Protein nutrition of growing lambs

2. Effect on nitrogen digestion of supplementing a low-protein–cellulosic diet with either urea, casein or formaldehyde-treated casein

BY T. J. KEMPTON, J. V. NOLAN AND R. A. LENG

Department of Biochemistry and Nutrition, Faculty of Rural Science, University of New England, Armidale, NSW 2351, Australia

(*Received* 9 *June* 1978 – *Accepted* 9 *May* 1979)

1. Lambs with cannulas in the duodenum and ileum were allowed free access to one of four diets: a basal diet of oat hulls and solka floc, or the basal diet supplemented with either urea, urea plus casein or urea plus formaldehyde-treated (HCHO)-casein. Mean nitrogen intake was 1·9 g N/d for the basal diet and 15·0, 32·4 and 36·9 g N/d respectively for the other diets.

2. The rate of irreversible loss of ammonia from the rumen pool estimated using $^{15}NH_4^+$ was highest on the casein diet (33 g NH_3-N/d) by comparison with 18 g NH_3-N/d for the urea and HCHO-casein diets and 7 g NH_3-N/d for the basal diet.

3. The proportions of bacterial and protozoal N in the rumen derived from rumen ammonia did not differ significantly between the supplemented diets and were 0·66 and 0·52 respectively.

4. Estimation of ^{15}N flowing to the duodenum during continuous infusions of $^{15}NH_4^+$ into the rumen indicated considerable ammonia absorption from the rumen on all the diets. Greatest absorption of ammonia (21 g N/d) apparently occurred in animals on the diet supplemented with urea and casein.

5. The estimated microbial non-ammonia-N (NAN) flowing out of the rumen per unit organic matter fermented in the rumen (FOM) was similar on all diets, i.e. 21·3 (±1·09) g N/kg FOM. The requirement for dietary fermentable N for microbial N production on these diets was 1·2 (±0·07) g N/MJ ME.

6. The flow of NAN into the duodenum and through the ileum, and total N in the faeces was significantly influenced by the form of N supplementation. The flow of NAN into the duodenum for the HCHO-casein diet (27 g N/d) was more than twice that for the other diets (11 g N/d). The flow of NAN through the ileum and excretion of total N in the faeces was also greater with the HCHO-casein diet than with all other diets. The apparent digestibility of NAN in the small intestine ranged between 0·62–0·66 for all diets.

7. Urea and casein supplements were apparently completely degraded in the rumen. In contrast, the HCHO-casein was almost completely resistant to degradation in the rumen and only 65% of the HCHO-casein was digested in the small intestine.

8. Protein absorbed : energy absorbed (expressed as NAN digested in the small intestine/MJ ME) was calculated to be 5·5 (±0·70) for the basal, urea and urea-plus-casein diets, and 11·6 (±1·71) for the urea-plus-HCHO-casein diet.

In the previous paper in this series it has been demonstrated that, in lambs given a low-protein–cellulose-based diet, responses in food intake and growth were obtained to supplementation with N forms that are rapidly degraded to ammonia in the rumen (i.e. urea or casein) and greater responses were obtained when a protein was given in a form which escaped rumen fermentation (as formaldehyde-treated (HCHO)-casein, Kempton & Leng, 1979). The growth responses were attributable to an increased food intake and the greatest response resulted from a supplement containing both HCHO-casein and urea. Although food intake was increased by supplementation, the proportion of the dietary organic matter (OM) that was digested in the rumen was unchanged (Kempton & Leng, 1979).

It was concluded that the increases in food intake and growth in lambs under such conditions are probably attributable to an increased supply of amino acids to the animal. It is not known how much of the increased supply of amino acids is derived from the supplement and how much is from an increase in microbial outflow from the rumen. As well as altering the amount of dietary protein that enters the duodenum, provision of

0007-1145/79/3173-0905 $01.00

soluble or insoluble proteins may also affect the efficiency of microbial protein synthesis and the flow of microbial protein from the rumen (Hume, 1970). For example changes in the microbial associations in the rumen as a result of changes in the flow through the rumen, and also changes in the rumen environment which may be brought about directly by supplementation or indirectly by increased food intake can increase the availability of microbial protein.

The objectives of the present study were to examine quantitatively the relationship between the food intake and growth response of lambs in relation to the availability of protein for digestion and absorption and also to examine some aspects of N digestion in the rumen and small intestine.

EXPERIMENTAL

The details concerning experimental animals, diets, sample collection procedures and proximal analysis methods are given by Kempton & Leng (1979).

An intraruminal infusion of $(^{15}NH_4)_2SO_4$ (97 atom %, 0·013 mg/ml; 0·65 ml/min) was begun at 09·00 hours on day 1 (i.e. day 11 in the schedule for Expt 3 described by Kempton & Leng, 1979). Samples of rumen fluid (10 ml) were obtained at 12 h intervals on days 1 and 2 and at 6 h intervals on day 3. The samples were transferred to centrifuge tubes and stored in ice until procedures for the isolation of microbes were commenced (within 15 min). Additional rumen fluid samples for analysis for enrichment of ammonia were obtained at 30 min intervals of the final 4 h of the infusion. Blood and urine samples (the latter collected over 1 h) were obtained at approximately 20.00 hours on day 3 and were stored at −20°. Glacial acetic acid (20 ml/d) was used as a urine preservative.

