs in group A have been given by Jagusch *et al.* (1970) and those for lambs in group B by Norton (1968). When the changes in the N content of individual organs were less than these values they were not considered significant.

Analytical methods

The chemical composition of the dietary constituents, faeces and urine was determined by the methods of Walker & Faichney (1964*a*).

RESULTS AND DISCUSSION

In Table 1 details are given of the experimental design, with the live weights of the lambs at the beginning of each period, the mean live weights per period, and the N content of each lamb at the beginning of the N-free period. In the preliminary period the apparent digestibilities of the dietary constituents for lambs in group B were: dry matter, 97·9 ± 0·4%; energy, 98·0 ± 0·4%; protein, 95·6 ± 0·4%. These values were in good agreement with the digestibility coefficients for this diet determined in previous experiments (Walker & Faichney, 1964*a*, *b*). In the N-free period the digestibility coefficients were much lower than those reported in the earlier experiment (Walker & Faichney, 1964*a*). The values for groups A and B, respectively, in this experiment and, in parentheses, for the lambs in the previous experiment ($n = 6$), were: dry matter, 82·9 ± 1·6 % for group A, 74·8 ± 3·6 % for group B, (92·7 ± 0·7 %); energy, 78·3 ± 2·0 % for group A, 68·0 ± 5·0 % for group B, (95·0 ± 1·0 %). As a result of the low digestibility of the N-free diet, the intakes of digestible energy were also low. Mean values with their standard errors (kcal/d per $kg^{0.73}$) for the intakes of gross energy and, in parentheses, digestible energy were: preliminary period (group B), 208 ± 3 (202 ± 3); N-free period (group A), 188 ± 2 (147 ± 6); N-free period (group B), 190 ± 11 (130 ± 4). In the previous experiment the energy intakes in the preliminary period were 164 ± 3 (158 ± 3), and in the N-free period 169 ± 3 (159 ± 2). As a result of these low intakes of digestible energy the lambs in group B lost more weight when given the N-free diet than lambs in the previous experiment. It is axiomatic that, when measurements of the endogenous urinary N excretion are being made, the animal must consume sufficient of the N-free diet to prevent the breakdown of body protein as a source of energy. Analyses of the bodies of the lambs in groups A and B at the end of the N-free period showed that they had gained fat during the N-free period. Thus, it was assumed that their intakes of digestible energy were adequate and that the urinary N losses were truly endogenous.

Table 1. *Initial age of lambs 1–3 (group A) and 4–6 (group B), live weight at the beginning of each period, mean live weight per period, and total body nitrogen at commencement of the N-free period*

	Group A			Group B		
	1	2	3	4	5	6
Age at commencement of expt(d)	3	3	3	3	3	3
Preliminary period (d)	0	0	0	14	14	14
Initial live weight (kg)	—	—	—	4·96	5·01	4·34
Mean live weight (kg)	—	—	—	5·65	6·04	5·21
N-free period (d)	7	7	7	7	7	7
Initial live weight (kg)	5·22	4·41	4·25	6·78	6·98	5·99
Mean live weight (kg)	5·34	4·49	4·31	6·67	6·76	5·87
Initial total body N (g)	155·1	132·0	127·5	194·4	199·7	174·1

The mean values for the metabolic faecal N excretion (MFN), with their standard errors ($n = 6$), were 0·26 ± 0·01 g N/100 g dry-matter intake and 1·29 ± 0·16 g N/100 g faecal dry matter. The comparable values of Walker & Faichney (1964*a*) were 0·29 and 3·86 respectively.

Endogenous urinary N

The daily urinary N excretions of all lambs are given in Table 2. The individual N balances given in Table 3 are based on a 4 d collection during the preliminary period (group B) and on a 3 d collection during the N-free periods (groups A and B). Urine was collected in the N-free period for the estimation of the endogenous urinary N (EUN) excretion when it was judged that the daily urinary N excretion had reached a low, yet constant, level. The mean value for EUN of 119 ± 3 mg N/d per kg mean live weight (range 111–128) in the present experiment, was not significantly different from the mean value of 112 ± 10 (range 80–156) reported in the previous experiment with Merino lambs (Walker & Faichney, 1964*a*). The mean value for the EUN excretion of all lambs ($n = 12$), in this and the previous experiment, was 117 ± 5 mg N/d per kg mean live weight.

