NITROGEN RETENTION IN RAPIDLY-GROWING STEERS GIVEN UREA SUPPLEMENTED DIETS

O. A. IKWUEGBU

National Veterinary Research Institute, Vom

SUMMARY

Four calves equipped with permanent rumen and T-piece duodenal cannulas were given four diets in a Latin square experiment carried out at two stages of development. DM flow was measured by dual-phase markers. The basal diet of hay and concentrate was calculated to be low in rumen degradable N (RDN) and tissue N. Additional RDN was provided by adding 6, 12 or 18g urea/kg concentrate.

Supplementary urea did not affect OM digestion either in the stomach or in the entire digestive tract. At the older age OM digestion in the stomach and the entire tract was significantly higher. On the basal diet, N retention was low. The flow of microbial N measured by the DAPA technique was not affected by diet and it was calculated that between 3 and 7g N/d were recycled to the rumen. The addition of supplementary urea increased N retention particularly at the lowest level of supplementation due to a concomitant decrease in urinary N.

INTRODUCTION

In mature animals the demands for tissue N are less than the supply of microbial protein N from the rumen, hence when such animals are given diets low in rumen degradable N (RDN), there is frequently an excess of tissue N that can be recycled to the rumen to satisfy the demands of micro-organisms and thereby maintain microbial fermentation and cell growth. With fast-growing or lactating animals there is a high demand for tissue N which exceeds the supply from microbial proteins alone (Roy, Balch, Miller, orskov and Smith, 1977). Thus, there may well be no excess of tissue N available to recycle to the rumen. Little is known about the effects of a high tissue N demand on the ability of such animals to recycle N to the rumen or whether indeed absorbed amino acids will be preferentially used to synthesise tissue protein or recycled as urea to the rumen if the diet is deficient in RDN. The purpose of the present experiment was to examine the effects of feeding a diet low in RDN on microbial activity and N retention in calves at two stages of development and consequently with different tissue N demands. These studies were carried out at the National Institute for Research in Dairying, Shinfield, Reading and were funded in part by the Agricultural Research Council, United Kingdom.

MATERIALS AND METHODS

Animals:

Four Friesian male calves equipped with permanent rumen and simple T-piece duodenal cannulas when 6 weeks old were used and measurements were made when they were aged 3 to 6 months (liveweight 83 to 130kg) and 8 to 11 months (liveweight 184 to 228kg). The animals were kept in galvanised steel pens (1.8 × 1.2m) with expanded metal floors in an environment of 14 to 19°C. They were allocated at random to dietary treatments in a Latin Square design and the same sequence was used at both stages of development.

Diets:

The basal diet was designed to be low in RDN and this was achieved by choosing feed ingredients expected to be low in degradability of protein. By using the factors outlined in the new protein scheme (ARC, 1980) and estimates of degradability based on a literature survey, the theoretical supply of RDN and tissue N was calculated. These factors are 0.8, 0.6, 0.3 and 1.0 for flaked maize, barley, sprat meal and urea respectively. On the supplemented diets urea provided additional RDN and comprised 6, 12 and 18g

TABLE 1

Composition of Concentrate cubes (g/kg total air dry weight) and estimated contribution (g) in the dry matter (DM) of total N, rumen degradable N (RDN), undegradable N (UDN) and tissue N from the four diets fed to Friesian steer calves

Diets	*A*	*B*	*C*	*D*
Flaked maize	560	556	553	550
Barley	354	352	349	347
Sprat meal	76	75	75	75
Minerals[1]	9	10	10	10
Vitamins[2]	1	1	1	1
Urea[3]	—	6	12	18
Estimated (g/kg DM)				
Total N	23.6	23.3	29.0	31.7
RDN	11.7	14.5	17.3	20.0
UDN	11.8	11.8	11.8	11.7
Tissue N	11.1	12.3	13.4	14.5

[1] Supermindiff VM5 (Boots Farmsales, Nottingham) contained per kg DM:

Ca, 40g; Cu, 1.2g; Se, 8mg; Vit A, 1200,000 iu; Vit D_3, 600,000 iu; Vit E, 100iu.

