Estimation of flows of organic matter and nitrogen components in postruminal digesta and effects of level of dietary intake and physical form of protein supplement on such estimates

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1. Steers fitted with simple rumen, abomasal and sometimes duodenal cannulas were given isonitrogenous and isoenergetic diets consisting of pellets containing equal amounts of rolled barley and chopped straw together with tapioca alone (B), or with some tapioca replaced by coarse soya-bean meal (M) or finely ground soya-bean flour (F). Diet B had an estimated rumen degradable nitrogen (RDN): metabolizable energy (ME) value of approximately 0·8 g/MJ. The corresponding value for each of the supplemented diets was 1·3–1·4. Diets were given at two levels of intake designed respectively to provide ME intakes to support weight gains of 0·5 kg/d (L) and 1·0 kg/d (H). Chromic oxide and polyethylene glycol were given as indigestible markers. Values were calculated for flows (g/24 h) at the abomasum of organic matter (OM), non-ammonia-N (NAN) and microbial (bacterial)-N (MN). Estimates of MN were based on RNA and α-ε-diaminopimelic acid (DAP) measurements. The steers had low rumen protozoal counts throughout the whole experiment with fewer than 8000 ciliate protozoa/ml of rumen fluid.

2. Proportions of OM apparently digested between the mouth and abomasum were significantly lower for H than for L intakes but showed no other significant differences. Mean estimated efficiency of microbial protein synthesis was approximately 17 g N/kg OM apparently digested in the rumen for the LB diet, but was significantly greater at approximately 26 g N/kg OM apparently digested for the HB diet. The protein-supplemented diets gave values of 31–32 which were significantly greater than the value for the LB but not the HB diet.

3. Amounts of feed nitrogenous constituents surviving between the mouth and the abomasum were estimated from NAN minus MN corrected for endogenous N. Values for the proportions of feed N degraded, derived from these estimates, were 0·71, 0·66, 0·72, 0·69, 0·67 and 0·70 for diets LB, HB, LM, HM, LF and HF respectively. There were no significant differences due to level of feeding, supplementary protein or the physical form of that protein.

4. Estimates of MN flows at the abomasum based on RNA determinations in fresh samples did not differ significantly from estimates based on DAP determinations. However, storage of aqueous suspensions of mixed rumen bacteria by deep-freezing or freeze-drying resulted in significant losses of RNA. No such losses were observed for abomasal digesta. This meant that if samples were stored by these means before analysis, the use of RNA as a microbial marker was vitiated.

5. In some experiments digesta samples were taken from the duodenum as well as the abomasum. Estimates of total N flows did not differ significantly between these sites.

A number of recent reviews have dealt with factors affecting microbial synthesis in the rumen (Smith, 1979; Stern & Hoover, 1979; Armstrong, 1980; Harrison & McAllan, 1980; Tamminga, 1980). The numerous studies to determine the efficiency of synthesis in vivo in terms of energy supply or the closely-associated measurement of organic matter (OM) digested in the rumen show a mean value of approximately 30 g microbial (bacterial)-N (MN)/kg apparently digested OM, but values ranging from 14–61 have been reported (Agricultural Research Council, 1980). Part of the variation reported is undoubtedly due to the use of different microbial markers and other differences in techniques but real differences almost certainly occur (Stern & Hoover, 1979). Some factors which might cause these differences have been considered in the present work.

One factor which might lead to variation in microbial synthesis efficiency is the level of food intake which is known to affect the rates of disappearance of both liquid and solid digesta components from the rumen. Rumen fluid turnover rate can, in some circumstances, affect the efficiency of microbial protein synthesis (Harrison & McAllan, 1980). Such experiments as have been reported (Kennedy & Milligan, 1978; Tamminga *et al.* 1979) have

not indicated marked effects of level of intake on the efficiency of microbial synthesis, but the experimental information is scanty. Level of intake may also influence the extent of protein degradation between the mouth and the duodenum by affecting retention time in the rumen while the extent of fineness of the protein supplement may affect both the rate of protein degradation and residence time in the rumen. Such changes in N availability in the rumen may have secondary effects on microbial synthesis.

The effects of level of food intake and of the physical form of the protein supplements on protein degradation and microbial synthesis in the rumen have been examined in the present work.