Isolation of protozoa and bacteria-rich samples from rumen fluid

Samples of bacteria and protozoa were prepared from rumen fluid by differential centrifugation methods (see Nolan & Leng, 1972).

Isolation of microbial protein from duodenal digesta

Microbe-rich samples were isolated from bulked duodenal digesta collected on day 3 by the procedures of Harrison *et al.* (1972).

Analytical methods

Ammonia in rumen fluid or urea-N in plasma and urine were isolated by the methods described by Nolan & Leng (1972). Total N in the digesta and faeces samples was converted to $(NH_4)_2SO_4$ by Kjeldahl digestion using selenium as a catalyst. The ammonia (0·6–1 mg N) from these samples was isolated by steam distillation and the enrichment of the N in the samples was determined using a mass spectrometer and methods described by Nolan & Leng (1972, 1974).

The mass spectrometer (model MS10, GEC-AEI (Electronics) Ltd, Manchester, England) was fitted with a dual inlet system which makes it possible for each unknown sample to be immediately compared with a laboratory standard. With this method, the effect of some potential errors in the analysis (e.g. residual background peaks), that are common to both the standard and unknown samples, can be removed and precision increased. The repeatability of the ^{15}N analysis was checked by repeatedly analysing two enriched $(NH_4)_2SO_4$ samples during routine procedures i.e. low enrichment standard 0·001 (SE 0·0002, n 63); high enrichment standard 1·72 (SE 0·006, n 21).

Other analytical methods are described by Kempton & Leng (1979).

Calculations

The rate of irreversible loss of rumen ammonia was calculated from the plateau enrichments in rumen ammonia obtained during an $^{15}NH_4^+$ infusion as described by Nolan & Leng (1972). The proportion of N in secondary pools derived from the primary pool (rumen ammonia) was also calculated by methods previously described (see Nolan & Leng, 1972).

Enrichment ratio

The value, mean enrichment of isolated microbial N from either rumen or duodenal digesta: enrichment of total NAN in the duodenal digesta (enrichment ratio) is used in the same way as the M:D ratio of Harrison, *et al.* (1972).

Estimation of digesta flow

The flows of digesta into the duodenum and through the ileum were estimated by reference to the recovery of non-absorbable markers, i.e. ^{51}Cr-EDTA and ^{103}Ru-P (see Tan *et al.* 1971; Faichney, 1975), as described by Kempton & Leng (1979).

Microbial N outflow from the rumen

The flow of microbial non-ammonia-N (NAN) into the duodenum was given by the product of enrichment ratio and the flow of total NAN. Microbial OM flows were calculated assuming that 1 g microbial NAN is contained in 12 g microbial OM (Hungate, 1966).

Dietary and endogenous N outflow

The dietary (and endogenous) N outflow from the rumen was calculated by subtracting the microbial NAN from the total NAN outflow from the rumen.

N digestion in the small intestine and hind gut

Since the flows of nitrogenous components into the duodenum and through the ileum were measured 3 d apart (see Kempton & Leng, 1979), and food intake was sometimes variable over this period of time, it was not possible to directly estimate, in any one animal, the digestibility of N in the small intestine. A regression approach based on equations given in Table 5 was used to allow for variations in digesta flows in relation to metabolizable energy (ME) intake. Using the mean food intake for the 3 d period during which the measurements were made, the apparent digestibility of the various components was calculated as:

$$\frac{\text{predicted duodenal flow} - \text{predicted ileal flow}}{\text{predicted duodenal flow}}$$

Rate of ammonia absorption from the forestomachs

This was estimated from the results of the $^{15}NH_4^+$ infusion assuming that steady-state conditions applied and that there was a balance between the ^{15}N infused into the rumen and the ^{15}N leaving by either absorption from the forestomach or flow in the digesta to the duodenum. The latter was estimated directly by multiplying the digesta flow by its ^{15}N content, and the ^{15}N absorption from the forestomachs was obtained by difference. It seemed reasonable to assume this absorbed ^{15}N was derived from $^{15}NH_4^+$ in rumen fluid and therefore ammonia absorption rate was calculated as follows:

$$\text{ammonia absorption (atoms N/d)} = \frac{^{15}\text{N not accounted for in flow to the duodenum (atoms } ^{15}\text{N/d)}}{\text{enrichment of rumen ammonia-N (atoms } ^{15}\text{N/total N atoms)}}$$

Dynamics of N metabolism in the rumen of lambs on each diet

Because no direct estimate of the rate of irreversible loss of rumen ammonia was available for animals on the basal diet, a value was obtained from the regression of irreversible loss *v.* rumen ammonia concentration for animals on the supplemented diets (see equation p. 307).

