DEGRADATION OF DUCKWEED PROTEIN IN THE RUMEN OF SHEEP

DAMRY AND J.V.NOLAN

School of Rural Science and Agriculture, University of New England, Armidale NSW 2351

SUMMARY

Degradation of plant N in the rumen results in the formation of ammonia-N and NAN. Rumen bacteria utilise these breakdown products to synthesise their cell proteins. This study was carried out to investigate the *in vivo* degradation of ^{15}N-labelled duckweed in the rumen and the utilisation of the degradation products by rumen bacteria. We found that duckweed protein was relatively resistant to rumen degradation, and therefore concluded that duckweed provides a potentially useful source of 'escape' protein for sheep. The results also indicated that plant N breakdown products more complex than ammonia-N were extensively assimilated by rumen bacteria. An appreciable intra-ruminal N recycling (48% of N intake) occurred in the sheep in this study.

Keywords: rumen ammonia, duckweed, ^{15}N, escape protein, sheep

INTRODUCTION

Ruminant animals obtain the majority of their requirement for amino acids by intestinal digestion of microbial protein synthesised in the rumen; the remainder comes from dietary proteins that pass through the rumen without being degraded by the action of plant or microbial enzymes (i.e. the so-called 'escape' or 'bypass' proteins). Rumen microorganisms can synthesise their proteins using materials such as peptides, amino acids or ammonia. Moreover, the ruminant host can survive and produce on diets that contain no true protein, provided their rumen microbes have access to non-protein nitrogen compounds such as urea or ammonia (Virtanen 1966).

Bryant and Robinson (1962) grew *in vitro* 89 species of bacteria that had been freshly isolated from the bovine rumen on defined media and found that 81% of these grew with ammonia as their main source of nitrogen: 25% would not grow without ammonia present, and 56% grew in the presence of either ammonia or amino acids from a casein hydrolysate. By labelling the rumen ammonia pool with ^{15}N in sheep given lucerne chaff and isolating and analysing rumen bacteria (Nolan and Leng 1972) found that about 54% of the bacterial nitrogen was derived from ammonia and concluded, by inference, that the remaining nitrogen came from non-ammonia nitrogen (NAN) compounds, e.g. preformed peptides or amino acids. There are few *in-vivo* studies in which the extent of rumen degradation of labelled plant protein has been directly investigated. However, one study by Chapman and Norton (1984) used ^{15}N - labelled Pangola grass and Siratro (harvested after 5 weeks' regrowth) showed that the plant protein degradability in the rumen was 95-99%.

In the present study, we administered dried ^{15}N-labelled duckweed (chosen as a convenient source of plant protein that is easy to label with ^{15}N) into the rumen of sheep to obtain a direct indication of the extent of forage protein breakdown to ammonia in the rumen. We also determined the extent of assimilation of the duckweed protein degradation products into microbial protein.

MATERIALS AND METHODS

Animals and diets

Duckweed (*Spirodella punctata*) was labelled with ^{15}N by growing it on a synthetic medium (pH 7) containing (mg/l) 24 N as $(^{15}NH_4)_2SO_4$ (4.66 % atom ^{15}N excess; Amersham International), 61.5 $MgSO_4.7H_2O$, 85.3 $CaCl_2.2H_2O$, 17.6 KH_2PO_4, 47.3 KCl, 8.3 micronutrient mixture and 9.9 FeEDTA. About two-third of the duckweed present was harvested every week. The harvested duckweed was washed 4-5 times with tap water, then dried (80°C, 24 h) and stored.

Two Merino x Border Leicester crossbreds (live weight 33.3 ± 0.09 kg, mean ± SEM) were fitted with rumen cannulas about four weeks before use. The animals were kept in individual metabolism crates in a continuously lit room and given a daily ration of 400 g oaten chaff and 400 g lucerne chaff (N = 1.31 and 3.16 % air dry, respectively). The feed was provided in approximately equal portions at hourly intervals by means of an automatic belt feeder. The sheep had a continuous access to drinking water.