METHODS

Animals, diets and collection of digesta

The main experiment was designed as a 6×6 Latin square but this could not be completed with the available food supplies and the experiment was therefore treated as an incomplete Latin square design with five animals and six treatments. At the beginning and end of the experiment respectively the five animals which completed all treatments had mean ($\pm$SE) weights (kg) of 148 ± 8 and 238 ± 8 and mean ($\pm$SE) ages (weeks) of $28{\cdot}9 \pm 1{\cdot}4$ and $46{\cdot}3 \pm 1{\cdot}4$. All had been fitted with rumen and simple abomasal cannulas at approximately 24 weeks of age as described by Smith & McAllan (1970). Three of the steers were also fitted with simple duodenal cannulas at the same time. The steers were virtually free of rumen protozoa; at no time did rumen ciliate counts exceed 8000/ml.

Diet B included rolled barley and chopped (6–12 mm) straw, pelleted together in equal proportions and containing 14·4 g N/kg dry matter (DM). These pellets were given with tapioca to bring the calculated metabolizable energy (ME) content of the diet to 10·5 MJ/kg DM. Two other diets were given in which part of the tapioca was replaced isoenergetically by heated soya-bean meal (M) or soya-bean flour (F) each of which contained 81·1 g N/kg DM. The soya-bean meal was from one single commercial batch (British Arkady Co. Ltd, Manchester) and the flour was prepared by grinding that batch of meal. Of the meal 70% was retained on a 1·5 mm mesh and none passed a 190 μm mesh sieve. Corresponding values for the flour were 0 and 64%. All three diets were given at two levels of intake calculated to support 0·5 (L) and 1·0 (H) kg weight gain/d. Diet components are presented in Table 1. The diets were given in two equal portions at 9.00 and 17.00 hours daily. Shredded paper impregnated with chromic oxide was introduced directly into the rumen at each feed at a rate of 0·9 g Cr_2O_3/kg DM intake together with 100 ml polyethylene glycol (PEG, molecular weight 4000, 300 g/l) throughout the experimental period. Particular experimental diets were given for 22 d and changed on day 23. The diets were given at a fixed level of intake during any one experimental period. The steers were started at approximately 150 kg weight and fed according to that weight for the first period as shown in Table 1. For subsequent periods, diets were calculated assuming an average weight gain of 0·75 kg/d. Thus period-2 diets were calculated assuming a starting weight of 166 kg, period-3 182 kg and so on. Actual mean weights for the animals at the beginning of periods 1, 2, 3, 4, 5 and 6 were 148, 170, 185, 201, 214 and 228 respectively.

On day 21 of each period, samples of abomasal (and in some instances duodenal) digesta were taken immediately before the morning feed and then at three-hourly intervals over the next 21 h. Samples were homogenized and sub-samples (100 g) were combined and stored for subsequent analysis. Samples of rumen digesta (approximately 250 g) were taken at 4, 8, 12, 16 and 24 h after the morning feed on day 22, combined, and mixed bacteria separated as described by Smith & McAllan (1974).

Table 1. *Amounts of the main dietary components (kg dry matter (DM)/d), nitrogen (g/d) and metabolizable energy (ME) (MJ/d) given to steers weighing 150 kg**

Diet...	LB	LM	LF	HB	HM	HF
Barley–straw cubes	3·00	3·00	3·00	4·40	4·40	4·40
Tapioca	0·34	—	—	0·49	—	—
Soya-bean flour	—	—	0·42	—	—	0·61
Soya-bean meal	—	0·42	—	—	0·61	—
N	43·6	77·3	77·3	63·6	112·9	112·9
ME†	35·1	35·1	35·1	50·8	50·8	50·8

* These amounts were increased by 5% for each succeeding experimental period (i.e. 16 kg weight gain).
† Calculated from energy values for individual components given in McDonald *et al.* (1975).

Analytical methods

DM, OM and α-ε-diaminopimelic acid (DAP) were determined as described by Smith *et al.* (1978). Total nitrogen (TN) and ammonia-N were estimated by the procedures of Smith & McAllan (1970). PEG and Cr_2O_3 were estimated as described by Smith & McAllan (1971) and Williams & Smith (1974) respectively. RNA was estimated as described by McAllan & Smith (1969) in fresh extracts of mixed bacteria, abomasal and duodenal digesta from three steers. For the other steers, digesta and bacterial samples were frozen and RNA extracts were prepared at a later date. This latter procedure, as will be demonstrated, proved unsatisfactory.

