Determination of nitrogen requirement for microbial growth from the effect of urea supplementation of a low N diet on abomasal N flow and N recycling in wethers and lambs

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1. Plasma urea entry rate, urinary urea excretion and, by difference, urea recycling in the body, together with the flow of non-ammonia N through the abomasum and digestion of dry matter (DM) before the abomasum were determined in both wethers and lambs receiving cereal-starch diets supplemented with urea to give 60–120 g crude protein (N × 6·25)/kg DM.

2. Lambs excreted less urea in urine than wethers given the same diet.

3. Relationships between plasma urea entry rate or urine urea excretion rate and plasma urea concentration were different for lambs compared to wethers suggesting greater conservation of body N by renal control in lambs.

4. Recycling of urea was not related to plasma urea concentration in wethers but was related exponentially in lambs, suggesting recycling is controlled rather than the result of simple diffusion from the blood to the gastro-intestinal tract.

5. Abomasal non-ammonia-N flow was similar for wethers and lambs and increased linearly with urea supplementation.

6. DM digestion prior to the abomasum was not significantly altered, although there was a tendency for decreased digestion of the basal diet given to lambs.

7. Maximum microbial N flow to the abomasum was estimated as 30 g N/kg organic matter (OM) fermented in the rumen.

8. This work and the literature reviewed suggested maximum net microbial production can be obtained when the diet supplies an amount of fermentable N equal to the microbial N output. It is calculated the diet should supply approximately 26 g fermentable N/kg digestible OM or 1·8 g fermentable N/MJ metabolizable energy. This corresponds to a fermentable crude protein supply varying from 65 to 130 g/kg DM as digestible OM content increases from 400 to 800 g/kg DM.

When considering the dietary protein requirements of ruminant animals attention should be given to supplying the needs of the rumen micro-organisms for fermentable protein or non-protein nitrogen (NPN) as well as supplying the needs of the tissues for amino acids (Miller, 1973). This paper deals in more detail with the needs of rumen micro-organisms for dietary N.

Qualitatively the rumen micro-organisms' needs are mainly for ammonia. Diets in which all the N is supplied as NPN have been shown to support maintenance and some production of either growth or milk (Loosli, Williams, Thomas, Ferris & Maynard, 1949; Duncan, Agrawala, Huffman & Luecke, 1953; Virtanen, 1966) and these experiments indicate that substantial if not maximum microbial protein production must have taken place. Even where the diet supplies mainly protein N, isotope studies have shown the microbes to incorporate 60–80% of their N from the rumen ammonia pool while only a small percentage of labelled amino acids are incorporated directly

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(Pilgrim, Gray, Weller & Belling, 1970; Mathison & Milligan, 1971; Nolan & Leng, 1972; Portugal & Sutherland, 1966; Walker & Nader, 1970).

Very few estimates either in vitro or in vivo of quantitative N needs of rumen micro-organisms have been made. These have been reviewed by Mercer & Annison (1976). In the study by Hume, Moir & Somers (1970), in which a synthetic diet was used, maximum microbial production was not achieved (see Hume, 1970*a*, *b*) and, therefore, a N requirement for maximum production cannot be calculated. In the experiments of Ørskov, Fraser & McDonald (1971) with ewes, addition of urea to the barley diet gave no increment in non-ammonia N (NAN) flow to the abomasum and, therefore, the N supplied by the basal diet must be considered a maximum rather than minimum requirement. Owens, Knight & Nimrick (1973) also failed to obtain any increase in flow of N or amino acids through the abomasum when urea was infused into the rumen of 30 kg lambs given a high-energy diet, and again the basal diet must have supplied sufficient N for maximum microbial growth. In studies with lambs, Ørskov, Fraser & McDonald (1972) obtained an increased fermentation of organic matter (OM) in the rumen and a 7% increase (not significant) in NAN flow when the barley diet was supplemented with urea to give 124 g crude protein (N × 6·25)/kg dry matter (DM). Sharma, van't Klooster & Frens (1968) obtained maximum NAN flow through the duodenum when 10–20 g urea were added to a low-N diet of oat straw and concentrates. However, since these authors did not report any measurement of available energy, their data cannot be used to calculate N requirements related to energy supply. Further quantitative estimates both of the N required to sustain maximum microbial production per unit of available energy and of the effect of limiting levels of dietary N on the extent of microbial protein synthesis would be valuable data for determining minimum dietary N requirements and conditions for the effective used of NPN and aldehyde-treated protein supplements.

In the experiments of Hume *et al.* (1970) and Ørskov *et al.* (1971, 1972) the requirement for fermentable N was assessed from the response in NAN flow through the omasum or abomasum to increments of urea in the diet. However, a complicating factor is the possibility of a variable endogenous contribution to the rumen ammonia pool. This may arise from either a variable saliva flow dependent upon the nature of the diet or a variable content of N, particularly urea, in the saliva and a variable extent of urea diffusion across the rumen wall dependent upon the physiological status of the animal and the concentration of urea in the plasma. In the experiments to be described here we have studied the effects of increments of urea to a low-protein, high-energy diet simultaneously on both NAN flow through the abomasum and N recycling within the body. Further we considered that a comparison of these responses in both adult animals and young growing lambs given the same diet and in the same quantities would indicate whether the recyling component and, therefore, the dietary need for fermentable N, varied with physiological state.