Experimental procedure

Sixty ml of a solution containing 42.3 mmol $(^{15}NH_4)_2SO_4$ (4.737 mol ^{15}N /100 mol N) was injected over about 0.5 min through the cannula into the rumen of Sheep 1 and directed to different sites in the rumen. Similarly, finely ground ^{15}N-labelled duckweed (N = 2.861 % air dry; 3.08 mol ^{15}N /100 mol N) was dosed into the rumen of Sheep 2. The duckweed (30g) was first made slurry with 200 ml of tap water in a beaker. The slurry was gently swirled and delivered slowly through the rumen cannula via a funnel and plastic tube. The tube was directed to different locations in the rumen to dissipate the labelled duckweed and assist it to mix more rapidly through the rumen contents. The beaker and tube were washed with a minimum amount of water while the tube withdrawn carefully from the rumen contents.

Strained rumen fluid samples (25 ml) were taken from both sheep 0.3, 0.6, 1.1, 1.8, 2.8, 4.4, 6.1, 8.3, 12.4, 22.8 and 32.1 h after ^{15}N-labelled materials were injected. These samples were transferred into centrifuge tubes placed in ice.

Sample preparation

The rumen fluid samples were immediately centrifuged (20,000 x g, 15 min, 4°C) to obtain a supernate which was acidified with 0.25 ml 18M H_2SO_4 and stored at -18°C until analysed. Bacteria were isolated from the residue by twice resuspending the residue in 20ml physiological saline (9 g NaCl/l), re-centrifuging and removing the top (bacteria-rich) layers of residue. The final bacterial isolate was suspended in about 3 ml saline solution and stored at -18°C.

Sample analysis

Ammonia-N (NH_3-N) was removed from the rumen fluid supernate (3 ml) by steam distillation after the rumen fluid was made alkaline with saturated Na-tetraborate (4 ml). The NH_3-N was collected into 3 ml 0.025 M H_2SO_4 and titrated to pH 5 with 0.025 M NaOH. It was then re-acidified to pH 3 and dried in an analytical oven at 95°C. The non-ammonia-N (NAN) left in the rumen fluid was subjected to micro-kjehdahl digestion using concentrated H_2SO_4 (3.5 ml) and a low Se catalyst tablet. The bacterial isolate (0.5 ml) was similarly digested and the digests were distilled after being made alkaline with 40 % NaOH and then dried as for NH_3-N. Ethanol was distilled between all samples to avoid ^{15}N cross-contamination. To prepare samples for ^{15}N analysis, the dried $(^{15}NH_4)_2SO_4$ was re-dissolved with double distilled H_2O and a solution containing 80-100 μg N was transferred into an 8x5 mm tin capsule (Elemental Microanalysis Ltd.). The solution was then re-dried in a vacuum desiccator and the capsule was closed and folded. The ^{15}N enrichment was analysed using a mass spectrometer (Tracermass; Europa Scientific) linked to an N analyser (Carlo Erber).

Calculations

All enrichment v. time curves were normalised to represent 2 mmol ^{15}N administered into the rumen. The resulting data for ammonia and NAN in rumen fluid supernate as well as bacterial N were fitted by curves with two exponential functions using GraphPad Prism version 3.02 for Windows (Motulsky 1999). Rumen fluid ammonia compartment size, rates of total flux and irreversible loss of ammonia and the fraction of bacterial N derived from rumen ammonia were calculated (Nolan and Leng 1972). The fraction of ^{15}N in the duckweed administered to Sheep 2 that subsequently entered the ammonia compartment in this sheep was used as an index of ruminally degradable protein in duckweed. The fraction was taken to be the product of the area under the bacterial curve and the rate of irreversible loss of ammonia (which was assumed to be the same as that determined Sheep 1).