Methods for obtaining models of N dynamics in the rumen of lambs

From the measurements of N metabolism in the rumen and with some assumptions (see later) the results have been translated into relatively simple models which summarize the flows of N in the rumens of the animals on the four diets. These models are included to allow the major differences in N metabolism between diets to be readily depicted and the values should be interpreted cautiously because of the assumptions used to simplify and also to balance the models. In particular it was necessary to assume that there were endogenous inputs of N in order to balance each model. The minimum amount of N needed was assumed. The procedures and the assumptions used are as follows: (*a*) dietary N was partitioned into protein and non-protein-N (NPN) (urea). It was assumed that all the NPN entered the ammonia pool; (*b*) the flow of N into the duodenum was partitioned into flows of ammonia, microbial NAN and dietary NAN. It was assumed that all undigested endogenous secretions entering the duodenum were included in the outflow of unfermented dietary NAN; (*c*) the proportion of microbial N derived from the ammonia pool (Table 3) was used in conjunction with the rate of outflow of microbial NAN to obtain an estimate of the quantity of ammonia-N entering the microbial pool; (*d*) endogenous inputs were calculated in two stages: first, it was assumed that the input of endogenous N into the protein pool was zero and a minimum estimate of the entry of N from protein into the ammonia pool was calculated, then using values for the rates of absorption of ammonia as calculated previously, another minimum value for endogenous input into the ammonia pool was calculated in order to balance the inflows and outflows of that pool.

Statistical analyses

Differences between means adjusted by covariance techniques to the same ME intake were analysed statistically as described by Kempton & Leng (1979).

RESULTS

N balance

When all diets were compared at the same ME intake by covariance techniques, N stored in the body was not significantly ($P > 0{\cdot}05$) different between diets and was therefore apparently not affected by the form of the N supplement. When the results were adjusted to the same ME intake there was a significantly ($P < 0{\cdot}01$) greater faecal N excretion in lambs on the diet containing HCHO-casein. The mean values for N intake (g N/d) and urinary and faecal losses of N are given in Table 1.

Rate of irreversible loss of rumen ammonia

In the supplemented animals, a 'plateau' enrichment in rumen ammonia was obtained after approximately 20 h infusion of $^{15}NH_4^+$ and irreversible loss was calculated from samples taken during the last 4 hours of infusion. In animals on the basal diet rumen ammonia concentrations were extremely low (< 6 mg N/l) and satisfactory estimates of enrichment and the rate of irreversible loss of ammonia could not be obtained.

In the supplemented lambs the rate of irreversible loss of ammonia from the rumen

Table 1. *Nitrogen balance (g/d) for lambs given a basal low-protein diet (A), plus urea (B), and either urea plus casein (C) or urea plus formaldehyde-treated (HCHO)-casein (D)*†

(Each value is the mean of four observations)

	Diet				Significance of difference between means	SEM
	A	B	C	D		
N intake	1·9[a]	15·0[b]	32·4[c]	36·9[c]	*	2·94
Faecal N	2·5[a]	4·2[ab]	6·2[b]	11·0[c]	*	1·04
Urine-N	1·1[a]	6·5[a]	15·0[b]	12·4[b]	*	1·83
N balance	−1·7[a]	4·3[a]	11·2[b]	13·6[b]	*	2·26

a, b Values within the same horizontal row with different superscripts are significantly different ($P < 0·05$).
* $P < 0·01$.
† For details of diets, see Kempton & Leng (1979).

Table 2. *Rate of irreversible loss of ammonia (gN/d) and ammonia concentration (mgN/l) in rumen fluid of lambs given a basal low-protein diet (A), plus urea (B) and either urea plus casein (C) or urea plus formaldehyde-treated (HCHO)-casein (D)*†

(Each value is the mean of four observations)

	Diet				Significance of difference between means	SEM
	A	B	C	D		
NH_3 production	6·5[a]‡	17·5[b]	32·7[c]	17·9[b]	*	3·06
NH_3 concentration	5·2[a]	190·3[b]	518·7[c]	252·0[b]	*	42·4

a, b, c Values within the same horizontal row with different superscripts are significantly different ($P < 0·05$).
* $P < 0·01$.
† For details, see Kempton & Leng (1979).
‡ Predicted from rumen NH_3 concentration (see calculations p. 307).

ammonia pool was highest in lambs given the diet containing urea plus casein (Table 2). The regression of the rate of irreversible loss of ammonia (Y, g N/d) *v.* ammonia concentration (X, mg NH_3-N/l) for lambs on the diets supplemented with urea, urea plus casein and urea plus HCHO-casein was as follows:

$$Y = 0·05\ (\pm 0·008)\ X + 6·28, \quad R^2\ 0·82, \quad \text{residual SD } 4·30.$$

This regression equation was used to predict the rate of irreversible loss of rumen ammonia in lambs on the basal diet (Table 2).

The proportions of bacterial and protozoal N in the rumen and also of plasma urea-N and urinary urea-N arising from rumen ammonia are given in Table 3. There were no significant differences between diets in the proportions of bacterial N (0·66) or protozoal N (0·52) derived from rumen ammonia.