A preliminary report of part of this study has been published (Allen & Miller, 1972).

Table 1. *Composition of the diets given to wethers and lambs (g/kg dry matter)*

	Diet			
	1	2	3	4
Barley	458	458	458	458
Maize starch	309	301	293	285
Chopped barley straw	100	100	100	100
Molassine meal*	100	100	100	100
Urea	0	8	16	24
Vitamins and minerals†	30	30	30	30
Chromic oxide	3	3	3	3
Estimated crude protein	60	80	100	120
Determined crude protein	59·8	82·1	100·3	116·2

* Molasses–sphagnum moss, 75:25, w/w: The Molassine Company Ltd, London SE10 0PU.

† The vitamin-mineral mixture contained (g/kg): NaCl 150, limestone flour 250, $CaHPO_4$ 250, MgO 40, $FeSO_4.2H_2O$ 5, ZnO 3, $CoSO_4.7H_2O$ 0·1, Na_2MoO_4 1·0, stabilized vitamin A equivalent to retinol 0·105; and (mg/kg): stabilized vitamin D_3 equivalent to cholecalciferol 1·25 and stabilized vitamin E supplying DL-α-tocopheryl acetate 40. The mix was made up to 1 kg with toasted cereal.

EXPERIMENTAL

Design

Two experiments were carried out, one with wethers, the second with lambs. The same diets were given in identical manner and amount in the two experiments. In the wether experiment, four diets were given to each of two wethers according to a randomized block design. In the lamb experiment, four diets were given to each of four lambs according to a Latin Square design.

Animals

The wethers were Finnish Landrace × Clun Forest; they weighed approximately 45 kg and were each fitted with a rumen cannula and a simple abomasal cannula. Ewe lambs of (Welsh half-bred × Suffolk) × (Welsh half-bred × Suffolk) breeding were weaned off the ewe at 4 weeks of age and were fitted with simple abomasal cannulas when weighing approximately 14 kg. The lambs weighed from 15 to 25 kg over the period of the experiment. The mean weight gain for the 8 week period was 8·3 kg.

Diets

The diet compositions are shown in Table 1. The basal diet contained a high proportion of starch to achieve a low N:energy ratio. Barley was included to supply some protein as well as energy so that microbial growth would not be limited by lack of precursors or cofactors other than ammonia. Barley straw was included as a source of roughage to stimulate saliva flow and rate of passage of digesta from the rumen. The remaining diets were supplemented with urea at the expense of starch, all other ingredients remaining constant. The diets were pelleted through a 15·3 mm die.

Experimental procedure

Each period consisted of 10 d adaptation to the diet followed by 4 d of experimental observations. For the first 7 d the animals were penned on slatted floors and given the daily ration of 720 g in two equal feeds. Then they were transferred to metabolism crates and were fed twenty-four times/d at a rate of 30 g fresh weight (870 g DM/kg)/h using an automatic feeding machine. Samples of abomasal digesta were obtained on two consecutive days in each period. Urea entry rate and urea excretion were determined on another two consecutive days. Diets were sampled daily and pooled in each period for analysis.

Abomasal sampling

Eight samples were taken from the abomasum at 1·5 h intervals on each day. About 20–25 ml digesta were collected by removing the bung and allowing the digesta to gush into a beaker. The initial flow of digesta was discarded as this tended to contain a high proportion of solid matter that had collected within the cannula. The sample was thoroughly mixed by rapid pumping with a syringe and 15 ml was dried at 80° to constant weight. The dried residue was analysed for N and chromic oxide. Since negligible amounts of ammonia remained after drying, these N values are referred to as NAN. A further aliquot of fresh digesta was used for ammonia determination. NAN and DM flowing through the abomasum were calculated from their respective ratios to chromic oxide in the digesta samples.

Determination of urea entry rate

This was calculated from the rate of infusion of [^{14}C]urea into the jugular vein divided by the calculated specific activity (SA) of plasma urea under steady-state conditions. Urea SA was calculated from the ratio of radioactivity to urea in 1 ml of plasma. Cocimano & Leng (1967) have shown this method gives the same value as determining the SA on urea isolated from the plasma. Catheters were fitted into each jugular vein. A solution of [^{14}C]urea containing 40 μCi and 9 mg urea in 100 ml sterile saline solution was infused into one jugular vein with a Perpex 10200 peristaltic pump (LKB Instruments Ltd, South Croydon, Surrey) at 10 ml/h for 9 h. A steady state of plasma urea SA was achieved after 6 h. Sometimes an infusion rate of 40 ml/h was used for the first 30 min to shorten the total infusion time and reach a steady state in 3–4 h. Six samples were withdrawn through the second catheter, one every 30 min during the last 3 h of infusion. Blood (10 ml) was taken into a syringe containing 500 i.u. heparin, then immediately centrifuged at 2000 *g* for 5 min at 0°. The plasma was stored at −20°.

