

## Degradation of maize protein in rumen contents Influence of ammonia concentration

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1. The influence of ammonia concentration on the distribution of nitrogen derived from opaque-2 maize uniformly-labelled with  $^{15}\text{N}$  has been investigated during short-term in vitro incubation of bovine rumen contents.
2. Less  $^{15}\text{N}$  derived from maize was found in the non-protein-N (NPN) fraction during incubation without added  $\text{NH}_3$  than with added  $\text{NH}_3$ , due entirely to differences in the amount of N derived from maize in the  $\text{NH}_3$  fraction.
3. From calculations based on the transfer of N derived from maize to the NPN pool and to a bacterial fraction, it was concluded that degradation of maize protein was not influenced by  $\text{NH}_3$  concentration within the examined limits.
4. The decrease in relative amount of N derived from maize in the  $\text{NH}_3$  fraction at low concentrations of  $\text{NH}_3$ , together with evidence for an increased fractional turnover rate of  $\text{NH}_3$ -N suggests that a deficient supply of  $\text{NH}_3$  is compensated for by increased catabolism of nitrogenous compounds derived from the rumen micro-organisms.

Since maize grain often accounts for a large part of the diet of beef cattle, information on the pattern of degradation of maize protein in rumen contents is important in order to improve the precision with which diets are compounded. In his review Chalupa (1978) only gave a gross estimate of 40% degradability for maize.

Some evidence was obtained that protein catabolism in rumen contents may be increased at low concentrations of ammonia-nitrogen (Nikolić, Jovanović *et al.* 1975). Other results suggested that the extra protein in the duodenal content of calves given a urea supplement may have been derived from both decreased degradation of dietary protein and more efficient synthesis of bacterial protein in the rumen (Nikolić, Pavličević *et al.* 1975). However, Ørskov *et al.* (1974) concluded that inclusion of urea in the diet did not have a sparing effect on the degradation of dietary protein (barley and fish-meal) in lambs.

In order to elucidate whether the concentration of  $\text{NH}_3$ , as an end-product of protein catabolism, affects the rate of breakdown of dietary protein, bovine rumen contents were incubated with maize, uniformly-labelled with  $^{15}\text{N}$ , in the presence and absence of added  $\text{NH}_3$ . The results obtained suggest that an excess of  $\text{NH}_3$  does not suppress hydrolysis of maize proteins within the limits studied.

### EXPERIMENTAL

#### *Maize labelled with $^{15}\text{N}$*

Maize (hybrid ZP-SK-72-O<sub>2</sub>) grown in two successive years on a plot fertilized with  $^{15}\text{NH}_4$ ,  $^{15}\text{NO}_3$  and  $(^{15}\text{NH}_4)_2\text{SO}_4$  respectively was used in the experiment. The ears were dried at 60° for 24 h before separation of the grain. The total and non-protein-N (NPN) contents of the two samples are shown in Table 1 along with the values for  $^{15}\text{N}$  content. Samples of maize used for incubation were ground to pass a 1.5 mm screen.

Table 1. Nitrogen content of  $^{15}\text{N}$ -labelled maize

Sample	Total N (mg/kg)	$^{15}\text{N}$ excess (g/kg N)	NPN (mg/kg)	NPN (g/kg N)	$^{15}\text{N}$ excess (g/kg NPN)
Maize 1	12.81	12.71	1.41	110.1	13.19
Maize 2	12.40	19.87	1.45	116.9	20.32

NPN, non-protein-N.

#### Animals

A young bull (about 15 months old) and a bull calf (about 4 months old), both of the Simmental breed, were used as sources of rumen content. The animals, fitted with rumen cannulas, were fed on a concentrate diet based on (g/kg) ground maize (660) and dried sugar beet pulp (300) supplemented with urea, minerals and vitamins at a total level of 6 kg and 3 kg/d respectively in two portions. They also received 0.5–1 kg lucerne hay/d except on the day before sampling.

#### Procedure

Samples of rumen contents (40 g) from each animal were incubated with each sample of maize (2 g) using the procedure described earlier (Nikolić, Jovanović *et al.* 1975) except that the flasks were gassed with carbon dioxide instead of  $\text{N}_2$ . In each incubation the flasks were divided into two series identical in all respects except for the addition of sufficient 1.42 M-ammonium chloride solution to increase the  $\text{NH}_3$  concentration in series B flasks by 15.2 mmol/l. At hourly intervals duplicate flasks of each series were removed from the water-bath and metabolic processes stopped by the addition of 2.5 M-sodium hydroxide solution (5 ml). Protozoal motility was checked and the pH measured in the contents of an extra flask examined without fixative after incubation for 5 h.