TN, NH_3-N (where appropriate) and RNA and DAP were determined in the washed bacterial sample and the corresponding abomasal sample. The proportion of non-NH_3-N (NAN) in the abomasal contents which was of microbial origin was estimated as:

$$\frac{\text{TN (bacteria)}}{\text{Marker (bacteria)}} \times \frac{\text{Marker (abomasal digesta)}}{\text{NAN (abomasal digesta)}}.$$

Amounts of digesta constituents entering the abomasum or duodenum in 24 h were estimated from their ratios to the non-absorbed markers Cr_2O_3 and PEG in the appropriate digesta samples and the 24 h intakes of these markers. Analysis of variance was carried out according to Cochran & Cox (1962).

RESULTS

Estimation of RNA

In the original procedure for the estimation of RNA described by McAllan & Smith (1969) and in subsequently reported studies from this laboratory (e.g. Smith *et al.* 1978), RNA was determined in dried fat-extracted residues prepared from mixed rumen bacteria or digesta samples within 2 h of their collection. In the present experiment this procedure was followed exactly with samples obtained from the first three animals. However, samples from the last two animals were frozen and stored at −30° for 4–6 weeks before the extraction and estimation of RNA. On analysing the stored samples it was observed that RNA-N:TN values in mixed bacteria were markedly different from those of the earlier steers.

An investigation was made, therefore, of the effects of storage on samples of digesta or mixed bacteria before the extraction and estimation of RNA. Samples were collected from steers receiving a variety of diets all of which contained approximately equal weights of roughage and concentrates. It was found that deep-frozen or freeze-dried bacterial samples contained significantly less RNA than samples extracted when fresh (Table 2). Greatest

Table 2. *Effect of treatment before extraction on the RNA and α-ε-diaminopimelic acid (DAP) contents of mixed rumen bacteria or abomasal digesta of steers receiving approximately equal proportions of roughage concentrate diets*

(Results are mean values for samples from five (RNA) and four (DAP) steers)

Treatment before extraction	mg RNA-N/g dry matter (mean)	Statistical significance of difference between untreated and treated ($P <$)	mg DAP-N/g dry matter (mean)
Mixed bacteria			
None	9·89	—	0·570
Freeze-dried	7·47	0·001	0·548
Deep-frozen			
1 week	7·69	0·05	—
4 weeks	7·04	0·05	—
8 weeks	6·82	0·05	—
4 weeks (with thawing and refreezing at weekly intervals)	4·02	0·01	0·582
SE	1·23	—	0·162
Abomasal digesta			
None	2·34	—	
Freeze-dried	2·31	NS	
Deep-frozen			
1 week	2·15	NS	
8 weeks	2·16	NS	
4 weeks (with thawing and refreezing at weekly intervals)	2·18	NS	
SE	0·19	—	

NS, not significant.

losses were obtained in samples which had been frozen and thawed a number of times. There was no evidence of similar changes of DAP concentration in bacteria when samples were stored.

Corresponding samples of abomasal digesta from the same animals as were used to supply the bacterial samples were subjected to similar treatments for storage. There were only small and non-significant losses of RNA after either deep-freezing or freeze-drying, amounting to 1–9% of the total RNA contents (Table 2).

Comparison of total flow markers

Amounts of digesta constituents entering the abomasum in 24 h were estimated as described earlier. Mean values (±SE) based on PEG for NAN flow (g/24 h) for diets LB, HB, LM, HM, LF and HF were 55±17, 79±26, 84±10, 97±10, 89±16 and 109±35 respectively. Corresponding values based on Cr_2O_3 were 46±12, 76±18, 78±8, 95±13, 75±9 and 101±30. The differences between markers were not statistically significant and it was considered that sampling was not greatly biased towards solid or liquid fractions of digesta. It was concluded that it was reasonable to assume that true values would lie somewhere between those found for each marker. Thus mean values based on Cr_2O_3 and PEG are reported subsequently (Table 3).

Table 3. *Daily intakes of organic matter (OM), total nitrogen (TN) and estimated metabolizable energy (ME) and rumen-degradable-N (RDN) together with daily abomasal flows of non-ammonia-N (NAN), α-ε-diaminopimelic acid-N (DAP-N) and estimated microbial (bacterial)-N(MN). Also shown are proportions of OM digested between mouth and duodenum both apparent (ADOM) and true (TDOM) and values for DAP-N:TN in corresponding samples of rumen bacteria* *(see p. 124)*

(The information was used to calculate MN yields and proportions of feed-N degraded before the abomasum. Results are mean values with standard errors for five steers each given the six diets (except for a missing value for diet LM) described in Table 1)