[2] Beta 103 (BP Nutrition, Northwich, Cheshire) contained per kg DM: Vit A, 8 million iu; Vit D_3, 2 million iu.

[3] Urea contains 46.21% N.

urea/kg air-dry concentrate. The roughage in the diet was provided by lucerne hay and fish meal was included to provide most of the undegraded N (UDN) necessary to support liveweight gain. The composition of the diets used is shown in Table 1.

Feed allocation was designed to allow 0.75kg/d gain for steers of large breeds receiving a diet of metabolisability $\left(\frac{ME}{GE}\right)$ of 0.7 (i.e. 13.3 MJ ME/kg DM) based on the ME requirement (MJ ME/$Wkg^{0.75}$) for 50, 100, 200kg animals which are 16.7, 24.3 and 37.1 MJ ME/d respectively (ARC, 1980). This was achieved by feeding 64g/$Wkg^{0.75}$ at 50kg body weight and 56g/$Wkg^{0.75}$ at 100kg (ARC, 1980) and assuming that the requirements at higher weights were linearly related to this. The basal concentrate provided 13.4 MJ ME and 23.5g N/kg DM. The chemical composition of the diets is shown in Table 2. Feeding was at 08.25h and 14.45h daily. Water was always available.

Experimental routine:

A 4 × 4 Latin Square design with four 2—week periods was used. Diets were changed abruptly at the beginning of each period. Each period consisted of a 6—day

TABLE 2

The mean concentration of some major nutrients in the dry matter of basal concentrates alone (A) or supplemented with 0.6% (B), 1.2% (C) or 1.8% urea (D) and in the lucerne given to four calves at two stages of development.

	A	B	C	D	*Lucerne*
	Stage 1,	*age 3*	*to 6*	*months*	
Organic matter (g/kg DM)	964	963	965	963	988
N (g/kg DM)	23.5	26.8	29.4	32.3	24.8
Starch (g/kg DM)	593	585	581	572	41
Acid detergent fibre (g/kg DM)	39	39	39	38	362
	Stage 2,+	**age 8**	**to 11**	**months**	
Organic matter (g/kg DM)	963	963	964	964	882
N (g/kg DM)	24.0	27.0	29.3	32.6	25.4

\+ Stage 2 analytical values for organic matter and N were similar to stage 1 values which therefore were used for the calculation of the intake of nutrients for both stages.

adaptation period. The animals were weighed on day 6 and day 13. The markers used were Cr-EDTA (Binnerts *et al.* 1968) and tris — (1, 10 phenanthroline) — Ru (III) Chloride (Tan *et al.* 1971). Marker solutions (67mg Ru/1 or 930mg Cr/1) were infused into the rumen at the rate of 15 and 30ml./h at the first and second stages respectively. Marker infusion was begun on day 4 and lasted for 6 days. Faeces was collected by a harness and hag arrangement and a sample was stored at — 20°. Urine was collected into 200ml. HCl (30/v/v) daily and a sample was retained for analysis. Duodenal and rumen samples were taken at 3 hourly intervals on day 11 and bulked. Rumen samples for separation of bacterial matter was collected at 11.30 and 22.00h on day 13 and 08.00h on day 14 and bulked. Bacterial matter was separated by differential centrifugation (Smith and McAllan, 1974).

Measurements:

The flow of duodenal digesta was measured by spot-sampling using Cr-EDTA and rumen indigestible acid-detergent fibre (ADF) as markers. Duodenal DM flow using ruthenium was unrealistic. Bacterial protein synthesis (BPS) was measured by the diaminopimelic acid technique (Smith, McAllan, Hewitt and Lewis, 1978). Bacterial samples were also analysed for DM, N and OM.

Chemical Analysis:

OM was determined by ashing at 550° for 4h, N by the microkjeldahl technique and ADF by the method of Van Soest (1973). Cr in duodenal fluid was determined by atomic absorption spectrophotometer (Pye 2900) according to the method of Whiteside (1976). Rumen ammonia was determined by the method of Conway (1955).