When expressed in relation to the body N content of each lamb at the start of the N-free periods, the EUN values in this and in the previous experiment were 397 ± 13 and 393 ± 32 mg N/d per 100 g body N respectively. The initial body N contents of

Table 2. *Urinary nitrogen excretion* (g/24 h) of lambs 1–3 (group A) and 4–6 (group B) given respectively a N-free diet immediately after being taken from the ewe at 3 d of age, and reconstituted dried-milk diet during the preliminary period, followed by a N-free diet*

	Group A			Group B		
Day	1	2	3	4	5	6
			Preliminary period			
11	—	—	—	2·37	2·29	2·20
12	—	—	—	2·26	1·99	2·01
13	—	—	—	2·20	2·20	2·04
14	—	—	—	2·48	2·41	2·35
			N-free period			
1	**2·63**	**2·15**	**2·46**	**1·26**	**1·21**	**1·08**
2	**2·13**	**1·49**	**1·91**	**0·82**	**0·94**	**0·81**
3	**1·13**	**0·97**	**0·97**	**1·08**	**1·00**	**0·73**
4	**0·94**	**0·91**	**0·90**	**0·96**	**0·91**	**0·84**
5	0·71	0·54	0·66	0·71	0·83	0·59
6	0·66	0·54	0·50	0·72	0·68	0·63
7	0·69	0·47	0·47	0·74	0·79	0·80

* Values boxed are for the samples taken for N balance and for endogenous N. Values in boldface type are those used for the calculation of protein reserves.

Table 3. *Nitrogen balance (g/24 h) and live-weight change (g/24 h) of lambs 1–3 (group A) and 4–6 (group B) given respectively milk from the ewe in the first 3 d of life, and a reconstituted dried-milk diet in the preliminary period, followed in both groups by a N-free diet*

Lamb no.	N intake	Faecal N	Urinary N	N balance	Live-weight change
		Preliminary period			
4	6·03	0·28	2·33	+3·42	+122
5	6·08	0·18	2·22	+3·68	+137
6	5·49	0·26	2·15	+3·08	+110
Mean	5·87	0·24	2·23	+3 40	+123
		N-free period			
1	0·00	0·26	0·69	−0·95	+9
2	0·00	0·17	0·52	−0·69	+4
3	0·00	0·15	0·54	−0·69	+6
Mean	0·00	0·19	0·58	−0·77	+6
4	0·00	0·26	0·72	−0·98	−38
5	0·00	0·28	0·77	−1·05	−70
6	0·00	0·26	0·67	−0·93	−48
Mean	0·00	0·27	0·72	−0·99	−52

lambs nos. 108 and 109 in the previous experiment were estimated from body-weight, by using regression equations derived from values for lambs that had been given a diet with a protein-calorie concentration of 10% (Norton, Jagusch & Walker, 1970). The EUN excretion in the present experiment was more closely related to live weight (kg) and to total body N (g N) than to metabolic body size ($kg^{0.73}$).

Labile protein reserves

The labile protein reserve of each lamb was calculated, by subtracting the value for the EUN excretion from the total urinary N excretion during the first 4 d of the N-free period. In making the calculations it was assumed that some tissue proteins were depleted very rapidly when the lambs were given a N-free diet. Such an effect,

Table 4. *Labile protein reserves of lambs given diets of different protein content in the period immediately preceding the nitrogen-free period*

Protein-calorie concentration of preliminary diet	No. of lambs	Labile protein reserves g N/4 d	mg N/kg	As % of initial body N
Colostrum (high)	3	3·9	827	2·8
29 % (medium)	7*	1·9	325	1·1
10 % (low)	2†	0·9	202	0·7

* Results include values for four lambs in an earlier experiment (Walker & Faichney, 1964*a*).
† Results calculated from values for lambs in an earlier experiment (Walker & Faichney, 1964*a*).

which has been seen in the young rat after 12 h on a starvation diet, has led to the procedure suggested by Allison (1964), which we have adopted, for determining labile protein reserves. It is known that the size of the labile protein reserve in rats (Henry, Cormack & Kosterlitz, 1961) and in children (Chan, 1968) is affected by the previous protein intake.