	Diet							Significance of effects		
	LB	LM	LF	HB	HM	HF	SE	Level	Supplement	Interaction
Intake										
OM (kg)	3·30	3·14	3·21	4·52	4·55	4·66	0·33	***	NS	NS
ME (MJ)‡	37·9	38·5	40·3	54·6	55·7	57·1	3·3	***	NS	NS
TN (g)	43·6	76·7	77·4	64·6	111·5	115·9	3·9	***	***	***
RDN (g)‖	31·0	55·2	51·9	42·6	76·9	81·1	—	—	—	—
Abomasal flow										
NAN (g)	50·0	80·8	81·8	77·4	96·3	104·8	9·7	**	***	NS
DAP-N (g)	0·21	0·33	0·29	0·35	0·38	0·38	0·09	†	NS	NS
MN (g)	31·6	49·2	57·6	59·3	59·6	63·1	6·9	*	**	NS
ADOM/OM intake	0·56	0·52	0·54	0·50	0·46	0·47	0·04	*	NS	NS
TDOM/OM intake	0·69	0·64	0·73	0·64	0·62	0·59	0·04	*	NS	NS
Bacterial DAP-N:TN	0·0069	0·0068	0·0058	0·0069	0·0065	0·0056	0·0029	NS	NS	NS
MN yield (g/kg ADOM)	17·1	31·2	31·5	26·1	31·4	31·0	3·8	NS	*	NS
MN yield (g/kg TDOM)§	13·9	25·1	23·9	19·9	22·7	24·1	2·3	NS	**	NS
Proportion feed N degraded¶	0·71	0·72	0·67	0·66	0·69	0·70	0·09	NS	NS	NS

‡ Calculated from energy values for individual components (McDonald *et al.* 1975).
‖ Calculated from feed-N degradability values obtained in this experiment.
§ Corrected for microbial contribution at the abomasum.
¶ Assuming 0·03 g endogenous-N secretion/kg live weight.
Significant differences: NS, not significant; † $P < 0·10$; * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

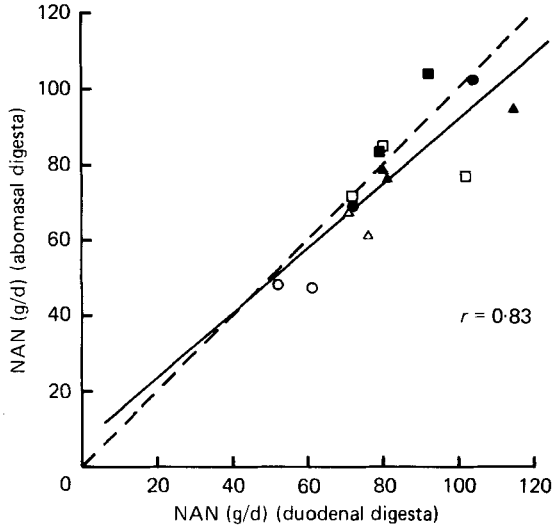

Fig. 1. Relationship between estimated amounts of non-ammonia-N (NAN) in abomasal and duodenal digesta from steers. Four animals received one or more of the following diets: (○), low basal; (●), high basal; (△), low soya-bean meal; (▲), high soya-bean meal; (□), low soya-bean flour; (■), high soya-bean flour. (---), The line of equality; (——), fitted by least squares analysis.

Comparison of sites of digesta sampling

A comparison was made of estimated NAN flows (g/d) obtained with digesta collected from the abomasal and duodenal cannulas. Results presented in Fig. 1 show a strong correlation between results obtained from the different sampling sites with no significant difference between them. Respective RNA-N:TN values in digesta samples from the abomasum duodenum (mean values ± SE) were 0·064 ± 0·004 and 0·061 ± 0·003 and showed no significant difference between them. However, corresponding values for DAP-N: TN were 0·0040 ± 0·00051 and 0·0036 ± 0·00040 and these were significantly different ($P < 0·05$) when a paired t test comparison was made.

Comparison of bacterial markers RNA and DAP

Satisfactory results for RNA were available for only three of the steers out of the five that constituted the incomplete Latin square and comparison of bacterial markers RNA and DAP was restricted to these animals. RNA values were adjusted (× 0·85) to allow for feed contamination in abomasal digesta (Smith *et al.* 1978). The results are shown in Fig. 2. It can be seen that there were no significant differences between calculated daily flows of microbial N at the abomasum based on RNA or DAP as the microbial marker.