Data Analysis:

The data were analysed as a Latin square with the two stages of development as sub-plots (Cochran and Cox, 1957). The SE of a difference between diets at a given stage is based on a pool of the main plot Latin Square (6df) and the interaction between stages, calves and diets (8df). The standard error of a difference between two stages for a given diet is based on the interaction between stages and calves within diets (11df.) Linear and quadratic responses were calculated and were related to the concentrations of urea in the concentrates.

RESULTS

Disappearance of nutrients between the mouth and duodenum and between the mouth and in faeces will be referred to as digestion in the stomach and in the total tract respectively.

Digestion of OM:

The effects of dietary treatments on the amounts of OM at the duodenum and in the faeces, or digestibility in the stomach and throughout the tract and on the ratio of digestibility in the stomach to digestibility throughout the tract were small and for individual stages, non-significant (Table 3). The only dietary effect to attain significance was a quadratic response in faecal OM output for both stages combined ($P \angle 0.05$). The coefficients of digestibility in the stomach and throughout the tract were, on average, 10% ($P \angle 0.05$) greater at the second stage.

TABLE 3

The mean amounts (g/d) of organic matter (OM) in the feed, at the proximal duodunem and in the faeces and the digestibility of the OM consumed by four calves at two stages of development (100kg and 200kg) and receiving a basal diet of lucerne hay and concentrates alone (O) or supplemented with 6, 12 or 18g urea/kg concentrates

	Stage	*g urea/kg concentrates*				*SED+*
		0	6	12	18	
Amounts (g/d) Feed	1	1983	1972	2003	1981	26.0
	2	2860	2839	2850	2851	(30.9)
Duodenum	1	870	874	915	862	66.3
	2	1087	1019	1101	1207	(76.9)
Faeces	1	362	348	330	364	27.3
	2	431	365	395	392	(33.7)
Digestibility	1	0.559	0.554	0.542	0.565	0.0267
Stomach	2	0.618	0.640	0.613	0.577	(0.0311)
	Significance of difference (SED: 0.0281 ±)	NS	*	*	NS	
Total	1	0.816	0.823	0.835	0.816	0.0103
	2	0.0848	0.868	0.861	0.863	(0.0126)
	Significance of difference (SED: 0.0131 ±)	*	*	NS	*	
Stomach: Total	1	0.683	0.673	0.649	0.692	0.0292
	2	0.728	0.736	0.712	0.669	(0.0330)
	Significance of difference (SED: 0.0262 ±)	NS	NS	*	NS	

NS, not significant

*, $P < 0.05$

±, the SED shown applied to all comparisons within a stage except those involving diet 6 in stage 2 when the value shown in parentheses should be used.

±, SED between two stages for a given diet except for diet 6 when the value shown should be multiplied by 1.155.

TABLE 4

The mean amounts (g/d) of nitrogen in the feed, faeces and urine and retained and the digestibility of the nitrogen consumed by four calves at two stages of development (100kg and 200kg) and receiving a basal diet of lucerne hay and concentrates alone (O) or supplemented with 6, 12 or 18g urea/kg concentrates

		g urea/kg concentrates				*SED*$^{+}$	Response per g urea/kg concentrates	
	Stage	0	6	12	18		*Linear*	*Quadratic*
Feed	1	48.8	54.8	60.5	65.5	1.01	—	—
	2	70.5	79.1	84.5	94.2	(1.16)	—	—
Faeces	1	13.8	13.8	13.3	14.7	0.75	NS	NS
	2	16.5	15.6	15.7	15.9	(0.93)	NS	NS
	Significance of difference (SED: 0.96±)	*	NS	*	NS			
Urine	1	21.7	15.1	21.2	26.8	4.74	NS	0.85 NS
	2	52.1	36.3	34.1	44.7	(5.42)	NS	1.83 **
	Significance of difference (SED: 6.24±)	***	*	NS	*			
Retained	1	13.2	25.9	26.0	24.0	5.22	0.54 NS	-1.02 NS
	2	1.9	27.1	34.7	33.7	(5.93)	1.72 ***	-1.82 *
	Significance of difference (SED: 6.21±)	NS	NS	NS	NS			
Digestibility	1	0.715	0.746	0.780	0.776	0.0119	0.0036 ***	NS
	2	0.764	0.799	0.813	0.832	(0.0137)	0.0036 ***	NS
	Significance of difference (SED: 0.0118±)	**	**	*	***			

NS, not significant

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

±, the SED shown applied to all comparisons within a stage except those involving diet 6 in stage 2 when the value shown in parentheses should be used.

