

EVALUATION IN VITRO OF AMMONIA NITROGEN REQUIREMENTS FOR RUMEN FERMENTATION AND PROTEIN SYNTHESIS WITH MATURE TROPICAL FORAGE

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SUMMARY

A rumen simulation device was utilised to identify whether the ammonia-nitrogen requirements for maximal rates of rumen fermentation and microbial protein synthesis differ. Using a mature *Heteropogon contortus* hay as the test feed, microbial fermentation and plant associated nitrogen reached maxima with ammonia-nitrogen concentrations of the order of 25 mg/L. However, non ammonia nitrogen synthesis in the fluid phase increased throughout the range of ammonia concentrations tested. These findings are discussed in the context of their potential influences upon voluntary feed consumption.

Keywords: ammonia, microbial activity, tropical grass

INTRODUCTION

Minimum requirements of ammonia-nitrogen (NH₃N) for rumen microbes are uncertain in zebu-crossbred animals fed tropical forages. Levels of 50 mg NH₃-N/L appear adequate for fermentation, but not for maximum forage intake (Boniface et al. 1986); and with diets such as these feed intake often increases in response to protein supplementation. Presumptive evidence exists that NH₃-N may enhance microbial protein synthesis (e.g. Elliot and Armstrong 1982; McAllan and Smith 1984) and, hence, improve the supply of amino acids to the host tissues.

In animal production systems in which it is difficult to maintain rumen ammonia levels above 40-50 mg N/L, despite the addition of urea to the diet, it is clearly important to establish the reasons for improved animal performance with rumen ammonia levels higher than those needed for maximum fermentation. This paper reports the results of investigations with a rumen simulator of the effect of changes in ammonia levels on forage fermentation and nitrogen metabolism by anaerobic rumen microbes.

MATERIALS AND METHODS

An eight chamber rumen simulation device (Ridgway and Radcliffe 1984) was inoculated with rumen contents from a crossbred zebu cow using the procedure of Czerkawski and Breckenridge (1977). The test diet was mature Spear grass (*Heteropogon contortus*) hay which contained 910 g OM, 4.4 g N and 0.4 g S per kg DM. The hay was ground to pass a 2.5 mm screen and two dacron bags (230 x 100 mm, 45 μ m pore size) each containing 6.0 g air dry material were suspended in each chamber daily. A continuous infusion of artificial saliva (McDougall 1948) and trace minerals (Morrison et al. 1986) was maintained with each chamber. Appropriate quantities of urea and sodium sulphate (to provide an infusate N:S ratio of 10) were included to achieve daily intakes of approximately 4, 6, 8, 10, 12, 14, 15 and 18 g N/kg OM. Data from chambers receiving 12 and 15 g N/kg OM are not presented as none of the variables measured differed significantly from those trends shown with treatments of 10, 14 and 18 g N/kg OM.

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Following an incubation period of 48 h two of the dacron bags were replaced with two new bags. Fluid pH was measured at this time and this procedure was maintained for 15 d. All dacron bags were washed and dried following the procedure described by Morrison et al. (1986) with the exception of one dacron bag removed from each chamber on days eight and 15. These bags were washed and frozen prior to freeze drying for total N analysis of the feed residue.

The fluid outflow from each chamber was collected daily in separate bottles containing 10 ml 10 N HCl. A 50 ml subsample was taken and frozen (-20°C) prior to analyses for total-N, ammonia-N (NH₃-N) and volatile fatty acids (VFA).

Microbial fermentation of the test diet was assessed to be the amount of DM lost in 48 hours that could not be accounted for by washing **unincubated bags** of the test diet under running tap water. Total N of dacron bag residues and total N of fluid outflow samples were determined by the Kjeldahl technique and NH₃-N was also determined by a steam distillation procedure. Non-ammonia nitrogen (NAN) was calculated as the difference between total N and NH₃-N concentrations. Concentration and relative proportions of VFAs were determined by gas liquid chromatography as described by Morrison et al. (1986). Data have been statistically analysed by analysis of variance and significant differences between treatment means identified by least significant difference.