#### Analyses

The concentration of  $\text{NH}_3$  was determined by the microdiffusion procedure of Conway (1962), total N by a standard Kjeldahl method and NPN by the Kjeldahl technique after precipitation of proteins in 0.61 M-trichloroacetic acid (TCA) solution. Total volatile fatty acid (VFA) production was determined volumetrically after steam distillation.

Determinations of  $^{15}\text{N}$  in the  $\text{NH}_3$  and non- $\text{NH}_3$ -NPN fractions were carried out as described previously (Nikolić, Jovanović *et al.* 1975). A bacterial fraction was prepared by differential centrifugation (750 g and 20000 g).

The sediment was washed twice with phosphate buffer (pH 7).

## RESULTS

### General conditions

The protozoa were found to be alive and motile at the end of each of the four incubations. Rumen contents from both animals contained many oligotrich protozoa, mainly small entodiniomorphs. A few holotrich protozoa were observed in the digesta from the calf. The mean ( $\pm$ SE) initial pH in the medium was  $7.10 \pm 0.18$  and the final pH after 5 h incubation was  $5.89 \pm 0.20$ . Mean ( $\pm$ SE) VFA production was  $9.8 \pm 1.9$  and  $9.9 \pm 2.4$  mmol/l per h in series A and B flasks respectively. The considerable variation, between incubations implied by the above values for SE was not related to the animal donor or maize sample. However, addition of  $\text{NH}_4\text{Cl}$  had no apparent effect on these factors.

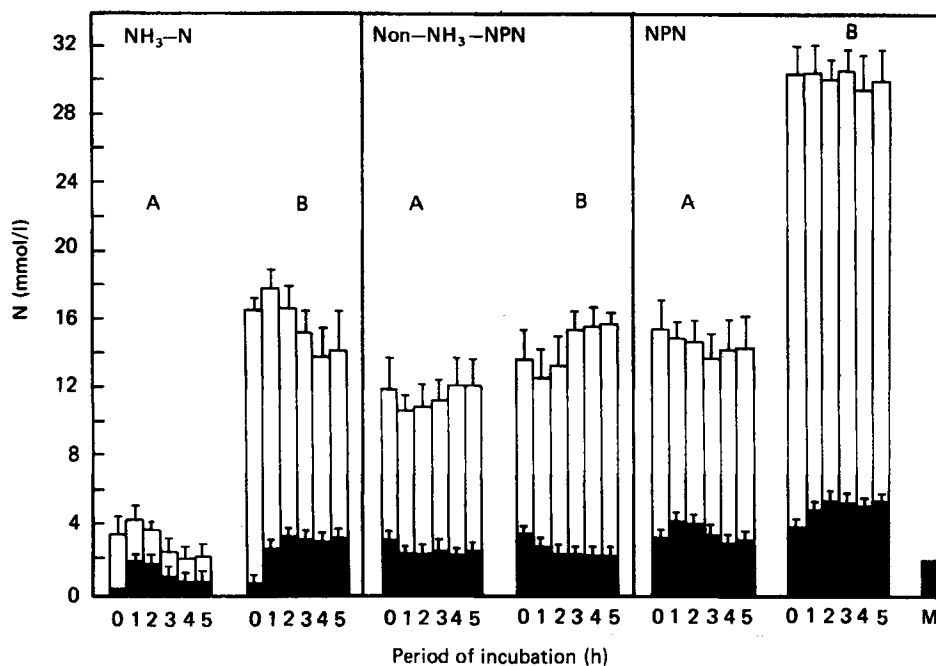


Fig. 1. Content of nitrogen derived from maize in different N fractions of rumen contents incubated for 5 h *in vitro* (series A, without added ammonia; series B, with added ammonia). Vertical bars represent the estimated SE of the mean ( $n$  4). M, added non-protein-N (NPN) derived from maize.