Urine collection

Wether experiment. Urine was collected by means of a rubber cup held firmly over the penis by a harness. A tube from the bottom of the cup delivered the urine into an aspirator containing sulphuric acid. Air was continuously drawn through the system by a diaphragm pump (Charles Austen Ltd, Weybridge, Surrey) so that the urine was quickly transferred into the acid. Collections were made for 24 h periods on the days

of urea entry rate measurements. The volume of urine collected was measured and a sample stored at −20°.

Lamb experiment. Lambs were fitted with urethral catheters at the same time as the jugular catheters. Urine was collected into sulphuric acid for the duration of each [^{14}C]urea infusion and also overnight between the two infusions. The volume of urine was measured and a sample stored at −20°.

Analytical procedures

N in the dried abomasal digesta was determined by micro-Kjeldahl digestion followed by colorimetric determination of ammonia in an autoanalyser system (Varley, 1966). Dietary and faecal N were determined in the same way except that the digestion was carried out on ten times the scale and the digest diluted ten times more before estimation of ammonia.

Chromic oxide in the diets was estimated by the method of Milner described by Owen, Davies, Miller & Ridgman (1967). The procedure was used on one-fifth of the scale for determination of chromic oxide in dried abomasal digesta.

Ammonia in the abomasal fluid was determined after the digesta was mixed with an equal volume of 1 M-HCl and centrifuged. The supernatant fluid was analysed in an autoanalyser system using the reaction described by Fawcett & Scott (1960).

Radioactivity was determined by adding 1 ml deproteinized plasma to 15 ml scintillation fluid (10 g butyl PBD (CIBA Chemicals Ltd, Duxford, Cambridge), dissolved in and made up to 1 l with toluene, mixed with 500 g Triton X-100) and counting in a Corumatic 2000 scintillation spectrometer (Tracerlab, Weybridge, Surrey).

Statistical analysis

Initially each experiment was analysed separately. Before statistical analysis, replicate values within and between days were averaged to give a single estimate of each variable for each sheep in each period. Standard errors of treatment means were calculated by analysis of variance. However, since there was some variation in the N content of batches of the same diet given in different periods, the data were also analysed by regression analyses using the determined N intake instead of the added urea (g/kg) as the independent variable. All linear and quadratic regressions were calculated on a 'within sheep' basis, that is with the sums of squares for the average differences between sheep removed. The exponential regressions were calculated omitting the classification according to sheep. In the lamb experiment, period effects were not significant and the experiment was reanalysed as a randomized block with the period sum of squares included in the error term.

Comparisons between experiments were complicated by the greater variability of the wether experiment and by small differences in the analysed N content of the same diet fed to different animals. Some comparisons were made between wethers and lambs by considering each diet singly, and assuming equivalent intake of N.

Table 2. *The effect of levels of dietary urea on urea movements and abomasal nitrogen in wethers and lambs*

(Units: g N/24 h unless otherwise stated)

	Urea in diet (g/kg)	Plasma urea (mmol/l)	Plasma urea entry	Urine urea excretion	Urea recycling (A)	Dietary N (B)	A+B	Abomasal NAN flow	Abomasal NH_3-N (mmol/l)	Rumen NH_3-N (mmol/l)
Wethers	0	1·16	7·5	3·3	4·2	5·9	10·1	10·3	7·9	7·9
	8	1·39	9·0	5·0	4·0	8·8	12·8	10·6	7·6	9·8
	16	1·53	10·0	6·3	3·7	10·2	13·9	12·4	7·9	11·4
	24	3·17	15·6	12·9	2·7	10·8	13·5	12·8	11·9	15·8
	SE	0·83	4·16	2·98	2·28	0·41	2·28	0·88	0·67	5·86
Lambs	0	0·65	2·6	0·2	2·4	6·0	8·4	10·3	5·4	—
	8	1·83	6·6	1·3	5·3	8·8	14·1	12·1	6·9	—
	16	3·04	8·2	2·6	5·6	10·1	15·7	12·6	9·1	—
	24	4·66	9·9	3·9	6·0	12·1	18·1	14·2	11·2	—
	SE	0·42	0·53	0·45	0·41	0·61	0·73	1·02	0·88	—

NAN, non-ammonia N; NH_3-N, ammonia N.

Table 3. *Linear and multiple regressions of the form* $y = bx+c$ *and* $y = b_1x_1+b_2x_2+c$, *relating values measured in the experiment with wethers*

(Units: rates g N/24 h; concentrations mmol/l)

Y	*x*	*b*	SE	*c*	Residual standard deviation	*r*	*P*
Abomasal NAN flow	Dietary N intake	0·51	0·226	6·99	1·22	0·728	< 0·1
Abomasal NAN flow	Dietary N intake +recycled N	0·41	0·166	6·42	1·13	0·738	< 0·1
Abomasal NAN flow	Dietary N intake (x_1)	0·54	0·065	5·85	0·346	0·795	< 0·05
	Recycled N (x_2)	0·25	0·071				
Plasma urea entry rate	Plasma urea concentration	4·26	0·486	2·83	1·50	0·972	< 0·001
Urine urea excretion	Plasma urea concentration	3·96	0·390	−0·41	1·20	0·977	< 0·001
Rumen NH_3-N concentration	Plasma urea concentration	3·21	0·467	0·52	1·81	0·928	< 0·01
Rumen NH_3-N concentration	Dietary N intake +recycled N	1·86	0·404	−1·22	2·75	0·768	< 0·05

NAN, non-ammonia N; NH_3-N, ammonia N.