RESULTS

The dried duckweed contained 2.86% N (w/w) of which 8% was present as ammonia. The enrichment v. time results in the period after ^{15}N administration and the fitted two-exponential curves are given in Figure 1.

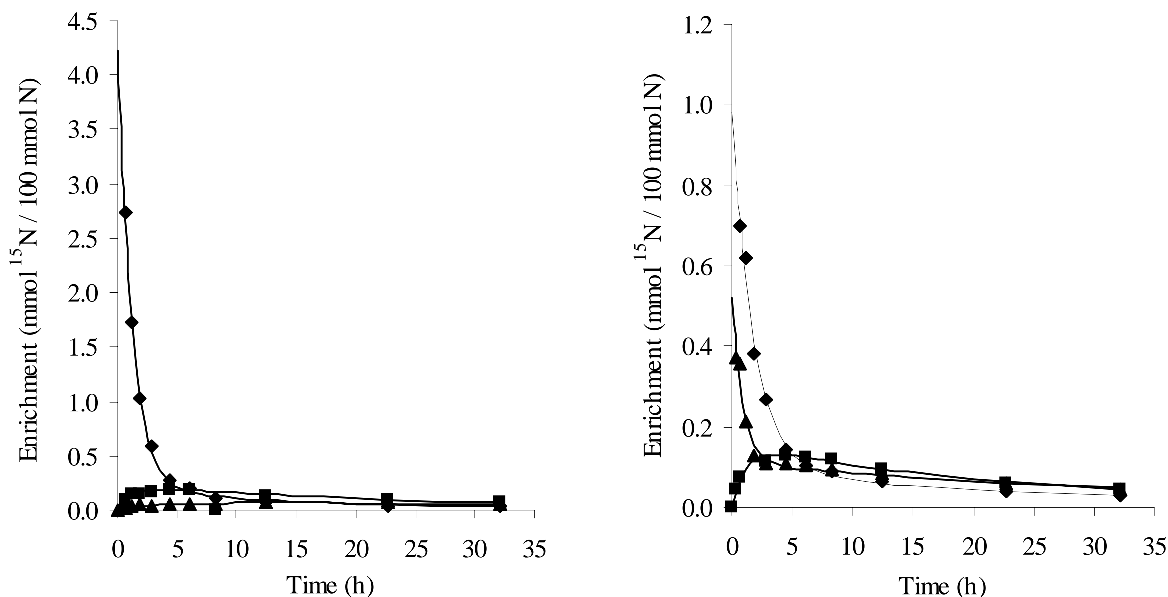

Figure 1. Enrichments in rumen fluid ammonia (♦), rumen fluid non-ammonia nitrogen ( ) and bacterial nitrogen ( ) over time after intraruminal administration of 2 mmol ^{15}N as ^{15}N-labelled ammonia (Sheep 1, left) or ^{15}N-labelled dried duckweed (Sheep 2, right). The lines represent fitted curves with two exponential functions

For Sheep 1, the ammonia concentration in rumen fluid was 161 mg N/l. The estimated rumen ammonia compartment size was 663 mg N and thus the volume of fluid in which this ammonia was distributed in this animal was 4.12 l. The total ammonia flux was 12.9 g N/d and the irreversible loss rate was 8.17 g N/d. About 4.8 g ammonia-N left the ammonia compartment and returned to it each day, i.e. recycled. The ratio of the area under the bacterial N curve to that under the rumen fluid ammonia curve for Sheep 1 was 0.70 indicating that 70% of bacterial N was derived directly from ammonia, and the remaining 30% was therefore derived from NAN. The corresponding ratio for Sheep 2 was considerably higher (0.92).

Sheep 2 had a lower rumen ammonia concentration than Sheep 1, i.e. 100 v. 161 mg N/l and after receiving the same dose of ^{15}N in duckweed as Sheep 1 (2 mmol), its ammonia compartment became labelled with ^{15}N, but to a far lower extent than occurred in Sheep 1 (see Fig. 1). However, the enrichments of rumen NAN and bacterial N relative to the ammonia enrichment were considerably higher than for Sheep 1. About 50% of the ^{15}N administered as duckweed to Sheep 2 subsequently entered the rumen ammonia compartment.