Synthesis and degradation between mouth and abomasum

Proportions of OM digested between the mouth and abomasum (both apparent and true) were greater for the lower level of feeding ($P < 0·05$) on all diets (Table 3).

Estimates of the microbial contribution to abomasal digesta flows given in Table 3 were made using DAP as marker. Estimated MN flows (g/d) were greater on high levels of intake of the basal diet (HB) and with soya-bean supplements (LF, LM, HF and HM) than on low levels of intake of the basal diet (LB) or those supplemented with soya-bean either as meal or flour. Efficiencies of microbial protein synthesis were greater at the higher level of intake than the lower but differences were not significant. Protein supplementation significantly increased the efficiency of microbial protein synthesis (Table 3) at both levels of intake.

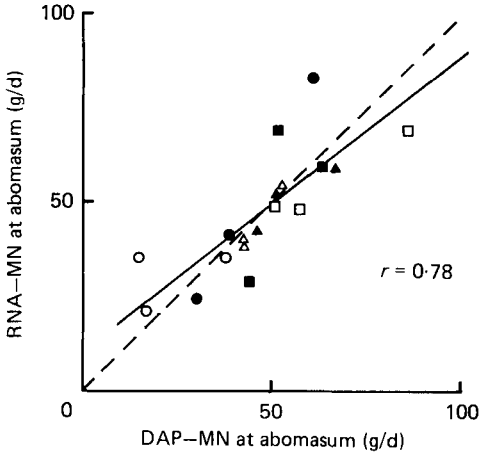

Fig. 2. Relationship between amounts of microbial (bacterial) nitrogen (MN) in abomasal digesta estimated using RNA and α-ε-diaminopimelic acid (DAP) as markers. Three steers received one or more of the following diets: (○), low basal; (●), high basal; (△), low soya-bean meal; (▲), high soya-bean meal; (□), low soya-bean flour; (■), high soya-bean flour. (----), The line of equality; (——), fitted by least squares analysis.

Approximate flows of residual dietary N at the abomasum were calculated by difference between NAN flows and estimated values for MN and taking assumed values for endogenous N of 0·03 g/d per kg body-weight (Harrop, 1974). The results are presented in Table 3. The estimated proportion of feed N degraded between mouth and abomasum was not significantly affected by the level of intake or protein supplementation.

DISCUSSION

In the original method for estimating RNA in microbial and digesta samples, McAllan & Smith (1969) prepared an acid-precipitated, fat-extracted dried residue from samples within 2 h of collection. This residue can be stored at 4° for periods of more than 1 year without deterioration (A. B. McAllan & R. H. Smith, unpublished observations). However, deep-freezing and freeze-drying are commonly-used procedures for storing samples before analysis (e.g. Siddons *et al.* 1979). In the present work these procedures have been shown to result in losses of RNA in suspensions of mixed rumen bacteria. Other experiments (D. N. Salter, unpublished observations) have shown that mechanical disruption of rumen bacteria results in a very rapid loss of RNA due, presumably, to autolytic degradation by intracellular enzymes. Deep-freezing of microbial cells undoubtedly results in rupturing of some of the cells and probably leads to loss of RNA in a similar manner. Whilst one would expect little enzyme activity at −30° some would certainly take place as the sample temperature rises during thawing for analysis. In the present work samples which had been frozen and thawed a number of times were those in which the greatest losses of RNA occurred. The marked variation in losses of RNA is not surprising as time of thawing would depend on the volume of sample and the procedure used. Losses occurring during freeze-drying were also presumably a result of cell disruption. Little or no loss was observed in the RNA content of frozen abomasal samples probably because their pH was approximately 2·5 and most bacterial nucleases have pH optima of approximately 7. Losses of this kind almost certainly explain some of the discrepancies between results based on RNA and other markers reported in the literature (e.g. Siddons *et al.* 1979). Elevation of the RNA-N:TN value in bacterial samples as a result of losses of RNA during storage would result in a variable over-estimate of the microbial contribution at the abomasum or duodenum. It is clearly of the greatest importance that samples are analyzed for RNA soon

after collection. When this is done the RNA method still suffers from the disadvantage that small amounts of feed nucleic acids survive to the abomasum and interfere with the estimation of the microbial contribution. A correction for this is necessary. Smith *et al.* (1978) found that for steers receiving diets containing approximately equal amounts of roughage and concentrates, dietary RNA contributed approximately 15% of the total. In the present work, using similar animals and diets, MN flows based on adjusted ($\times 0{\cdot}85$) RNA values were very similar to those obtained using DAP. Although RNA would also take account of any protozoal contribution at the abomasum while DAP would not, the good agreement found between the methods is not surprising as the steers used in this experiment were almost free of ciliate protozoa.