RESULTS

Wether experiment

Plasma urea concentration, urea entry rate and urea excretion. The mean values of these three measurements for the four diets are shown in Table 2. The values for the individual sheep on the same diet were very different and the differences between the sheep were not consistent on all diets. Thus, although the mean values for the two sheep appeared to increase with dietary N intake as might have been expected, the changes were not statistically significant even when considered at $P = 0{\cdot}10$. However, the variability was not entirely random nor due to experimental error, since urea entry rate into the plasma and plasma urea concentration were significantly correlated ($P < 0{\cdot}001$). The relationship is shown in Fig. 1 and the linear regression equation is given in Table 3. Also plasma urea concentration and urea excretion in the urine were closely correlated ($P < 0{\cdot}001$). This relationship is shown in Fig. 2 and the regression equation is given in Table 3.

Urea recycling. This was calculated as the difference between entry rate into the plasma and the excretion rate in urine. The mean values are given in Table 2. As with the two prime measurements, the difference value (urea recycling) varied between sheep. The amount of urea recycled did not differ significantly with increase in N consumed, nor was it related in any simple manner with urea entry rate. When the latter was low, recycling was also low but with diet 4 both sheep gave low values for recycled N despite high urea entry rates. Similarly, there was no simple relationship of urea recycling with urea concentration in the plasma.

Flow of DM *and NAN through the abomasum.* DM flow did not vary with N intake. The mean value, with SE, was $316 \pm 22{\cdot}7$ g/24 h. As the DM ingested was 626 g/24 h, the amount apparently digested in the forestomachs amounted to 310 g/24 h. The

Table 4. *Linear regression of the form* $y = bx + c$ *relating values measured in the lamb experiment*

(Units: rates g N/24 h; concentrations mmol/l)

y	*x*	*b*	SE *b*	*c*	Residual standard deviation	*r*	*P*
Urea concentration	Dietary N intake	0·59	0·073	−2·94	0·692	0·887	< 0·001
Plasma urea entry rate	Dietary N intake	1·09	0·070	−3·26	0·666	0·922	< 0·001
Urine urea excretion	Dietary N intake	0·55	0·094	−3·09	0·889	0·859	< 0·001
Abomasal NAN flow	Dietary N intake	0·48	0·161	7·78	1·519	0·551	< 0·05
Abomasal NAN flow	Dietary N + recycled N	0·31	0·136	8·01	1·840	0·502	< 0·10
Urine urea excretion	Plasma urea concentration	0·93	0·078	−0·335	0·485	0·965	< 0·001

NAN, non-ammonia N.

mean values of flow of NAN through the abomasum are given in Table 2. NAN increased linearly ($P < 0·1$) with dietary N intake and with the sum of dietary N and recycled N. Including the latter term did not increase the precision of the relationship. As only part of the recycled N might be returned to the rumen the multiple regression of NAN flow on dietary N intake and recycled N as independent variables was examined and found to give an improved correlation (Table 3).

Rumen ammonia concentration. This was not linearly related to either dietary N intake or to recycled N considered separately but was related to the sum of dietary N and recycled N ($P < 0·05$; Table 3). It was also correlated with plasma urea concentration ($P < 0·01$; Table 3).

Lamb experiment

Plasma urea concentration, urea entry rate and urea excretion. The mean values for the four diets are shown in Table 2. Plasma urea concentration and excretion of urea in the urine increased linearly with dietary N intake. Plasma urea concentration and excretion of urea in the urine were closely correlated ($P < 0·001$) as shown in Fig. 2. Regression equations describing these relationships are given in Table 4.

The relationship between urea entry rate and plasma urea concentration was curvilinear. Regression analysis for linear and quadratic components showed the latter to be significant ($P < 0·001$). However, a closer relationship, indicated by a lower residual mean square, was obtained when the data were fitted to an exponential model (Table 5). Similarly, exponential equations best described the relationships between urea entry rate and dietary N intake (Table 5).

Urea recycling. Little recycling took place when the basal diet was given. The first addition of urea resulted in a twofold increase in recycling. Further additions of urea had little effect, so that the over-all response to N intake was curvilinear, and was best described by an exponential equation (Table 5).

