Estimation of protein degradation in rumen by three methods

J.H.F. Meyer and S.I. van der Walt
National Chemical Research Laboratory,
P.O. Box 395, Pretoria 0001, Republic of South Africa

The rumen degradability of protein in diets containing maize straw, fish meal and 0, 30 and 60% maize grain was estimated in three ways:
(i) from the difference between the total non-ammonia N and microbial N entering the duodenum over a 24-hour period using ^{35}S and DAPA as microbial markers,
(ii) from the disappearance of N from nylon bags in the rumen and the rate of outflow of rumen solids, and
(iii) from the solubility of the feed N in a bicarbonate-phosphate buffer.

The values obtained by methods (i) and (iii) decreased as the proportion of grain in the diet increased. Those given by method (ii) showed no definite trend.

Voerproteïenafbraak in die herkouer rumen van diëte bestaande uit mieliestrooi, vismeel en 0, 30 en 60% mieliegruis is met behulp van die volgende metodes bepaal:
(i) die verskil tussen die totale NAN en mikrobe N in duodenale inhoud deur gebruik te maak van ^{35}S en DAPA as mikrobe merkers,
(ii) die verdwyning van N uit nylon sakkies in die rumen, gekorrigeer met die vloei van vastestowwe uit die rumen uit,
(iii) en die oplosbaarheid van voer N in 'n bikarbonaat-fosfaat buffer.

Die resultate verkry met metodes (i) en (iii) toon 'n afname in proteïen afbraak met 'n toename van mieliegruis in die dieet, terwyl metode (ii) geen definitiewe tendens toon nie.

Keywords: Rumen, protein degradation, estimation

Introduction

The increasing scarcity and price of protein feeds has made it necessary to optimize the utilization of protein in the diet of ruminants. This has focused attention on the extent of breakdown of feed protein and the synthesis of microbial protein in the rumen. A number of methods have been proposed for measuring the breakdown of feed protein. These include:

(a) The *in vivo* measurement of non-ammonia N (NAN) leaving the rumen and the proportion of microbial protein in this, and hence by difference the undegraded feed protein,

(b) the determination of rate of breakdown of feed protein in artificial fibre bags in the rumen and the rate of outflow of protein from the rumen,

(c) calculation of degradability from the solubility of the protein assuming that all the soluble protein and a fraction of the insoluble protein is degraded.

We have compared the values obtained by these three methods for diets containing maize straw, fish meal and 0, 30 and 60% maize grain fed to sheep. Microbial protein leaving the rumen *in vivo* was measured using both ^{35}S and diaminopimelic acid (DAPA) as markers.

Methods

Five mutton Merino wethers fitted with ruminal and re-entrant duodenal cannulae were used for the collection of digesta. The high (P), medium (R) and low (T) roughage diets (Table 1) were fed in turn in that order. At the conclusion of the experiment the animals were readapted to diet P and the experiment was repeated. Each sheep received 600 g of food twice daily. Collection of duodenal digesta, or nylon bag studies, was made after the animals had been on the diet for 6 – 8 weeks.

Duodenal digesta were collected from the sheep over a 24-hour period. ^{103}Ru-phenanthroline complex (Ru P) was infused for 6 days prior to, and during the collection period and its recovery was used to calculate the normal daily flow of digesta. Microbial protein in the digesta was measured using ^{35}S as the marker (Mathers & Miller, 1980). DAPA was determined on the same microbial and digesta samples using a Beckman model 120 Amino Acid Analyser. Undegraded feed protein was calculated as the difference between total digesta NAN and microbial NAN. No correction was made for endogenous NAN.

Table 1 Diets[a] fed to the sheep

Component	Composition of diets (%)		
	Diet P	Diet R	Diet T
Maize straw	75,0	46,4	17,7
Maize grain	0,0	30,3	60,8
Molasses	10,0	10,0	10,0
Fish meal	9,0	7,3	5,5
Mineral mix	6,0	6,0	6,0

[a]All diets contained *ca.* 10,3% crude protein

Samples of feed (5 g) were weighed into nylon bags (100 × 200 mm, 12,7 μm pore size), and placed in the rumen of 5 sheep fed the same diet as was being tested. One sample was removed from each sheep every 2 hours over a 12-hour period. The rate of flow of protein from the rumen was assumed to be equal to that of the solid portion of the digesta and was measured in the sheep from which the duodenal digesta collections were made. The infusion of Ru P was continued for one day after the digesta collection and then stopped. The rate of flow of digesta from the rumen was calculated form the rate of decline of the specific radioactivity of ^{103}Ru in the faeces.