Results obtained in this work using digesta samples from abomasal or duodenal cannulas agreed very well. The use of abomasal cannulas appears to have several advantages over the use of simple duodenal cannulas. Digesta samples are easier to obtain and the digesta appears more homogeneous. Problems of contamination of samples with bile and pancreatic juice due to backflow are avoided and in our experience, at least in growing steers, abomasal cannulas are easier to maintain and to keep clear and functioning. The small differences found in the DAP-N:NAN values in abomasal digesta compared with duodenal digesta are difficult to explain. They may reflect slight losses of DAP between the abomasum and the duodenum although this seems unlikely. It is possible that the differences were a result of slightly better recoveries of DAP from the more acid digesta of the abomasum during the analytical procedure. This slight discrepancy needs to be borne in mind but has little effect on the interpretation of the results shown in Table 3.

Flows of MN and estimates of the efficiencies of microbial protein synthesis obtained in the present work were similar to those obtained for other steers receiving soya-bean-supplemented diets (Smith *et al.* 1978). The low efficiency of microbial protein synthesis observed for the low level basal diet was very probably a result of growth restriction due to inadequate rumen-degradable-N (RDN). Similar depression has been observed previously (Smith *et al.* 1978). It has been proposed (Agricultural Research Council, 1980) that the RDN:ME (g/MJ) value required for maximum microbial protein synthesis in the rumen is 1·25. On the basal diets (LB and HB) estimated RDN:ME values were approximately 0·80 compared with the corresponding values in the protein-supplemented diets of approximately 1·3–1·4.

The difference in efficiencies of microbial protein synthesis between the low- and high-intake basal diets is more difficult to explain. It is possible that the higher level of intake may have had a stimulating effect on the amount of recycled N entering the rumen. The fact that a similar difference was not observed between the high- and low-intake supplemented diets makes it unlikely that the effect was due simply to the turnover rate of rumen digesta being greater with higher intakes (Kennedy *et al.* 1976; Kennedy & Milligan, 1978; Tamminga, 1978) and microbial efficiency being better for that reason (Harrison *et al.* 1975; Isaacson *et al.* 1975). Indeed the possibility of such effects predictably influencing microbial growth efficiency under the conditions in the rumen needs to be given critical consideration. As pointed out by Harrison & McAllan (1980), published results indicate that whilst a change of dilution rate from 0·02 to 0·06/h may result in increased microbial cell yield, an increase in dilution rate from 0·15 to 0·20/h is unlikely to have an appreciable effect. Fluid turnover rate was not determined in the present experiment but with other steers receiving diets of approximately equal proportions of roughage and concentrates, similar to the diets used here, fluid turnover rates ranging from 0·09–0·17/h have been reported (Merry *et al.* 1982). Moreover it is important to recognize that, in the rumen, solid and liquid turnover rates may be quite different from each other (Faichney, 1980) and microbial nutrient supply may be more closely related to the former than to the latter. More work is necessary to investigate the effect of level of dietary intake (in conjunction with rumen liquid and solid

outflow rates) on the efficiency of microbial protein synthesis, but under the conditions of the present experiment the idea of a simple relationship between greater intakes and greater efficiency of microbial synthesis was not supported.

Estimates of feed N degraded between mouth and abomasum were similar to those observed for other steers receiving diets containing soya-bean supplements which contributed approximately 45% of the N intake (Smith *et al.* 1978). Neither the physical form of the protein supplement nor the level of diet intake appeared to affect the extent of degradation of feed N between mouth and duodenum in the present work. It is known that the residence time of solid material in the rumen is inversely related to particle size (Mertens & Ely, 1979) and Netemeyer *et al.* (1980) reported that a finely-ground soya-bean supplement led to greater flows of NAN at the duodenum of steers than a coarse soya-bean supplement. This could be interpreted as being due to the former having a shorter residence time in the rumen and therefore being less degraded but the effect was not significant. It is possible that in the present work a shorter retention time of the flour in the rumen was offset by its greater susceptibility to degradation but in this area also it is necessary to have a greater understanding of the underlying processes before accurate prediction of N flows to the duodenum can be achieved.

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