RESULTS

Table 1 presents mean values of observations made between days six and 15 of the experiment, when steady state had been reached for all treatments. The **stepwise** increase (P < 0.05) in urea infusion gave rise to NH₃-N concentrations

Table 1. The effect of increasing fluid NH₃-N concentration upon in-vitro measurements of variables describing the fermentation of spear grass

Level of urea supplement (1=lowest 8= highest)	1	2	3	4	6	8	PSE*
NH ₃ -N concentration (mg/L)	13 ^a	16 ^a	25 ^a	52 ^b	109 ^c	155 ^d	1.9
Fluid dilution (vol/d)	0.40	0.38	0.43	0.45	0.44	0.44	0.01
Net DM loss in 48 hour (%)	17.8 ^{a+}	27.2 ^b	32.1 ^c	33.2 ^c	33.1 ^c	33.1 ^c	0.20
Total OM loss (g/d)	2.74	3.78	4.31	4.45	4.43	4.41	
VFA concentration (mmol/L effluent)	36.8 ^a	50.3 ^{bc}	54.2 ^c	57.1 ^c	55.2 ^c	53.5 ^c	0.70
Daily VFA output (mmol)	18.3 ^a	24.1 ^b	29.1 ^{bc}	33.2 ^c	30.5 ^c	29.7 ^c	0.67
mol VFA/kg DOM	6.68	6.39	6.76	7.47	6.89	6.73	
N supply (mg/d):							
Feed	50	50	50	50	50	50	
Total	60.2 ^a	80 ^b	97.7 ^c	117.1 ^d	152.5 ^e	191.5 ^f	1.61
N output (mg/d):							
NH ₃ -N	6.7 ^a	8.1 ^a	14.4 ^a	31.0 ^b	62.0 ^c	84.0 ^d	1.16
Plant-assoc N ₊₊	32.8 ^a	37.3 ^{ab}	40.4 ^b	39.7 ^b	36.1 ^{ab}	37.2 ^{ab}	0.73
NAN in fluid ⁺⁺	20.7 ^a	34.6 ^b	42.9 ^b	46.4 ^c	54.4 ^c	70.3 ^d	1.50
Efficiency of NAN synthesis in fluid:							
gNAN in fluid/kg OM loss	7.81	9.47	10.37	11.01	12.82	14.88	
gNAN in fluid/mol VFA output	1.16	1.49	1.54	1.48	1.86	2.21	

* PSE; Pooled standard error of observations

+ Rows containing values with **unlike** superscripts differ significantly (P<0.05)

++ Non-ammonia nitrogen

of 13 to 155 mg/L, but had no effect on fluid dilution rate (Table 1) or on pH which remained at 7.0 ± 0.1 . Increasing $\text{NH}_3\text{-N}$ concentration from 13 to 16 and to 25 mg/L increased OM loss, VFA production and the nitrogen associated with the washed feed residues in the dacron bags ($P < 0.05$).

The characteristics of VFA production were consistent with expectations for such a diet in vivo. With $\text{NH}_3\text{-N}$ concentrations between 16 and 155 mg/L, no significant differences were observed in the proportions of acetic, propionic, butyric and isovaleric plus valeric acids mean values for which were respectively 71.7, 20.2, 7.1 and 1.0 mmol/100 mmol. However, with the lowest $\text{NH}_3\text{-N}$ concentration, acetic propionic and isovaleric acid production were significantly ($P < 0.05$) altered with the respective proportions of VFA being 68.6, 22.1, 7.4 and 1.9 mmol/100 mmol. Daily output of VFA can be considered to represent VFA production relative to OM fermented in 24 hours and this was calculated to range between 6.39 and 7.47 moles VFA per kilogram OM fermented (Table 1). Total uptake of N by microbes cannot be estimated as the extent of breakdown of plant-N is not known. However net $\text{NH}_3\text{-N}$ uptake increased with increasing levels of $\text{NH}_3\text{-N}$ infusion. The amount of nitrogen attached to washed feed residues was approximately 65% of that supplied in the feed at the lowest $\text{NH}_3\text{-N}$ infusion, increased to 74% and then 81% at the next two levels of infusion and fluctuated between 72 and 79% thereafter. By contrast nitrogen in the fluid phase in forms other than ammonia, that is (NAN), increased ($P < 0.05$) with increasing ammonia supply; although total microbial N synthesis could not be estimated, the efficiency of NAN synthesis in the fluid phase alone expressed either in relation to OM fermented or VFA produced increased throughout the range of ammonia supplied.