#### *N metabolism*

The mean ( $\pm$ SE) concentration of total N in the flasks was  $79.2 \pm 7.3$  and  $95.2 \pm 8.2$  mmol/l in series A and B respectively, the difference corresponding to the amount of added NH<sub>3</sub>-N. As expected, total N concentration did not change during incubation while NPN (Fig. 1) tended to decrease, indicating net synthesis of protein, i.e. microbial synthesis of protein was greater than degradation of maize protein and turnover of microbial protein together.

Even at the start of the incubation the amount of N derived from maize, which was present in the NPN pool, was larger than the amount added (Fig. 1), suggesting rapid bacterial action on soluble proteins during the few minutes between addition of labelled maize to the rumen contents and fixation with NaOH solution and steam. During the first hour of incubation the amount of maize N in the NPN fraction increased in both series of flasks (Fig. 1). Thereafter, it reached a plateau in series B but decreased in series A. This reduction was entirely due to a decrease in the amount of N derived from maize in the NH<sub>3</sub>-N fraction (Fig. 1).

Thus, after 2 h incubation the amount of maize N in the non-NH<sub>3</sub>-NPN fraction remained constant in both series A and series B. In series A the concentration of non-NH<sub>3</sub>-NPN first decreased and then increased back to the initial level. This was accompanied by an increase in NH<sub>3</sub>-N concentration followed by a decrease to values below the initial level (Fig. 1). The pattern of change in these two fractions was similar in series B except that the final concentration of non-NH<sub>3</sub>-NPN was higher than the initial concentration and the drop in NH<sub>3</sub>-N concentration was nearly double that in series A. The difference in the concentration of non-NH<sub>3</sub>-NPN between the two series after 3 h incubation was statistically significant ( $P < 0.05$ ) supporting earlier findings that an excess of NH<sub>3</sub> may be partly

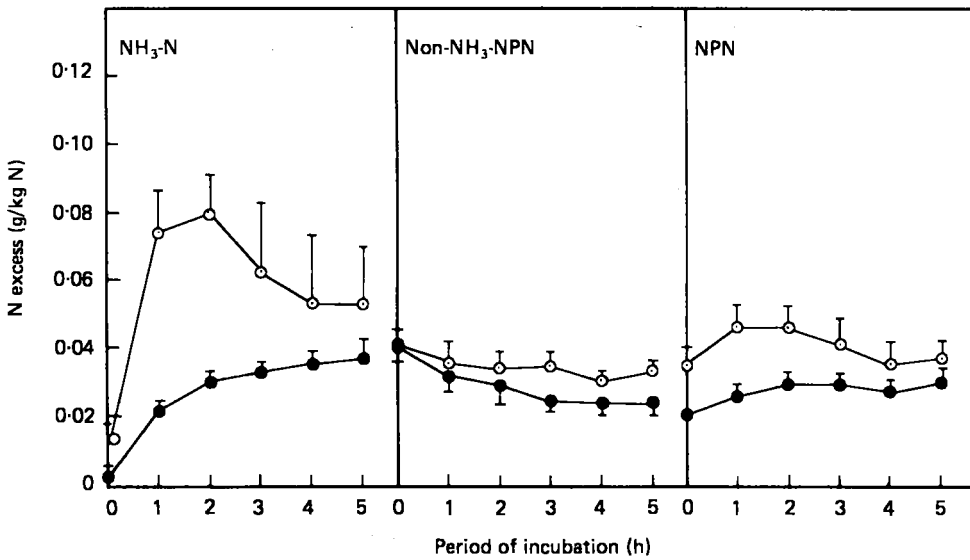


Fig. 2. Relative excess of  $^{15}\text{N}$  derived from maize in different N fractions of rumen contents incubated for 5 h in vitro. (○-○) series A, without added ammonia; (●-●) series B, with added ammonia. Vertical bars represent the estimated SE of the mean ( $n$  4). NPN, non-protein-N.

Table 2. Conversion of maize protein into microbial protein during incubation of bovine rumen contents in vitro with (series B) and without (series A) added ammonia (Mean values with their standard errors)

Incubation		Maize N in NPN (mmol/l)		Maize N in bacterial fraction (mmol/l)		Total degradation of maize protein* (mmol N/l)	Relative degradation of maize protein
		Mean	SE	Mean	SE		
Series A	0 h	5.07	0.40	3.58	0.58	13.17	0.56
	5 h	5.23	0.33	16.58	2.85		
Difference		0.17	0.73	13.00	2.28		
Series B	0 h	4.69	0.83	3.64	0.64	15.07	0.64
	5 h	8.33	0.71	15.05	0.06		
Difference		3.65	1.53	11.42	0.60		

\* Trichloroacetic acid-insoluble N.

converted into amides (Nikolić *et al.* 1971) or amino acids (Demeyer & Van Nevel, 1980). This effect can be seen more clearly in Fig. 2, where the relative excess of  $^{15}\text{N}$  in the non-NH<sub>3</sub>-NPN fraction decreased more steeply in series B than in series A, having started at approximately the same value.