The solubility of N in the feeds was measured at room temperature in a phosphate-bicarbonate buffer, pH 6,9 (Na HCO_3 9,24 g; Na $HPO_4 \cdot 12H_2O$ 7,12 g; NaCl 0,47 g and KCl 0,45 g/l). The rumen degradation of the N was calculated assuming that all the soluble N and 35% (Verité *et al.*, 1979) or 50% (Baldwin & Koong, 1979) of the insoluble N was degraded.

Results and Discussion

The proportion of microbial protein in the duodenal digesta estimated using DAPA as a marker (diet P 46%, diet R 38% and diet T 34%) was less than that found using ^{35}S (diet P 79%, diet R 61% and diet T 59%) (Table 2). This is to be expected because DAPA is present only in bacteria whereas ^{35}S is incorporated into both bacterial and protozoal protein. The DAPA values will thus underestimate the degradation of the feed protein, and in the present study gave lower values for this than were obtained by any of the other methods (Tables 2 and 3). The coefficient of variation of the results based on the use of ^{35}S as a microbial marker increased from 11% for diet P to 25% for diet T. This is probably less a reflexion of experimental error than of differences in the activities of the rumen microbiota in different sheep, since the microbial population tends to

become increasingly variable as the grain content of the diet increases.

Table 2 Microbial protein in duodenal digesta estimated with ^{35}S and DAPA and calculated degradation of feed protein. (Values represent the mean ± S.D.)

	% Microbial protein in digesta protein		Degradation of feed protein	
Diet	^{35}S	DAPA	^{35}S	DAPA
P(n = 10)	79 ± 7	47 ± 8	79 ± 9	46 ± 13
P(n = 9)	65 ± 6	45 ± 7	61 ± 10	38 ± 14
T(n = 8)	66 ± 7	45 ± 6	59 ± 15	34 ± 17

Table 3 Degradation of the dietary protein as estimated using the solubility and nylon bag methods

		N degradation (%)		
		Solubility methods[a]		Nylon bag method
Diet or feed component	N solubility %	35%	50%	(n = 5)
Diet P	35	58	68	71 ± 11
Diet R	30	55	66	55 ± 4
Diet T	27	52	63	73 ± 8
Fish meal	24	51	62	–
Maize grain	18	47	59	–
Maize straw	38	60	69	–

[a]Calculated on the assumption that all the soluble plus 35% or 50% of the insoluble protein is degraded

The solubility of N in maize grain was lower than that in fish meal and maize straw, and this is reflected in the decrease in solubility and hence the estimated degradability by this method, as the grain content of the diet increased (Table 3). This decrease was also seen in the results obtained from the digesta collections. Assuming that 35% of the insoluble protein was degraded, the solubility method gave lower values (diet P 58%, diet R 55% and diet T 52%) than were found *in vivo* with ^{35}S. If 50% degradation of the insoluble protein was assumed, the results (diet P 68%, diet R 66% and diet T 63%) were somewhat higher than the *in vivo* ^{35}S experiment, except in the case of diet P.

The coefficient of variation of values obtained by the nylon bag method was highest with the high fibre diet. This was probably due to the high solid content of the rumen ingesta of these animals which made mixing difficult. This method gave values in fair agreement with the *in vivo* ^{35}S results for diets P (71%) and R (56%) but gave a much higher figure than any other method for diet T (70%). The agreement between values for the degradation of proteins estimated by the nylon bag and duodenal collection techniques seems to vary owing to, at present, unknown factors. Some authors have found close agreement whilst others have found poor correspondence (ARC, 1980). It is clear that more work should be done on the comparison of values obtained by different methods and the sources of error in each.

The solubility method would seem to be the most suitable for routine examination of feed components. It is simple and rapid and gives a fair indication of the *in vivo* degradability of the proteins.

Acknowledgements

The authors are endebted to Dr J. Dijkstra for the DAPA determinations and to Mr I. Litster for the N determinations.

References

ARC, 1980. Requirements for protein. In: The Nutritional Requirements of Ruminant Livestock. (eds. K. Blaxter *et al.*) Commonwealth Agricultural Bureau. Farnham Royal. p. 121.

BALDWIN, R.L. & KOONG, L.J., 1979. Rumen Modelling. In: Digestive Physiology and Metabolism in Ruminants. (eds. Y. Ruckebusch & P. Thivend). MTP Press. Lancaster. p. 251.

MATHERS, J.C. & MILLER, E.L., 1980. A simple procedure using ^{35}S incorporation for the measurement of microbial and undegraded food protein in ruminant digesta. *Br. J. Nutr.*, 43, 503.

VERITÉ, R., JOURNET, M. & JARRIGE, R., 1979. A new system for the protein feeding of ruminants — The PDI system. *Livestock Prod. Sci.* 6, 349.