The 'plateau' enrichments of urinary urea-N or plasma urea-N were not significantly different for any one animal indicating that urinary urea was derived only from plasma urea. The proportions of plasma (and urinary) urea-N derived from rumen ammonia in sheep on the diet containing urea plus casein (0·66) were significantly greater than for sheep on other diets (0·42) (Table 3).

Microbial NAN leaving the rumen

There was no significant difference between the plateau enrichment of bacterial N in the rumen and microbial N isolated from the duodenum in any one animal. Therefore the mean enrichment of the two samples was used to calculate the enrichment ratio and hence the proportions of microbial NAN in total NAN in duodenal digesta.

Table 3. *Percentage of bacterial-N, plasma urea-N and urine urea-N derived from rumen ammonia in lambs given a basal low-protein diet (A), plus urea (B) and either urea plus casein (C) or urea plus formaldehyde-treated (HCHO)-casein (D)*†

(Each value is the mean of four observations)

	Diet				Significance of difference between means	SEM
	A	B	C	D		
Bacterial N	64·7‡	70·6	69·4	60·8	NS	7·25
Protozoal N	32·9‡	64·3	55·8	35·9	NS	9·05
Urine urea-N	35·2[a]	43·3[a]	69·9[b]	33·5[a]	**	6·73
Plasma urea-N	39·7[a]	48·9[a]	65·8[b]	36·1[a]	*	5·43

a, b Values within the same horizontal row with different superscripts are significantly different ($P < 0.05$).
NS, Not significant.
* $P < 0.05$, ** $P < 0.01$.
† For details, see Kempton & Leng (1979).
‡ These values required estimations of ammonia enrichment based on predicted rumen ammonia irreversible loss rate and tracer infusion rate. These values were not included in the statistical comparison.

In lambs given the basal diet, and the diets with urea and urea plus casein, the mean (± SE) value for the enrichment ratio was 1·07 (± 0·079) indicating that 93% of the NAN entering the duodenum was of microbial origin. In animals supplemented with HCHO-casein, the enrichment ratio was 3·3 (± 0·28) indicating that only 30% of the duodenal NAN was from microbes. The remainder was therefore unfermented dietary and endogenous NAN.

The rate of microbial NAN flow into the duodenum was significantly ($P < 0.01$) greater in lambs on all supplemented diets than those on the basal diet. Furthermore, the flow of microbial N in lambs supplemented with urea plus casein was significantly ($P < 0.01$) greater than for those receiving the other two supplements (see Fig. 1 for mean values). When adjusted to a common ME intake, the flows of microbial NAN in the lambs receiving either the urea, or the urea-plus-casein-supplemented diets were significantly ($P < 0.01$) greater than in those receiving the basal or urea + HCHO-casein-supplemented diets. The relationship between the microbial NAN flow to the duodenum and ME intake is given in Fig. 2.

The estimated net yield of microbial protein (NAN × 6·25) per unit dietary OM fermented in the rumen (FOM) did not differ significantly between diets, i.e. 21·3 ± 1·09 g N/kg FOM.

N flows in the intestines

The flows of NAN into the duodenum and also through the ileum, and the loss of N in faeces were significantly ($P < 0.01$) greater in lambs receiving the diet supplemented with urea and HCHO-casein than in lambs given the other diets, for which these measurements were not significantly different (Table 4). The flow of NH_3-N into the duodenum of sheep given urea plus casein was significantly ($P < 0.01$) greater than in sheep given the other diets for which the flows did not differ significantly (see Fig. 1). There were no significant differences in NH_3-N flow through the ileum in animals given these diets (i.e. mean 1·1 g N/d). The regression equations describing these and other relationships between ME intake and the flows of N in various components of digesta at the duodenum, ileum or in the faeces are given in Table 5. In lambs given the diet containing urea plus HCHO-casein the flows of total N through the duodenum (35 g N/d) and ileum (13·4 g N/d) were significantly ($P < 0.01$) greater than in lambs given the other diets for which the flows were