DISCUSSION

This study demonstrated the potential use of labelled duckweed in elucidating the kinetics of plant N metabolism in the rumen. The duckweed could easily be grown in a medium containing ^{15}N, and an appreciable amount of ^{15}N-labelled duckweed could be produced for use in *in vivo* studies.

The results for Sheep 1 lead to conclusions that are similar to those obtained previously e.g. Nolan (1975). Ammonia flux through the rumen fluid compartment was presumably derived from degradation of nitrogenous feed materials and microbial residues and from endogenous urea transfer via the saliva and

across the rumen wall (Nolan 1975). This flux (12.9 g N/d) was greater than the amount of N ingested in the feed (10 g N/d), and considerably greater than that likely to be released from the feed by microbial digestion in the rumen. Thus it seems clear that appreciable amounts of ammonia were derived from recycling processes some of which would have been urea transferred from the blood, but degradation of microbial protein from lysed cells within the rumen was probably also an important source of ammonia (Nolan and Leng 1972). Also, because more than 50% of the bacterial N was derived directly from ammonia, it can be deduced that the remaining bacterial N was derived by direct assimilation of NAN materials.

The results from ^{15}N-duckweed administration to Sheep 2 provide direct support for this deduction. It is clear that some bacterial N was again likely to have been derived from ammonia, but in this case the amounts appearing in ammonia were greater than that expected on the basis of the results from Sheep 1 (see Figure 1), even after allowing for the small input of ammonia which was 8% of the total N in the dried duckweed. The relatively high degree of ^{15}N labelling of bacterial N in Sheep 2 provides a direct indication that some of the rumen bacterial-N in Sheep 2 must have been derived from duckweed N that did not first pass through the rumen ammonia compartment. The areas under the respective enrichment vs. time curves in rumen fluid NAN and in bacterial-N for Sheep 1 and Sheep 2 support this conclusion. It is likely that this was due to the assimilation by bacteria of peptides and amino acids (Wallace 1996).

By assuming that the irreversible loss of ammonia from rumen fluid of Sheep 2 was similar to that determined for Sheep 1, we were able to calculate the fraction of the ^{15}N administered in duckweed that passed through the ammonia compartment of Sheep 2. The resulting value has been used to indicate the fractional degradation of plant protein in the rumen (Chapman and Norton 1984). In our experiment, the value was 50%, indicating that about half of the duckweed protein was degraded in the rumen and escaped rumen rumen fermentation. This supports our previous study (Damry *et al.* 2001) in which we concluded that duckweed could be used as a source of rumen 'escape' protein for ruminants.

REFERENCES

BRYANT, M. P. and I. M. ROBINSON, 1962. *J. Bact.* **82,** 605-14.
CHAPMAN, P. G. and B. W. NORTON, 1984. *Anim. Prod. Aust.* **15,** 286-9.
DAMRY, J. V. NOLAN, R. E. BELL and E. S. THOMSON, 2001. *Asian Aust. J. Anim. Sci.* **14,** 507-14.
MOTULSKY, H. J., 1999. GraphPad Software Inc., San Diego.
NOLAN, J. V., 1975. In *The IV International Symposium on Ruminant Physiology* (Eds, I. W. McDonald and A. C. I. Warner) pp. 416-31. The University of New England Publishing Unit, Armidale.
NOLAN, J. V. and R. A. LENG, 1972. *Brit. J. Nutr.* **27,** 177-94.
VIRTANEN, A. I., 1966. *Science* **153,** 1603-14.
WALLACE, R. J., 1996. *J. Nutr.* **126,** S1326-34.

Email: jnolan@metz.une.edu.au