DISCUSSION

The data presented indicate that whereas microbial fermentation of matured tropical forage reached maximal rates at $\text{NH}_3\text{-N}$ concentrations of 25 mg/L, synthesis of NH_3 into more complex molecules continued at higher concentrations. Observations of the distribution of NAN between washed feed residues and the fluid phase are consistent with the theory that the size of the microbial population closely associated with plant cell wall reached a maximum at ammonia levels of 25 mg N/L, whereas that of the population of other microbes expanded at higher ammonia levels. If this was so, the increased efficiency of NAN synthesis with increasing ammonia concentrations relates mainly to the fluid phase microbial population. This would imply that observations of increased intake of low quality roughages in response to additional ammonia (Boniface et al. 1986; Kennedy et al. 1987) are associated more with an enhanced supply of amino acids to the tissues of the host animal than to an accelerated fermentation of plant cell wall in the rumen. This enhancement of amino acid supply from NPN supplementation has been shown by Egan and Doyle (1985).

Evidence that the rate of fermentation was restricted at the lowest levels of ammonia raises the question of mechanisms involved. Presumably the size of the microbial population was affected, but in addition the change in pattern of individual VFA suggests a corresponding change in the distribution of microbial types. One nutritional factor, the branched chain VFA, known to be required by the major cellulolytic rumen bacteria, was presumably not limiting as the proportions and amounts of these VFA in effluent at the lowest ammonia supply were higher than at subsequent ammonia concentrations.

With this mature spear grass hay much of its DM, which is potentially fermentable, can be released by microbial action in 48 h and fluid dilution rates used in this work maintain microbial viability (Radcliffe and Ridgway 1984; Morrison et al. 1986). Therefore it seems the influences from $\text{NH}_3\text{-N}$ concentration

upon fibre fermentation reflected the relative changes in the number of micro-organisms which were actively fibrolytic and that released products of cell wall fermentation. The increasing amounts of NAN in fluid with higher $\text{NH}_3\text{-N}$ concentrations implies increased numbers of fluid borne microorganisms, of which some may possess cellulolytic action. Indeed, Kennedy et al. (1987) observed in cattle and buffalo maximal levels of cotton thread digestion with $\text{NH}_3\text{-N}$ concentrations greater than those necessary to maximise the digestion of matured spear grass. To achieve further improvement in fibre digestion, nutritional factors of plant material, such as the forms and number of attachment sites for rumen microorganisms, the range of microorganisms capable of particle fragmentation (such as the anaerobic rumen fungi) and the efficiency of chewing warrant further study.

Using the same in vitro device slow fluid dilution rates have been observed to increase the production of carbon dioxide (Czerkawski and Breckenridge 1977) and slightly depress the yield of VFA per kilogram OM digested without significantly altering their molar proportions (Stanier and Davies 1981). In response to decreasing fluid dilution rate from 0.9 to 0.45 volume per day, Stanier and Davies (1981) also observed a 43% decrease in microbial yield to 8.07 g N per kilogram OM fermented; the present operating conditions presumably then did not affect the validity of the conclusions drawn.

In applying the present data to an understanding of the regulation of roughage intake, it is recognized that an in vitro system while identifying minimal requirements for optimal nutrition of rumen microbes, cannot take into account physiological effects on the host. These could include effects of increasing ammonia levels with or without changes in VFA levels, on blood flow and on VFA metabolism in the rumen wall, on the propulsive effectiveness of rumen contractions and on endocrine changes in the animal's tissues. It seems probable though that increased feed intake reflects metabolic changes in the animal consequent on an improved supply to the tissues of amino acids arising from enhanced microbial protein synthesis.

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