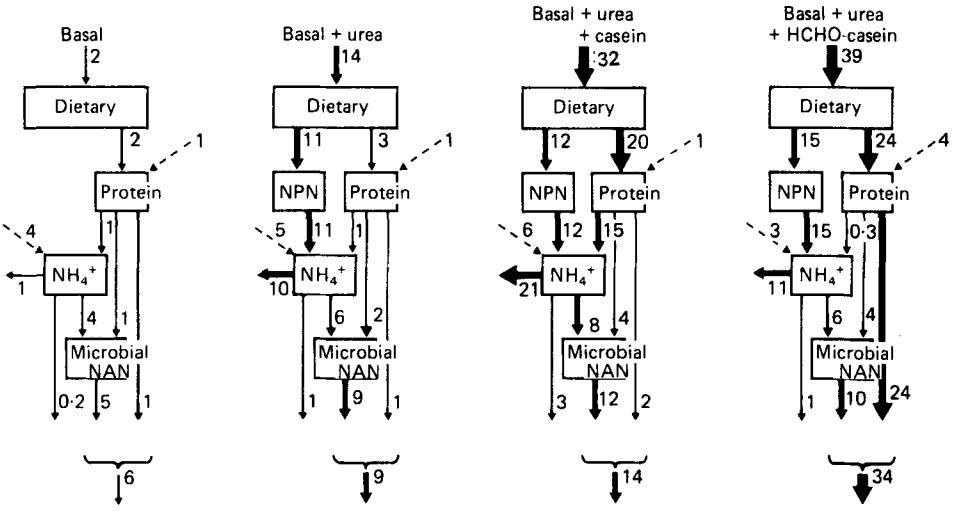

Fig. 1. Flows of nitrogen (g N/d) in the rumens of lambs given a basal low-protein diet (A), plus urea (B) and either urea plus casein (C) or urea plus formaldehyde-treated (HCHO)-casein (D) (for details of diets, see Kempton & Leng, 1979). Only the major pools and pathways of N transactions in the rumen are shown. NAN, non-ammonia-N; NPN, non-protein-N.

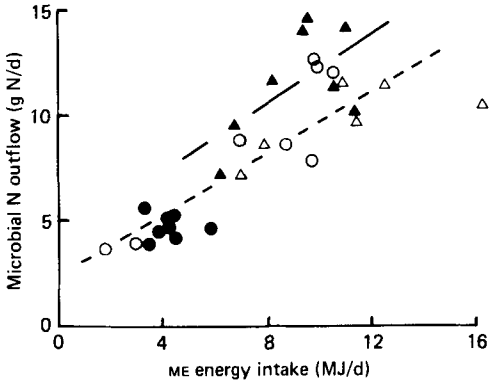

Fig. 2. Microbial nitrogen outflow (g N/d) from the rumen in relation to metabolizable energy (ME) intake (MJ/d) in lambs given either a basal low-protein diet (●), plus urea (○), and either urea plus casein (▲) or urea plus formaldehyde-treated (HCHO)-casein (△) (for details of diets, see Kempton & Leng, 1979). The regression equations (± SE) of the regression coefficients are given in Table 5.

not significantly different (i.e. 10·8 and 4·6 g N/d for duodenal and ileal N flows respectively).

Apparent N digestibility in the small intestine

The NAN flowing into the duodenum and through the ileum was predicted from the regression equations given in Table 5 using the mean ME intakes during the 3 d when NAN flows at each of these sites were being estimated. Using mean ME intakes of the lambs on the four diets i.e. 4·3, 7·5, 9·1 and 11·8 MJ/d respectively, the mean apparent digestibilities of NAN in the small intestine were calculated to be 0·63, 0·64, 0·65 and 0·68 for each diet respectively.

Table 4. *Flows of various fractions of N(g/d) in duodenal and ileal digesta of lambs fed a basal low-protein diet (A), plus urea (B) and either urea plus casein (C) or urea plus formaldehyde-treated (HCHO)-casein (D)*†

(Values adjusted to a common intake of metabolizable energy (ME) (MJ/d) by covariance analysis are also given; each value is the mean of eight observations)

	Diet				Significance of difference between means	SEM
	A	B	C	D		
Flow into the duodenum:						
Unfermented dietary NAN	0·2[a]	1·3[a]	1·8[a]	23·8[b]	**	1·83
NH_3-N	0·2[a]	1·0[ab]	2·7[c]	1·7[b]	**	0·35
Total NAN	5·0[a]	10·2[a]	12·2[a]	33·7[b]	**	2·03
Total N	5·2[a]	11·2[b]	16·1[b]	35·4[c]	**	2·09
Flow into the ileum:						
NAN	1·9[a]	5·6[b]	3·7[ab]	11·6[c]	**	0·83
NH_3-N	0·3	1·1	1·2	1·8	NS	0·28
Total N	2·2[a]	6·7[b]	4·9[b]	13·4[c]	**	0·92

	Diet				Significance of difference between		SE of difference of adjusted mean
	A	B	C	D	slopes	means	
Flow into the duodenum:							
Dietary NAN	3·0[a]	2·4[a]	2·7[a]	19·9[b]	NS	**	1·63
NH_3-N	0·6[a]	1·1[a]	2·5[b]	1·3[a]	**	not tested	0·39
Total NAN	10·6[a]	11·1[a]	12·8[a]	26·9[b]	*	not tested	1·31
Total N	11·2[a]	12·5[ab]	15·6[b]	28·6[c]	NS	**	1·77
Flow into the ileum:							
NAN	3·3[a]	5·1[a]	3·6[a]	10·3[b]	NS	**	1·30
NH_3-N	0·9	1·0	1·0	1·5	NS	NS	0·43
Total N	3·7[a]	6·8[a]	4·7[a]	12·0[b]	NS	**	1·47

a, b Values within the same horizontal row with different superscripts are significantly different ($P < 0·05$).
NS, Not significant.
* $P < 0·05$, ** $P < 0·01$.
† For details of diets, see Kempton & Leng (1979).