In both series the relative excess of  $^{15}\text{N}$  achieved a higher final value in the NH<sub>3</sub> fraction than in the non-NH<sub>3</sub>-NPN fraction suggesting that the whole of this heterogeneous fraction was not in equilibrium with the NH<sub>3</sub> fraction. However, the fact that the relative excess of  $^{15}\text{N}$  in the whole NPN fraction reached a plateau in series B after 2 h incubation indicated that entry and exit of N derived from maize was equal and constant.

The presence of a certain amount of N derived from maize in bacterial protein at the start of the incubation indicated that the fraction had some contamination (Table 2). However, assuming that the same quantity of maize protein is spuriously present after 5 h incubation, it is possible to calculate the total degradation of maize protein during incubation. The values obtained indicate that the extent of degradation was similar in series A and B, i.e. a low  $\text{NH}_3$  concentration did not stimulate degradation of maize protein while a high  $\text{NH}_3$  concentration did not inhibit it. Thus, taking the average of the values shown in Table 2, it appears that the relative degradation of maize protein under these conditions was 0.60. Similarly, taking into account the NPN fraction it can be calculated that the over-all mean value for relative degradation of maize crude protein ( $\text{N} \times 6.25$ ) was 0.6 g.

#### DISCUSSION

The incubation system used mimics the post-prandial situation in the rumen of beef cattle fed on maize, with or without a supplement such as urea, without the complications due to absorption and passage of metabolites and nutrients. Maize-N accounted for approximately 0.25 and 0.20 of the total N in the incubation medium in series A and B respectively. The uniformity with which maize-N was labelled with  $^{15}\text{N}$  was shown by the similarity in values obtained for  $^{15}\text{N}$  in different parts of the maize plant (Filipović, 1980) and in total N and NPN in grain (Table 1). Thus, the assumption that values obtained for  $^{15}\text{N}$  in various N fractions can be converted to values for N derived from maize would appear to be valid.

The mean results obtained for relative degradation of maize protein (0.69 for crude protein ( $\text{N} \times 6.25$ ) and 0.60 for TCA-insoluble material) were higher than those of Ørskov & Mehrez (1977) and Mehrez & Ørskov (1978), who used an *in vivo* incubation technique. In our calculation it has been assumed that the bacterial fraction is representative of the whole microbial population, whereas it may be expected that the protozoa would contain less  $^{15}\text{N}$  than the bacteria (Abe & Kandatsu, 1969; Pilgrim *et al.* 1970). This would introduce a small error into the calculation. However, the endosperm of normal maize hybrids contains half its protein in the form of the poorly-degradable zein (Ely *et al.* 1967; Hume 1970), while it is known that opaque-2 mutants have much less zein and relatively more albumin and globulin in the endosperm (Jiménez, 1966). It may be expected that the degradability of protein in the opaque-2 mutant used here would be greater than for the conventional hybrids examined by other authors.

Nevertheless, after 3 h incubation the mean concentration of  $\text{NH}_3\text{-N}$  (2.5 mmol/l) in series A flasks approached the level critical for optimal microbial protein synthesis (Satter & Slyter, 1974). Since at this time the relative excess of  $^{15}\text{N}$  in the  $\text{NH}_3$  pool had started to decrease (Fig. 2), one may assume that entry of non-maize-N to the  $\text{NH}_3$  pool is stimulated in preference to N derived from maize. The large standard error in the measurements at this time was due to the fact that the relative excess of  $^{15}\text{N}$  decreased very rapidly in two incubations (one from each animal), where the apparent number of protozoa was moderate and much less steeply in the two incubations where many active protozoa were observed. The rapid reduction in relative excess of  $^{15}\text{N}$  was accompanied by a fall in  $\text{NH}_3$  concentration (to 0.5 mmol/l at 4 h in one incubation).