Table 5. *Exponential regressions of the form* $y = a + b(R)^x$ *relating values measured in the lamb experiment*

(Mean values with their standard errors. Units: rates g N/24 h; concentrations mmol/l)

y	*x*	*a*	*b*	*R*	Residual standard deviation	*P*
Urea entry rate	Plasma urea concentration	11·6 ±0·86	−11·9 ±0·69	0·630 ±0·0608	0·633	< 0·001
Urea entry rate	Dietary N intake	16·9 ±9·58	−24·9 ±4·79	0·903 ±0·0864	1·53	< 0·001
Recycled N	Dietary N intake	7·0 ±1·54	−15·5 ±7·93	0·796 ±0·1138	1·02	< 0·001

Flow of DM *and NAN through the abomasum.* DM flow did not vary with N intake. The mean value, with SE, was 356 ± 29·3 g/24 h. Although the difference was not significant, the value obtained with the basal diet was 395, whereas the mean of the supplemented diets was 342 g/24 h. The mean values of flow of NAN through the abomasum are shown in Table 2. NAN flow increased linearly with dietary N intake ($P < 0·05$). Including recycled N either by simple addition to the dietary N or by considering dietary N and recycled N as independent variables in a multiple regression analysis did not give any closer relationship with NAN flow. The regression equations are given in Table 4.

DISCUSSION

Plasma urea concentration, urea entry rate and urea excretion

The excretion of urea in the urine was deliberately measured over short periods of time concomitant with the isotopic determination of urea entry rate. Therefore, the urinary excretion is only a very approximate guide to the long-term N balance. The variability of the urea measurements made with the wethers and their failure to vary with N intake indicates that changes were taking place in body protein reserves with the production of a very variable amount of endogenous urea which masked the effects of dietary supplements of urea. In contrast the lambs gained in weight throughout the experiment so that mobilization of tissue N reserves was less likely, and the urea measurements were less variable and were related to N intake. Less urea appeared to be excreted by the lambs than by the wethers on all four diets (Table 1) and this difference was also reflected in lower plasma urea entry rates with lambs. Therefore, the lambs appeared to be utilizing their dietary N more efficiently than the wethers The simplest explanation of this would be that the NAN flowing to the small intestine was more than adequate to meet the maintenance needs of wethers and the surplus protein was deaminated and contributed to the urea entry and urine excretion, while the supply was inadequate for the potential growth of the young lamb. However, the different relationships between urea entry rate and plasma urea concentration (Fig. 1) and between plasma urea concentration and urea excretion in the urine (Fig. 2) indicate that other mechanisms to conserve N in the lamb were also in operation. The smaller increase in urea excretion in the urine of lambs per unit increase in plasma

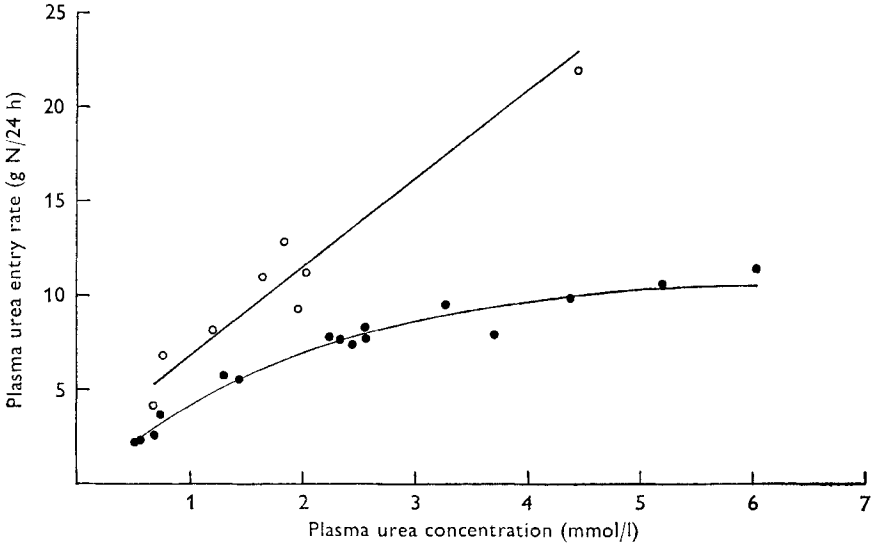

Fig. 1. The relationship between plasma urea concentration (mmol/l) and plasma urea entry rate (g N/24 h) for wethers (○) and lambs (●).

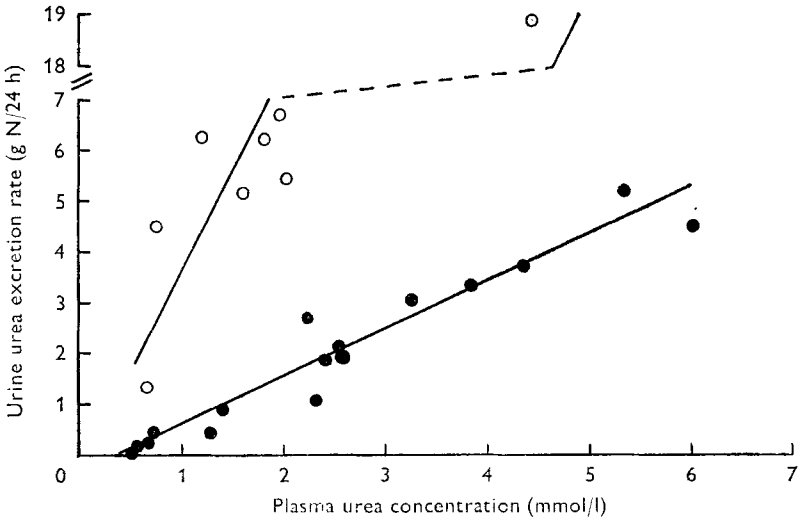

Fig. 2. The relationship between plasma urea concentration (mmol/l) and urinary urea excretion rate (g N/24 h) in the urine for wethers (○) and lambs (●).

urea concentration suggests the kidney was exerting control over the excretion of urea and preventing excessive body losses of urea. This would explain the observation at equal urea entry rates of a higher plasma urea concentration in lambs compared to wethers and also the exponential compared to the linear relationships that were observed (Fig. 1).