Ammonia absorption from the forestomachs

The proportion of $^{15}NH_4^+$ infused into the rumen that flowed out in duodenal digesta decreased with increasing dietary N intake (Fig. 3). Mean values for the apparent ammonia absorption from the forestomachs for animals on the four diets are shown in Fig. 1. These are slight underestimates of the true values since ammonia-N cycling exterior to the rumen has not been included in the calculations. Ammonia absorption occurred on all diets but was highest in animals given the diet containing urea plus casein. The relationship between apparent ammonia absorption and rumen ammonia concentration is given in Fig. 4.

DISCUSSION

To fulfil the aims of these studies, it was important that the animals were allowed to eat to appetite. This resulted in variation in the intake of individual animals both between days and between diets. Therefore, to assist in the interpretation of the results, a regression approach was adopted in conjunction with a covariance analysis technique to adjust for differences in food intake.

Table 5. *Regression equations describing relationships between flows of the various fractions of N in digesta* (Y, *gN/d*) v. *metabolizable energy* (ME) *intake* (X, *MJ/d*) *in lambs fed a basal low-protein diet* (*A*), *plus urea* (*B*) *and either urea plus casein* (*C*) *or urea plus formaldehyde-treated* (*HCHO*)*-casein* (*D*)*

(Pooled equations (±SE) of the regression coefficients are given for diets which are shown to be not significantly different by covariance analysis)

Duodenal flow of N	Diet	Regression equation	R^2	Residual
Microbial N	ABD	$Y = 0{\cdot}75\ (\pm 0{\cdot}096)\ X + 2{\cdot}27$	0·75	1·67
	C	$Y = 0{\cdot}85\ (\pm 0{\cdot}447)\ X + 3{\cdot}93$	0·37	2·27
Dietary NAN	ABC	$Y = 0{\cdot}21\ (\pm 0{\cdot}086)\ X - 0{\cdot}34$	0·21	1·24
	D	$Y = 2{\cdot}62\ (\pm 0{\cdot}518)\ X - 6{\cdot}71$	0·81	4·76
NH_3-N	ABD	$Y = 0{\cdot}14\ (\pm 0{\cdot}022)\ X - 0{\cdot}155$	0·65	0·44
	C	$Y = 0{\cdot}51\ (\pm 0{\cdot}160)\ X - 1{\cdot}95$	0·62	0·82
Total N	ABC	$Y = 1{\cdot}65\ (\pm 0{\cdot}158)\ X - 0{\cdot}69$	0·83	2·29
	D	$Y = 2{\cdot}69\ (\pm 0{\cdot}560)\ X + 4{\cdot}03$	0·79	5·14
Ileal flow of N				
NAN	ABC	$Y = 0{\cdot}44\ (\pm 0{\cdot}079)\ X + 0{\cdot}33$	0·60	1·18
	D	$Y = 0{\cdot}51\ (\pm 0{\cdot}415)\ X + 5{\cdot}67$	0·23	4·11
NH_3-N	ABCD	$Y = 0{\cdot}13\ (\pm 0{\cdot}033)\ X - 0{\cdot}03$	0·34	0·77
Total N	ABC	$Y = 0{\cdot}53\ (\pm 0{\cdot}127)\ X + 0{\cdot}86$	0·44	1·94
	D	$Y = 0{\cdot}39\ (\pm 0{\cdot}383)\ X + 8{\cdot}69$	0·14	4·19
Faecal flow of N				
	ABC	$Y = 0{\cdot}50\ (\pm 0{\cdot}163)\ X + 0{\cdot}52$	0·49	1·47
	D	$Y = 0{\cdot}95\ (\pm 0{\cdot}480)\ X + 0{\cdot}83$	0·66	2·48
Urinary flow of N				
	ABCD	$Y = 1{\cdot}49\ (\pm 0{\cdot}404)\ X - 3{\cdot}75$	0·49	4·75

* For details of diets, see Kempton & Leng (1979).

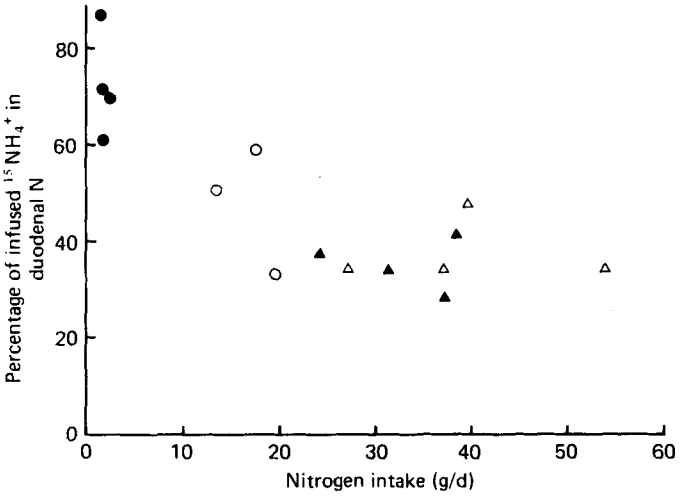

Fig. 3. Percentages of $^{15}NH_4^+$ infused into the rumen that entered the duodenum in relation to nitrogen intake (g N/d) in lambs given a basal low-protein diet (●), plus urea (○), and either urea plus casein (▲) or urea plus formaldehyde-treated (HCHO)-casein (△) (for details of diets, see Kempton & Leng, 1979).