Nikolić, Jovanović *et al.* (1975) showed that the fractional turnover rate of the  $\text{NH}_3$  pool increased as the  $\text{NH}_3$  concentration decreased, so that the over-all utilization rate of  $\text{NH}_3$  (flux) remained approximately constant. In this experiment accurate compartmental analysis to obtain rate-constants was not possible because the isotopic label,  $^{15}\text{N}$ , was neither supplied as a single dose nor at a known constant rate. The four different types of protein and the NPN fraction in maize may be expected to release  $^{15}\text{N}$  to the  $\text{NH}_3$  pool

at vastly differing rates. Nevertheless, an approximate calculation made over the first 2 h of incubation using a two-pool closed-system model indicated that the fractional turnover rate of the  $\text{NH}_3$  pool was much greater in series A than in series B which confirms earlier results (Nikolić, Jovanović *et al.* 1975). It appears from the values given in Figs. 1 and 2 and Table 2 that this may be related to an increased turnover rate of microbial N. In this connection Pine (1973) has shown that ammonia deficiency may immediately stimulate intracellular proteolysis in *Escherichia coli* up to double the normal level. Since it has been shown that the main pathway of  $\text{NH}_3$  fixation by rumen bacteria differs according to the prevailing concentration of ammonia (Erfe *et al.* 1977), it is suggested that catabolic processes in rumen bacteria may also be influenced by  $\text{NH}_3$  concentration. However, the results presented here suggest that low  $\text{NH}_3$  concentrations do not affect the rate of degradation of dietary protein, at least in the short term.

Nevertheless, it is possible that the rate of degradation of maize protein is limited by the accessibility of the protein within the particles of maize grain. Namely, the rate of digestion may depend on the rate for other components of the cereal rather than proteolytic activity per se. In this case the capacity for protein degradation could change without having any effect on the rate of  $^{15}\text{N}$  release. Only an experiment using purified maize protein would answer this question.

#### REFERENCES

- Abe, M. & Kandatsu, M. (1969). *Jap. J. Zootech. Sci.* **40**, 290.  
 Chalupa, W. (1978). *Proc. 3rd Wld Cong. Anim. Feeding, Madrid*, **8**, 211.  
 Conway, E. J. (1962). *Microdiffusion Analysis and Volumetric Error*, 5th ed. London: Crosby Lockwood.  
 Demeyer, D. I. & Van Nevel, C. J. (1980). *Proc. Nutr. Soc.* **39**, 89.  
 Ely, D. G., Little, C. O., Woolfolk, P. G. & Mitchell, G. E. (1967). *J. Nutr.* **91**, 314.  
 Erfe, J. D., Sauer, F. D. & Mahadevan, S. (1977). *J. Dairy Sci.* **60**, 1064.  
 Filipović, R. (1980). *Soil Nitrogen as Fertilizer or Pollutant*, Vienna: International Atomic Energy Agency.  
 Hume, I. D. (1970). *Aust. J. agric. Res.* **21**, 305.  
 Jiménez, J. R. (1966). *Proc. High Lysine Corn Conference, Lafayette*. p. 74 [E. T. Hertz and O. E. Nelson, editors]. Washington DC: Corn Refiners Assoc. Inc.  
 Mehrez, A. Z. & Ørskov, E. P. (1978). *Br. J. Nutr.* **40**, 337.  
 Nikolić, J. A., Jovanović, M. & Filipović, R. (1975). *Tracer Studies on Non-protein Nitrogen for Ruminants* vol. 2, p. 43. Vienna: International Atomic Energy Agency.  
 Nikolić, J. A., Jovanović, M., Stosic, D. & Pavličević, A. (1971). *Br. J. Nutr.* **26**, 237.  
 Nikolić, J. A., Pavličević, A. & Zebrowska, T. (1975). *J. agric. Sci., Camb.* **84**, 469.  
 Ørskov, E. R., Fraser, C., McDonald, I. & Smart, R. I. (1974). *Br. J. Nutr.* **31**, 89.  
 Ørskov, E. R. & Mehrez, A. Z. (1977). *Proc. Nutr. Soc.* **36**, 79A.  
 Pilgrim, A. F., Gray, F. V., Weller, R. A. & Belling, C. B. (1970). *Br. J. Nutr.* **24**, 589.  
 Pine, H. J. (1973). *J. Bacteriol.* **115**, 107.  
 Satter, L. D. & Slyter, L. L. (1974). *Br. J. Nutr.* **32**, 199.