In the present experiment it was not possible to determine the previous protein intake of lambs in group A. However, it is known that the protein concentration in ewe's colostrum is higher than that in the milk (Perrin, 1958) and higher labile protein reserves in the colostrum-fed lambs (group A) could be expected. The reserves of group A were 827 ± 58 mg N per kg live weight (2·8% of initial body N) and for group B 178 ± 18 mg N per kg live weight (0·6% of initial body N). In Table 4 mean values are given for the labile protein reserves of lambs in groups A and B in this experiment, and for the two groups of lambs in the previous experiment, calculated as described above. It is clear that the labile protein reserve of the lamb is significantly affected by the intake of protein immediately before the N-free period.

Changes in body and organ N content

In Table 5 mean values are given for the initial N content of the body, for the distribution of N in the separate organs, for the change in N content during the N-free period, for the significance of these changes in individual organs, and for the relative contribution of the individual organs to the total N loss. The skin and liver lost a

Table 5. *Mean values for the distribution of body nitrogen at the beginning of the N-free period, the N change and the distribution of the N loss of the three lambs per group given the N-free diet*

	Initial distribution of body N (%)†		Change in N content of individual organs (%)				Significance of change in organ N		Distribution of body N loss (%)	
	Group A	Group B	Group A		Group B		Group A	Group B	Group A	Group B
Total body	100·0	100·0	−9		−6		*	*	—	—
Carcass	61·4	61·9	−9		−7		*	*	53·9	60·4
Skin	14·2	12·4	−20		−18		*	*	27·7	30·2
Wool	8·0	10·0		+14		+12	*	*	—	—
Liver	2·0	1·8	−25		−14		*	*	5·0	3·6
Residual‡	6·0	5·8	−10		−6		*	*	5·7	5·0
Blood	5·3	5·6	−7			+3	NS	NS	3·5	—
Lung	1·6	1·3	−23		−2		*	NS	3·5	0·1
Heart	0·7	0·5		+7		+5	NS	NS	—	—
Kidneys	0·5	0·4	−16		−4		NS	NS	0·7	0·1
Spleen	0·2	0·2		+15	−16		NS	*	—	0·4
Pancreas	0·1	0·1	−5		−25		NS	NS	—	0·1
	Initial body N (g)		N loss (g)	N gain (g)	N loss (g)	N gain (g)				
	138·2±8·5	189·4±7·8	14·1	1·7	13·9	2·6	—	—	—	—

NS, not significant. * $P < 0·05$.

† Calculated from regression equations (see p. 8). ‡ Includes digestive tract, reproductive organs and mesenteries.

relatively greater proportion of their initial N content than did the other organs and, together with the carcass and digestive tract, contributed over 90% of the total N loss from the body.

Wool was the only N reservoir that showed a significant gain in N during the N-free period. This capacity of the wool follicle to maintain production under adverse nutritional conditions is well documented (Marston, 1955; Walker & Cook, 1967).

Table 6. *Mean values and ranges for the partition of the nitrogen loss (mg N/kg) in the urine of lambs during a 4 d starvation period*

N fraction	No. of lambs	Mean	Range
Total loss*	5	1788	1335–2218
Endogenous loss†	12	468	322–624
Labile reserve‡	7	325	145–531
Tissue catabolism (by difference)	—	995	—

* Lambs given a medium-protein diet immediately preceding starvation (Walker, 1967).

† Mean for six lambs from the present experiment and six lambs from the experiment of Walker & Faichney (1964*a*), given low-, medium- or high-protein diets before N starvation.

‡ Mean for three lambs from the present experiment (group B) and four lambs from the experiment of Walker & Faichney (1964*a*), given a medium-protein diet before N starvation.

Tissue catabolism during starvation

In Table 6 a calculation is made of the N derived from tissue catabolism during starvation, after allowance has been made for endogenous N losses and for the contribution of the labile protein reserves. These values lead to the conclusion that, when lambs that have been fed on a medium-protein diet are starved, about one-half of the urinary N excreted during the first 4 d of starvation is derived from tissues catabolized as a source of energy, about one-quarter is from endogenous sources, and one-fifth is contributed by the labile protein reserves.

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