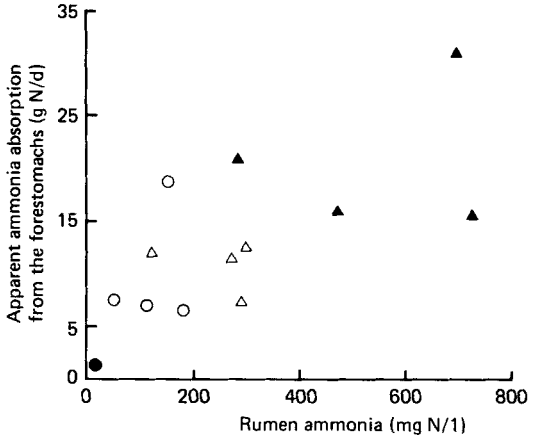

Fig. 4. Apparent ammonia absorption (g N/d) from the forestomachs in relation to rumen ammonia concentration (mg N/l) in lambs given a basal low-protein diet (●), plus urea (○), and either urea plus casein (▲) or urea plus formaldehyde-treated (HCHO)-casein (△) (for details of diets, see Kempton & Leng, 1979).

As a means of depicting N transactions in the rumens of animals on each diet, much of the information has been summarized in flow diagrams (Fig. 1). In these diagrams, the rumen system has been simplified to show only major pools and pathways. Endogenous inputs have been included in order to balance the model and the values assigned probably underestimate the true values because recycling has been assumed to be negligible.

The primary effect of supplementing diets with urea plus HCHO-casein was to increase the quantity of dietary (as distinct from microbial) amino acids available for absorption by the animal. The amount of unfermented dietary protein in the duodenal digesta of the lambs given the HCHO-casein indicates that this supplement was highly resistant to fermentation (see Fig. 1). Although 100% of the dietary HCHO-casein reached the duodenum only 65% of this material was apparently absorbed from the small intestines. A value of 65% was also calculated by MacRae *et al.* (1972) for the digestibility of formaldehyde-treated casein in the small intestines of sheep given a dried-grass diet. Formaldehyde treatment of the dietary casein also increased faecal N excretion, indicating that some of the dietary NAN that was not digested in the small intestine was also indigestible in the large intestine. The apparent digestibility of total NAN (including microbial, dietary and endogenous NAN) in the small intestine was between 0·63 and 0·68 for all diets and thus the apparent digestibility of NAN was largely independent of its source. The true digestibility of NAN in the small intestine would have been somewhat higher, depending on the amount of endogenous NAN that was secreted into and not reabsorbed from the small intestine.

Estimation of the rate of irreversible loss of rumen ammonia

The estimate of the rate of irreversible loss of rumen ammonia provides a reasonable approximation of the ammonia arising directly from dietary N. The measured rates of irreversible loss of rumen ammonia (and rumen ammonia concentrations) indicate that the urea and the untreated-casein supplements were degraded almost quantitatively whereas none of the HCHO-casein supplement was degraded to ammonia in the rumen (see Fig. 1).

In lambs given the basal diet, the rumen ammonia concentrations were extremely low (< 6 mg N/l) and the measured 'plateau' enrichment of the N in rumen ammonia was

less than that in isolated bacteria. This is not possible if a single well-mixed ammonia pool existed in the rumen. It seems likely therefore that the infused $^{15}NH_4^+$ was rapidly assimilated by micro-organisms near the infusion site before it could be mixed. It is also possible that because the quantity of rumen ammonia isolated for analysis was so small, that contamination with nonspecific N produced an underestimate of ammonia enrichment. These problems were not significant in animals on the supplemented diets where rumen ammonia concentrations were at least 30 times higher (> 190 mg N/l) and an identifiable ammonia pool existed. Nolan & Stachiw (1979) obtained information supporting the validity of tracer mixing and steady state kinetics in the rumen of sheep given low quality diets where rumen ammonia concentrations were lower than for supplemented animals in the present study but were 10 times higher than in animals on the basal diet.

Apparent absorption of ammonia from the rumen

In animals on all diets, the flow of ^{15}N into the duodenum during the intraruminal administration of $^{15}NH_4^+$ was less than that infused, suggesting there was a net absorption of ^{15}N, presumably as ammonia, from the forestomachs. In lambs given the basal diet, and where rumen ammonia concentrations were low (6 mg N/l), approximately 25% of the ^{15}N infused was apparently absorbed. In animals given the supplemented diets, where rumen ammonia concentrations were much higher (190–520 mg N/l) up to 60% of the infused ^{15}N was apparently absorbed (see Fig. 3). From these results, and a knowledge of rumen ammonia enrichment, apparent ammonia absorption was estimated. At least 1–2 g N/d was absorbed in lambs on the basal diet and absorption increased with increasing rumen ammonia concentrations. Up to 21 g N/d was absorbed in animals on the supplemented diets (see Figs 1 and 4). These values underestimate the true values because allowances were not made for the small quantity of ^{15}N which enters the duodenum and is subsequently recycled to the rumen (see Nolan & Stachiw, 1979).

