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Digesta entering the duodenum of ruminants is composed of material of feed, microbial and endogenous origin. The contribution of any one component can be estimated by marker dilution by measuring the concentration in whole digesta of a marker which is specific to, and of known concentration in, the component. The purpose of the present study was the comparison of four markers (diaminopimelic acid (DAPA), ribonucleic acid (RNA), ^{35}S and ^{15}N) and the amino acid profile method (Evans *et al.,* 1975) for estimating microbial amino acid flow to the duodenum of sheep fed grass silage or dried grass.

Suffolk x Halfbred wethers, 2-3 years old, and fitted with a rumen cannula and re-entrant cannulae in the proximal duodenum and distal ileum, were used. The diets (600 g DM/d) were given continuously and ruthenium phenanthroline (7 mg Ru/sheep per d) was infused into the rumen for 6 d and $Na_2{}^{35}SO_4$ and $(^{15}NH_3)_2SO_4$ for 36 h prior to making collections of duodenal digesta, and taking samples of rumen digesta from each animal for the preparation of a microbial sample. The duodenal digesta and microbial samples were analysed for amino acids, DAPA, RNA, ^{35}S-methionine specific activity and ^{15}N enrichment. The proportion of microbial amino acids in duodenal digesta was determined and was used, in conjunction with total amino acid flow to the duodenum (based on 100% recovery of Ru), to calculate microbial amino acid flow. The results are given in table 1.

Although total amino acid flow to the duodenum was higher on dried grass (79.1 g/d) than on silage (65.0 g/d), microbial amino acid flow as estimated by any one method was similar for both diets. However, there were considerable differences between methods ; RNA gave higher microbial amino acid flow than DAPA, ^{15}N, ^{35}S, and the amino acid profile. The results obtained with the amino acid profile method were very low and the reliability of this method is questionable when forage diets are fed, because of the similar amino acid composition of the feed, microbes and resultant duodenal digesta. The RNA method is dependent on the complete degradation of feed RNA in the rumen, and the high microbial amino acid flows (on the silage it was higher than the total amino acid flow) given by this method suggest that feed RNA does escape rumen degradation. The higher results obtained using the DAPA method as compared to the two isotope methods was unexpected since DAPA is specific to bacteria and would not therefore take into account the contribution of the protozoa to duodenal microbial protein. However, if bacterial turnover in the rumen involved preferential degradation of cell contents (DAPA being present in cell walls), it is possible that DAPA could over-estimate microbial amino acid flow. The two isotope markers are not without criticism since they suffer from the limitation, as do the other methods, that the rumen microbial sample, apart from the possibility of contamination

Table 1. - Flow of amino acids (g/d) of microbial origin to the duodenum of sheep estimated by different methods. Mean values ± standard error for 6 sheep.

	Diet	
	Grass silage	Dried grass
Amino acid profile	15.9 ± 3.11	14.4 ± 2.52
^{35}S-methionine	32.5 ± 2.36	28.8 ± 2.37
^{15}N	35.4 ± 1.58	38.4 ± 1.82
Diaminopimelic acid (DAPA)	60.4 ± 6.07	65.8 ± 5.96
Ribonucleic acid (RNA)	73.5 ± 5.88	66.2 ± 4.88

with feed particles, is unlikely to be representative of the microbial population which passes out of the rumen. This is particularly important if protozoa contribute significantly to microbial flow at the duodenum. The microbial sample is prepared by differential centrifugation and is essentially free of protozoa which are likely to have a lower methionine specific activity or ^{15}N enrichment than bacteria due to the utilization of preformed feed amino acids.

The results support the view that the considerable variation in the energetic efficiency of microbial protein synthesis in the rumen (g microbial protein/kg organic matter apparently digested) reported in the literature may, to a large extent, be a consequence of techniques used. The question as to the most suitable method of measuring microbial flow to the duodenum remains unanswered although the use of isotope markers would seem to be less subject to criticism than other methods. Of the two isotopes used in the present study the coefficient of variation associated with the ^{15}N method was lower than with the ^{35}S method.

References

EVANS R.A., AXFORD R.F.E., OFFER N.W., 1975. A method for estimating the quantities of microbial and dietary proteins flowing in the duodenal digesta of ruminants. *Proc. Nutr. Soc.* **34**, 65A.