±, SED between two stages for a given diet except for diet 6 when the value shown should be multiplied by 1.155.

Nitrogen Metabolism:

The intake of N together with the amounts in faeces and urine, and that retained are shown in Table 4. At neither stage of development did the addition of urea have a significant effect on faecal N excretion, with the result therefore that there was a significant ($P < 0.001$) linear increase in the apparent digestibility of dietary N both at stage 1 and 2. For all the diets, the apparent digestibility of N was higher at stage 2. The addition of 6g urea/kg concentrate to the basal diet reduced urine N excretion by 30% at both stages; higher levels of urea inclusion resulted in increase in urine N although the quadratic relationship reached significance only during stage 2 ($P < 0.01$).

Nitrogen retention, as measured by N balance, was lowest in calves given the basal diet and increased significantly at both stages when the diet was supplemented with 6g urea/kg concentrate. At stage 1 there was no further response of higher levels of urea inclusion, but at stage 2 there was both a linear ($P < 0.001$) and quadratic ($P < 0.05$) response. Nevertheless, for any given level of urea inclusion in the diet there was no significant difference between the two stages in the amount of N retained. The increase in N retention with the addition of 6g urea/kg concentrate to the basal diet, 12.6 and 25.2g/d at the two stages respectively, exceeded the amounts of urea N, 6.0 and 8.6g/d respectively, added to the diets (Figs 1 and 2).

The daily amounts of total N, ammonia N and non-ammonia N (NAN) passing through the duodenum and the digestibility of total N in the different sections of the digestive tract are shown in Table 6. Over both stages combined, there were

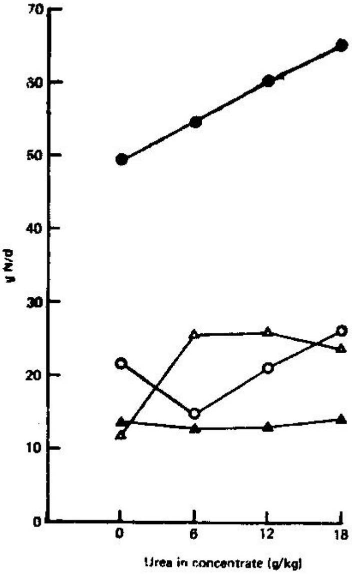

Fig. 1 Nitrogen transactions showing (g/d), mean N intake (●—●), excretion in urine (○—○) and faeces (▲—▲) and retention (△—△) in four calves aged 3 to 6 months and receiving a basal diet of hay and concentrates alone or supplemented with 6, 12 and 18g urea/kg concentrate

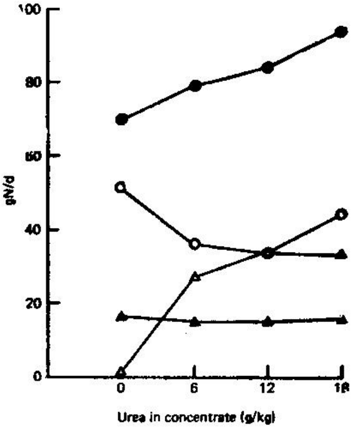

Fig. 2 Nitrogen transactions showing (g/d), mean N intake (●—●), excretion in urine (○—○) and faeces (▲—▲) and retention (△—△) in four calves aged 8 to 11 months and receiving a basal diet of hay and concentrates alone or supplemented with 6, 12 and 18g urea/kg concentrate

significant linear increases in duodenal NAN (P 0.001) and in the amount of N digested in the intestines (P 0.01) with increasing urea concentration in the diet, but when each stage was considered separately, the response was not significant at stage 1. At stage 2, the linear responses ($P < 0.01$) were 0.82g duodenal N per g urea/kg concentrate and 0.9g N digested in the intestines per g urea/kg concentrate, although for both variables the quadratic responses were significant ($P < 0.05$). The increase in duodenal ammonia N was significant at both stages, increases of 0.097g per g urea/kg concentrate ($P < 0.01$) being calculated for stage 1 and 0.065g per g urea/kg concentrate ($P < 0.05$) for stage 2. In general the levels of ruminal ammonia rose markedly a few hours after feeding and were significantly higher during the second stage for any dietary treatment. (Figs. 3 and 4).