In similar studies, Smith *et al.* (1976) postulated that ^{15}N in urea given in the diet was not absorbed as ammonia but might be taken up irreversibly by bacteria sequestering at the rumen wall. In the study reported here ^{15}N was infused into the rumen for 3 d. All pools would therefore be close to maximally labelled and the ^{15}N entering these pools would have been balanced by a similar amount of ^{15}N leaving them. The majority of ^{15}N not accounted for at the duodenum was therefore almost certainly absorbed as ammonia across the wall of the forestomachs.

Microbial protein availability

Voluntary food intakes were higher in all supplemented animals than in animals given the basal diet and hence FOM and microbial outflow from the rumen were also higher in the supplemented animals. However, the net efficiency of microbial synthesis (expressed as microbial N outflow from the rumen/unit FOM) was not significantly different between diets. This is of interest because several factors which are likely to affect the efficiency of microbial protein synthesis were probably different between these diets, e.g. dilution rate (Hobson & Summers, 1967; Sutherland, 1976); turnover of micro-organisms in the rumen (see Thomas, 1973); availability of peptides and amino acids in addition to ammonia (Portugal & Sutherland, 1966; Wright & Hungate, 1967; Hume, 1970; Maeng *et al.* 1976) and availability of branched-chain and higher fatty acids (Hemsley & Moir, 1963; Hume 1970). For instance, in lambs given the diet containing HCHO-casein, mean voluntary food intake was 80% greater than in lambs given the basal diet, and therefore digesta turnover and dilution rate in the rumen must have been substantially increased. Microbial outflow per unit FOM however was not significantly different from that of the lambs on the basal diet. Similarly, although branched chain and higher fatty acids (and presumably

also free peptides and amino acids) were present in relatively high concentrations in the rumens of lambs given the diets containing untreated casein, there was no apparent effect on microbial outflow from the rumen per unit FOM. Without estimates of the extent of turnover of microbes within the rumen (Abe & Kandatsu, 1969; Nolan & Leng, 1972) the efficiency of total microbial protein synthesis in the rumen cannot be determined. The net efficiency, expressed in terms of microbial outflow from the rumen, was 21·3 g N/kg FOM for all diets.

Requirements for rumen fermented N

If it is assumed that the fermentable N requirement for microbial growth equals the quantity of microbial N leaving the rumen (Allen & Miller, 1976), then in this study 1·2 g fermentable N/MJ ME was the apparent requirement. This is similar to the value of 1·3 g N/MJ ME adopted by Roy *et al.* (1977), but is less than the value of 1·8 calculated by Allen & Miller (1976). This approach however considerably underestimates the true dietary requirement for fermentable N if ammonia absorption exceeds fermentable endogenous N entry into the rumen.

Protein: energy (P: E) of absorbed nutrients

For practical purposes, it is useful to know the amounts of digestible protein and energy nutrients available from a diet. This has been referred to as P:E (Egan, 1974). Values reported for P:E for both sheep and cattle vary between 7 and 10 g digestible protein/MJ ME (Egan, 1974, 1976, 1977; Egan & Walker, 1975; Preston, 1976). In this study the calculated P:E for lambs on the diets which contained no supplementary rumen-undegraded protein was 5·5 g (±0·7) digestible protein/MJ ME. Supplementation with such a protein in the form of HCHO-casein increased the apparent absorption of amino acids from the intestines and the calculated P:E increased to 11·6 (±1·7) g digestible protein/MJ ME. The latter value is higher than the majority of estimates previously reported. However these reported estimates were often obtained with animals with restricted food intakes.

Conclusions

From these studies it appears that the primary factor limiting voluntary food intake and growth of lambs given low-N–cellulose-based diets was the availability of amino acids for absorption from the small intestine. When lambs were offered this diet, food intake was stimulated apparently in responses to provision of extra amino acids post-ruminally.

Although the basal diet used in these studies was not highly lignified, responses in voluntary food intake to supplementation with proteins which escape degradation in the rumen have also been obtained in sheep on highly-lignified–dry-cellulosic materials such as pasture hay and straw chaffs (less than 30 g crude protein (N × 6·25)/kg, digestibility 0·50, Egan, 1977). Taken together, our results and those of Egan (1977) suggest that intake of low-quality lignified feedstuffs may be stimulated by supplementing with rumen-undegraded protein. The application of these concepts to ruminants grazing on low-quality pastures will have immediate application in those areas of the world that have pronounced wet–dry seasons where pasture protein content may decrease to levels as low as 20 g/kg DM. The findings will also have implications for production from ruminant animals given low-quality feeds such as agro-industrial by-products.

The authors are indebted to the Australian Wool Corporation Trust Fund and the Australian Meat Research Committee. Messrs F. Ball and V. Scollen and Mrs W. Minnis gave valuable assistance, and also Dr V. Bofinger gave advice on statistical analysis. T.J.K. was in receipt of an Australian Wool Corporation Postgraduate scholarship.

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Printed in Great Britain