The method used to determine urea recycling measures the total recycling taking place within the body. It is not known what proportion of that recycling is directed to the rumen as opposed to other parts of the gastro-intestinal tract. Therefore, the calcu-

lated dietary N intake plus recycled N is only an approximate guide to the amount of N made available to the micro-organisms. Certainly the estimate of urea-N recycled in the body is the upper limit of the contribution that could be made to rumen microbial needs from this source. From studies using ^{15}N with adult sheep given high-protein lucerne hay, Nolan & Leng (1972) showed that only 1·2 g urea N/24 h was returned to the rumen, representing 19% of the urea recycled. From the same results Nolan, Norton & Leng (1973) have estimated that all the urea N recycled to the rumen could be accounted for by the normal saliva flow, that little entered by diffusion across the rumen wall (Hecker & Nolan, 1971) and that the majority of recycling took place to the intestines and caecum.

In contrast to the ^{15}N studies, a number of studies of N flow from the rumen or through the abomasum on medium- to low-N diets indicate substantial endogenous contributions of N. In addition to urea, other N compounds may enter the rumen in the form of saliva, muco-proteins and sloughed cells of oesophageal and ruminal epithelia. However, there are no quantitative estimates of this contribution. Hume *et al.* (1970) report an increase between diet and omasum of 4·0 and 3·1 g N/d on the two N-deficient diets. Clarke, Ellinger & Phillipson (1966) report increases from diet to duodenum of 3·9 and 4·9 g N/d for diets of hay and hay + maize respectively. They further estimated that secretions of the abomasum might account for only 2·0 g of this increase. In the present work the NAN flows through the abomasum of wethers and lambs given the basal diet were 4·4 and 4·3 g greater than N intakes respectively. In addition, assuming a liquid flow rate of 3 l/d through the abomasum, an additional 0·3 or 0·2 g N respectively passed as ammonia. McIntyre (1971) infused ewes given a low-N diet intravenously with urea and observed an increase in N balance of 4·3 g/d which could represent an additional synthesis of 6·7 g microbial N/d. Each of these indirect estimates of endogenous contributions are probably minimal values since they take no account of ammonia losses by absorption from the rumen or omasum.

The apparent conflict between the conclusions as to the extent of recycling to the rumen arising from the isotope studies with high-N diets and the N-flow and -balance studies with low-N diets might be resolved if there are physiological controls that prevent excessive recycling which is energetically wasteful (Martin & Blaxter, 1965). In the present experiments the recycling was not linearly related to plasma urea concentration, as would be expected if recycling took place by simple diffusion from the blood to the gastro-intestinal lumen. For both wethers and lambs recycling was between 1 and 3 g N/d when plasma urea concentrations were around 0·65 mmol/l. Recycling increased to 5–6·5 g N/d at plasma concentrations of 1·33–2·0 mmol/l. At greater plasma concentrations the amount decreased for the wethers and remained unchanged for the lambs. While changes at low levels of blood urea concentration might be interpreted as a passive diffusion process as proposed by Englehardt & Nickel (1965) and Houpt & Houpt (1968), the lack of any further increase in recycling at the plasma concentrations greater than 2·0 mmol/l favours a controlled carrier mechanism as proposed by Decker, Hill, Gartner & Hornicke (1960) and Gartner, Decker & Hill (1961). Várady, Bőda, Havassy & Bajo (1969) have also produced evidence of slower

urea passage across the rumen wall from blood to rumen in sheep when given a high- compared to a low-protein diet.

A second explanation of the apparently low extent of recycling of urea-N to the rumen obtained in the isotopic experiments (Nolan *et al.* 1973) in which high protein lucerne diets were given is that urea entering the rumen by diffusion is degraded to ammonia in the epithelium and the [^{15}N]ammonia is reabsorbed without equilibrating with the rumen ammonia pool. There is a need to determine the extent of recycling of ^{15}N from the plasma to the rumen ammonia pool under conditions of low dietary N and rumen ammonia concentration that would encourage rapid microbial uptake of recycled nitrogen rather than rapid reabsorption. Even where recycled ammonia does not equilibrate with the rumen pool, a high, localized, concentration of ammonia in the rumen epithelium would create a barrier to the diffusion of ammonia from the rumen and maintain a higher concentration of ammonia in the rumen. Under these circumstances recycling of ^{15}N to the rumen ammonia pool would underestimate the importance of recycling to the maintenance of the N supply for microbial growth.