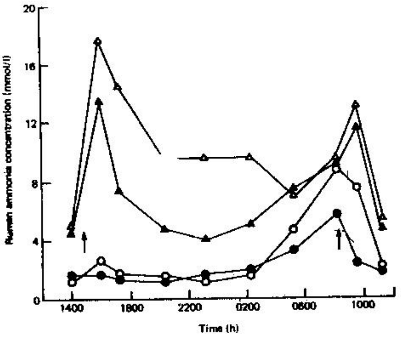

Fig. 3 Mean rumen ammonia concentrations (mmol/l) in four calves aged 3 to 6 months and receiving a basal diet of hay and concentrates alone (●—●) or supplemented with 0.6% (○—○), 1.2% (▲—▲) or 1.8% urea (△—△). Feeding time is indicated by arrows.

Bacterial N Flow:

No protozoa were found in rumen fluid at either stage of development. At both stages of development bacterial N flow was lowest on the diet containing 6g urea/kg but dietary effects were not significant. The proportion of bacterial N in total duodenal N averaged 0.36 at the first stage and 0.49 at the second stage and was unaffected by diet.

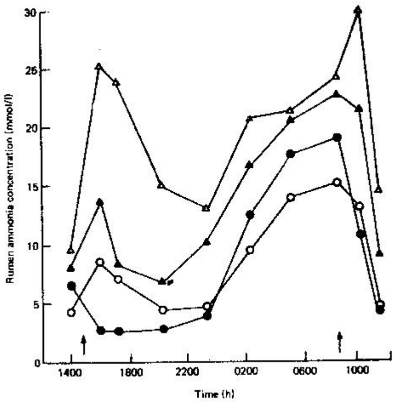

Fig. 4 Mean rumen ammonia concentrations (mmol/l) in four calves aged 8 to 11 months and receiving a basal diet of hay and concentrates alone (●—●) or supplemented with 0.6% (○—○), 1.2% (▲—▲) or 1.8% urea (△—△). Feeding time is indicated by arrows.

DISCUSSION

Measurements of nutrient flow at the duodenum are complex and subject to many different errors. Young animals growing rapidly were used and therefore simple cannulas were employed instead of re-entrant cannulas which have been shown in some cases to exert greater stress (MacRae, 1975; MacRae and Wilson, 1977). Becuase samples obtained from simple cannulas are generally reported not to be representative of digesta passing to the duodenum (Hogan and Weston, 1967), dual-phase markers were used to calculate flow of digesta according to the principles described by Faichney (1975). The estimates of DM and ADF flow to the duodenum based on Ru, the solid phase marker, appeared improbable; ADF flow was higher than intake in the diets. The causes of this remain unknown. The use of rumen-indigestible ADF as a particulate phase marker was a matter of expendiency, but it can be justified. It relies primarily on the assumption that the digestion of ADF in the rumen represents a relatively constant proportion of measured digestion in the total tract. Armstrong and Beaver (1969) concluded that the percentage of digestible cellulose apparently digested in the stomach of cattle, sheep, and goats was relatively constant at about 90%. Based on a survey of the literature, but excluding values for ground and pelleted forages and for lipid-supplemented diets which tend to be lower, Ikwuegbu (1981) concluded that 88.1 could be used. Recently Waller, Merchen, Hanson and Klop-Fenstein (1980) compared the adequacy of chromic oxide, indigestible neutral detergent fibre and indigestible ADF as particulate markers in steers with simple abomasal cannulas and concluded that indigestible ADF was the most suitable of the markers used.

In the present experiment supplementary urea had no effect on the amounts of OM digested in the stomach or in the entire digestive tract even when based on Ru-based DM flow. This is in agreement with the findings of Allen and Miller (1976) who found no increase in DM digestion in the stomach of wether sheep or lambs when 6 to 24g urea/kg were added to a diet containing still lower amounts of N (10g N/kg DM). In contrast Orskov *et al* (1972) found increases of 25% in $ADOM_R/ADOM_T$ in young lambs as a result of adding 7 to 21g urea/kg to a basal ration containing 16g N/kg DM. Since at each stage of maturity in the present studies the amounts of OM truly digested in the stomach were similar on all diets, it can be concluded that energy available for bacterial protein synthesis was similar. Thus, even though the supply of RDN in the basal diet in the present experiment was theoretically insufficient to support maximum microbial protein synthesis according to ARC (1980), neither OM digestion nor BPS was increased by supplementary urea. On the basal diet ruminal levels of ammonia were low and it is reasonable to assume that with these highly digestible diets energy availability from OM digestion did not limit BPS and that the low levels of rumen ammonia at stage 1 result partly from rapid incorporation of recycled urea into bacterial proteins (Fig. 3).

The increase in NAN flow to the duodenum in response to urea supplementation was very small and not significant ($P > 0.5$). However at the higher liveweight, the calculated average increase was about 0.8g per g urea/kg concentrate and at the highest level of supplementation, the flow of NAN was 18% greater than on the basal diet. Since urea supplementation had no detectable effect on OM digestion or bacterial protein synthesis in the rumen, it could be concluded that the basal diet provided sufficient RDN but this clearly was not so. The amount of N entering the duodenum exceeded that consumed with diets containing up to 12g urea/kg concentrate at stage 1 and with the basal diet only at stage 2. The amount of N apparently added in the stomach when the basal diet was given amounted to 10.4 and 9.4g N/d at stages 1 and 2 respectively. Assuming that the daily endogenous abomasal N secretion amounted to 0.03g/kg body weight (Harrop, 1974), it can be calculated that on average about 3 and 6g N/d were secreted at stages 1 and 2 respectively. Thus there was clearly a requirement for additional RDN with the basal diet and it appears this need was adequately met by recycling. However this recycling was associated with large losses of urinary N. It appears that the principal effects of the supplementary RDN in the form of urea were to reduce the amount of N recycled and, by reducing losses of urinary N, improve N retention.

The results of the present experiment not only confirm the general conclusions of Orskov et al (1972) and Allen and Miller (1976) that very young ruminants are able to recycle N to maintain rumen metabolism as are mature ruminants; they also suggest that such young calves possess a mechanism that allows them to maintain rumen fermentative activity at the expense of reduced tissue protein deposition.

The results of the experiment further show that non-protein N sources such as urea can be profitably included as cheap dietary sources of N provided that readily digestible sources of OM are provided and that the diets used do not contain readily degradable protein. For this reason proteins are treated with heat, tannins or formaldehyde (Ferguson, 1975) such that they pass largely undegraded through the rumen but can be digested and absorbed in the small intestines. In Nigeria there is an urgent need to determine the protein degradability of feed ingredients of Tropical origin particularly those from agro-based industries in order to be able to feed ruminants correctly and profitably. The expertise is simple and the technique using Dacron bags (Mehrez and Orskov, 1977) is not labour-intensive. By the use of less degradable feed proteins, the use of non-protein N sources such as urea and biuret can be maximised with appreciable economic returns for the farmer.

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REFERENCES

Agricultural Research Council (1980). *The Nutrient Requirements of Ruminant Livestock.* Commonwealth Agricultural Bureaux: Slough.

ALLEN, S.A. & MILLER, E.L. (1978). 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. *Br. J. Nutr.* **36**, 353—368.

ARMSTRONG, D.G. and BEEVER, D.E. (1969). Post abomasal digestion of carbohydrate in the adult ruminant. *Proc. Nutr. Soc.* **28**, 121—131.

BINNERTS, W.T., van't KLOOSTER, A. TH. & FRANS, A.M. (1968). Soluble chromium indicator measured by atomic absorption in digestion experiments. Vet. Rec. 82, 470.