Abomasal NAN flow, microbial synthesis and N requirement

Ideally the microbial contribution to the NAN flow should be determined and methods for determining this are being developed (Smith, 1969). The method developed by us using $^{35}SO_4$ infused into the rumen (Roberts & Miller, 1969) was not used in this experiment as we wished to avoid cannulation of the developing rumen in the very young lambs that were used. More recent developments of this method (Harrison, Beever & Thompson, 1972; Beever, Harrison, Thompson, Cammell & Osbourn, 1974; Hume, 1974) were not availble to us at the time of these experiments. However, if the assumption is made that urea supplements affect neither the proportion of the dietary protein reaching the abomasum undegraded nor the NAN secretions into the abomasum, any increase in NAN flow with urea supplementation can be assumed to represent increased microbial N. Further, previous studies with wethers given a barley-urea diet, similar in all respects to that used here except for the replacement of maize starch by additional barley, showed the microbial N accounted for 93 % of trichloroacetic acid-precipitable N in the rumen and 96 % of NAN flow through the duodenum (J. R. Mercer, S. A. Allen & E. L. Miller, unpublished results). Therefore, in these experiments it was expected the NAN flow would consist of mainly microbial N together with a smaller but unknown contribution from abomasal secretions.

The response of NAN flow to increased N intake by urea supplementation was closely similar in both wethers and lambs, as shown by the regression equations (Tables 3 and 4). Since the response was linear over the whole range of N intake studied it cannot be concluded that maximum microbial production had been achieved. Differences between wethers and lambs in urea recycling, excretion and plasma concentration had no apparent effect on NAN flow. NAN flow exceeded N intake on all four diets for both wethers and lambs. Phillipson (1964) estimated between 1 and 2 g N are secreted into the abomasum. Subtracting 1·5 g N/24 h from the NAN flow still leaves increases over N intake ranging from 2·9 g N/24 h on the basal diet to 0·5 g N/24 h at the highest level of supplementation. If all the recycled N is assumed

to return to the rumen then NAN flow only exceeds N supply on the basal diet. As shown below, the estimated yields of microbial N/unit fermented energy at the highest N intake are within the range of reported values determined under conditions where N supply was unlikely to be limiting. Therefore, the N supplied by the diet supplemented with 24 g urea/kg (116 g crude protein/kg DM) is likely to be close to the minimum requirement for maximum microbial growth. This value is intermediate between those of Ørskov *et al.* (1971, 1972) of 105 and 124 g crude protein/kg DM needed to sustain maximum NAN flow through the abomasum of ewes and lambs respectively given diets of similar digestible energy content. The present experiments provide no evidence of a different flow of NAN in lambs compared to adult animals when given the same amount of the same diet nor of any difference in N requirement to sustain the microbial growth.

The present study and those of Hume *et al.* (1970) and Ørskov *et al.* (1971, 1972) all indicate that maximum microbial production or NAN flow to the abomasum is achieved when the diet supply of fermentable N is approximately equal to the microbial N output. It is not implied that the micro-organisms trap the N with 100% efficiency but that the recyled N is sufficient to equate with the inevitable losses of ammonia N from the rumen. Continuous in vitro culture experiments indicate maximum microbial growth is achieved when the ammonia N concentration is between 20 and 50 mg/l (Satter & Slyter, 1974) or 64 mg/l (Allison, 1970). In vivo, Hume *et al.* (1970) obtained maximum rumen tungstic acid-precipitable N at a rumen ammonia-N concentration of 88 mg/l but maximum precipitable N flow at the omasum when the concentration was 133 mg/l. In the present study, rumen ammonia-N levels in the wethers were very variable between 116 and 312 mg/l on the high-urea diets, but abomasal ammonia-N was more consistent, at about 160 mg/l, for both wethers and lambs.

Under maintenance conditions of feeding, less than maximum microbial protein production might provide sufficient amino acids to meet tissue needs and this in turn would mean a lower dietary N requirement. From the slope of the response in NAN flow to N intake with both wethers (Table 3) and lambs (Table 4) it is seen that the reduction in NAN flow is only 0·5 g N/24 h for each g N/24 h reduction in intake over the range from 12 to 6 g N intake/24 h. A very similar slope can be calculated from the data of Hume *et al.* (1970) for those diets where N supply was limiting. Presumably the relatively small effect on NAN flow is due to more efficient use of the recycled N by the rumen micro-organisms. However, although the reduced yield of microbial protein might suffice to meet tissue needs for maintenance, the reduced microbial growth may result in less fermentation of dietary carbohydrate and less energy supply to the tissues. With the readily fermented diets used here, a depression in DM fermented was only noted with lambs given the basal diet, and even then the difference did not achieve statistical significance. Similarly Hume *et al.* (1970) did not observe any depression in fermented organic matter of their N-deficient synthetic diets. In contrast, Ørskov *et al.* (1972) have reported that urea supplements to barley diets given to lambs improved fermentation of organic matter in the rumen. Loosli & McDonald (1968) have reviewed a number of reports where urea supplements have increased the rate of

Table 6. *Estimates of microbial nitrogen yield related to digestion in the rumen of wethers and lambs.*

Dietary urea (g/kg) ...	0	8	16	24
Microbial N (g/kg apparently digested DM)				
Wethers	31	28	44	41
Lambs	38	37	39	46
Microbial N (g/kg apparently digested OM)				
Wethers	29	26	41	39
Lambs	35	35	36	43
Microbial N (g/kg FOM)				
Wethers	23	22	31	29
Lambs	27	27	28	31

DM, dry matter; OM, organic matter; FOM, fermented organic matter.

digestion, thereby permitting greater food intake, of poor quality roughages. Further work is required to delineate the minimum dietary N requirement to sustain maximum energy digestion of different quality diets.

Estimates of the amount of microbial N flowing to the abomasum are given in Table 6. Microbial N was estimated by subtracting 1·5 g N/24 h from the NAN flow to allow for endogenous secretions and any small contribution of unfermented dietary N. Values are expressed per kg DM apparently disappearing prior to the abomasum. We made no measurements of ash flow, but in order to compare our results with published estimates related to apparent OM digestion, it was assumed that the ash content of the diets was 50 g/kg and the flow of ash through the abomasum as a proportion of intake was 1·57 (Ørskov *et al.* 1971). A further calculation of microbial yield per kg fermented OM (FOM) was made by correcting for microbial OM flow assuming that bacteria contain 117 g N/kg OM (Lindsay & Hogan, 1973).

Yield of microbial N per unit energy appeared to be depressed for wethers given the two lowest-N diets since there was less flow of NAN without any depression in disappearance of DM. In contrast, the lambs given the basal diet had both lower NAN flow and lower apparent DM digestion in the rumen and the yields of microbial N per unit energy were not greatly affected by low dietary N. Averaging the values for wethers and lambs at the highest-N diets gives an estimate of maximum microbial N yield of 30 g N/kg FOM. This value is in good agreement with published estimates of microbial N production under conditions where only energy would appear to be a limiting factor. Published values for microbial N/kg FOM are: Hume (1970*b*) 27·3, 37·2, 31·7, 36·0; Hume & Bird (1970) 29·6, 30·4, 28·3, 32·3; Lindsay & Hogan (1972) 35·2, 40·5; Hume & Purser (1975) 30·9, 35·7, 32·3, 28·5. The mean of these values is 32·6. Bucholtz & Bergen (1973), from in vitro production adjusted for a 25% turnover of microbial protein in the rumen, estimated net yield as 30 g N/kg FOM. Leibholz (1972) reported values, determined in vivo from the SA ratio of bacterial and duodenal [^{35}S]cystine, ranging from 9·9 to 18·6 g N/kg FOM. These low values may be due to the methods used, since digesta flow rates were unadjusted

for markers, care is needed to separate residual $^{35}SO_4$ from cysteic acid during ion-exchange chromatography, and the method of calculation determines the proportion of duodenal cystine that is of microbial origin and assumes this ratio also applies to the N. Differences in cystine content of dietary and endogenous proteins compared to microbial protein are not taken into account.

Estimates of bacterial protein synthesis, which being based on the flow of diaminopimelic acid exclude unknown quantities of protozoal protein, have also been reported. For forage diets, values varied from 20·4 to 36·8 with a mean of 27·4 g N/kg FOM (Hogan & Weston, 1970, 1971; Lindsay & Hogan, 1972; Hogan, 1973). Ørskov *et al.* (1972) report values ranging from 23·0 to 26·1, mean 24·9 g N/kg FOM, for all-concentrate diets. Hagemeister & Pfeffer (1973) determined bacterial N flow in cows given mixed roughage and concentrate diets. On converting their values of apparent OM digested to FOM, assuming bacteria to contain 117 g N/kg OM, the bacterial yields varied from 24·8 to 33·7, mean 27·6 g N/kg FOM. In general these estimates of bacterial N are only slightly lower than the estimates of total microbial N and support the findings of Weller & Pilgrim (1974) that, through sequestration within the rumen, protozoa make only a small contribution to the microbial N flowing from the rumen.

The following assumptions were made: a microbial yield of 30 g N/kg FOM, which is equivalent to 40 g N/kg OM apparently digested in the rumen; that the proportion of OM digestion apparently taking place in the rumen is 0·65 (range 0·60–0·70 for long roughages and roughage plus concentrate diets); that the dietary supply of fermentable N should equal microbial N leaving the rumen; and that 1 kg digestible OM contains 14·8 MJ metabolizable energy. The requirement for dietary fermentable N is calculated as 26 g N/kg digestible OM or 1·8 g N/MJ metabolizable energy. This corresponds to a fermentable crude protein supply varying from 65 to 130 g/kg DM as digestible OM content increases from 400 to 800 g/kg DM. Although the literature values of microbial protein production do not show marked differences between concentrate and forage diets, these studies were conducted with highly digestible diets. Further work is required with roughages of low digestibility since there are indications of reduced microbial yields with the slower rates of fermentation (Egan, 1974), and this may further reduce the requirement for fermentable N in diets of low digestibility.

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