COCHRAN, W.G. & COX, G.M. (1957). Experimental Deslgns. 2nd Edition pp. 293—304. Wiley: New York.

CONWAY, E.J. (1955). Microdiffusion analysis and volumetric error. MacMillan Co. New York.

FAICHNEY, G.J. (1975). The use of markers to partition digestion within the gastro-intestinal tract of ruminants In: *Digestion and Metabolism in the Ruminant* pp 277—291. England Publishing Unit, Armidale, NSW, Australia.

FERGUSON, A.K. (1975). The protection of dietary proteins and amino acids against microbial fermentation in the rumen. In: *Digestion and Metabolism in the Ruminant.* pp 448—464 (Eds. I. W. McDonald & A.C.I. Warner). Univ. of New England Publishing Unit, Armidale, NSW, Australia.

HARROP, C.J.F. (1974). Nitrogen metabolism in the ovine stomach. 4. Nitrogenous components of the abomasal secretions. *J. Agric. Sci., Camb.* **83**, 249—257.

HOGAN, J.P. & WESTON, R.H. (1967). The digestion of chopped and ground roughages by sheep. II. The digestion of nitrogen and some carbohydrate fractions in the stomach and intestines. *Aust. J. Agric. Res.* 18.

IKWUEGBU, O.A. (1981). *Nitrogen metabolism in ruminant animals at different stages of maturity.* Ph.D. Thesis University of Reading.

MACRAE, J.C. (1975). The use of re-entrant cannulae to partition digestive function within the gastro-intestinal tract of ruminants. In: *Digestion and Metabolism in the Ruminant* pp 261—276 (Eds. I.W. MacDonals & A.C.I. Warner). Univ. of New England Publishing Unit, Armidale, NSW. Australia.

MACRAE, J.C. & WILSON, S. (1977). The effects of various forms of gastro-intestinal cannulation on digestive measurements in sheep. *Br. J. Nutr.* **38**, 65—71.

MEHREZ, A.Z. and ORSKOV, E.R. (1977). A study of the artificial fibre bag technique for determining the digestibility of feeds in the rumen. *J. Agric. Sci. Camb.* 88, 645—650.

ORSKOV, E.R., FRASER, C., & MACDONALD, I. (1972). Digestion of concentrates in sheep. 4. The effects of urea on digestion, nitrogen retention and growth in young lambs. *Br. J. Nutr.* **27**, 491—501.

ROY, J.H.B., BALCH, C.C., MILLER, E.L., ORSKOV, E.R. & SMITH, R.H. (1977). Calculation of the N-requirement for ruminants from nitrogen metabolism studies. In: *Protein Metabolism and Nutrition* pp. 126—129 (Ed. S. Tamminga). Centre for Agricultural Publishing and Documentation: Wageningen, The Netherlands.

SMITH, R.H. & McALLAN, A.B., HEWITT, D. & LEWIS, P.E. (1978). Estimation of amounts of microbial and dietary nitrogen compounds entering the duodenum of cattle. *J. Agric. Sci., Camb.* **90**; 557—568.

SMITH, R.H. & McALLAN, A.B. (1974). Some factors influencing the chemical composition of mixed rumen bacteria. *Br. J. Nutr.* **31**; 27—34.

TAN, T.N., WESTON, R.H., & HOGAN, J.P. (1971). Use of ^{103}Rulabelled (tris 1, 10 phenanthroline) ruthenium (II) chloride as a marker in digestion studies with sheep. Int. J. app. Radiat. Isotopes **22**; 301—308.

VAN SOEST, P.J. (1973). Determination of acid-detergent fibre. *AOAC* **56**; 23—27.

WALLER, J., MERCHEN, N., HANSON, T., & KLOPFENSTEIN, T. (1980). Effect of sampling intervals and digestive markers on abomasal flow determination. J. Anim. Sci. **50**; 1122—1126.

WHITESIDE, P.J. (1976). *Pye Unicam Atomic Absorption Data Book.* 2nd Edition. Pye Unicam Ltd., Cambridge.