MANIPULATION OF NITROGENOUS SUPPLY TO INCREASE EFFICIENCY OF NET MICROBIAL CELL SYNTHESIS IN THE RUMEN

A thesis submitted to the University of New England for the degree of Doctor of Philosophy

by

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PREFACE

The studies presented in this thesis were completed by the author whilst a postgraduate student in the Department of Animal Sciences, Faculty of Science, The University of New England, Armidale, NSW, Australia. Any assistance received is acknowledged in the text or in the list of acknowledgments. All references cited are included in the bibliography. The work is otherwise original.

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I certify that the substance of this thesis has not already been submitted for any degree and is not being currently submitted for any other degree.

I certify that, to the best of my knowledge, any help received in preparing this thesis, and all sources used, have been acknowledged in this thesis.

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I dedicate this work to my father who never saw my success.

ABBREVIATIONS

min	minute
h	hour
d	day
w	week
mg and µg	milligram and microgram
g	gram
kg	kilogram
cm, mm, µm and nm	centimetre, millimetre micrometre and nanometre
ml	millilitre
l	litre
M, mM, µM and nM	molar, millimolar, micromolar and nanomolar
DNA	deoxyribonucleic acid
RNA	ribonucleic acid
ATP	adenosine triphosphate
Y_{ATP}	the molar growth yield (g cells per mole ATP)
M_{ATP}	the maintenance coefficient (mmoles ATP per g cells per h)
NPN	non protein nitrogen
N	nitrogen
DM	dry matter
OM	organic matter
OMDR	organic matter digested in the rumen
MCO	microbial outflow from the rumen
ENMS	efficiency of net microbial cell synthesis
P:E ratio	protein:energy ratio
NH_3-N	ammonia nitrogen
EDTA	ethylenediamine tetraacetate
S.E.	standard error
VFA	volatile fatty acids
C2	acetic acid
C3	propionic acid
C4	butyric acid
C5	valeric acid
IsoC4	isobutyric acid
IsoC5	isovaleric acid
S	sulphur
P	phosphorus
DMI	dry matter intake

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Summary

1) The Effects of Dietary Urea on Rumen Function of Sheep (Chapter 4).

Two experiments were conducted to examine the effects of a range of concentrations of ammonia in the rumen on forage digestibility, microbial growth efficiency and the mix of microbial species. Four cross bred Merino x Border Leicester and eight Merino wethers were given a restricted ration of 800 and 750 g/d of oaten chaff (0.6 and 0.8% N), respectively. In the first experiment, 6 amounts of urea (0-17.36 mg urea/min) were continuously infused into the rumen of sheep given oaten chaff that was presented in equal portions at hourly intervals over 24 h. In the second experiment, 5 amounts of urea (0-20 g/d) were sprayed onto oaten chaff and presented to sheep once daily. The design of the two experiments was a 4x6 and a double 4x5 Latin Square design with a 14-d and 21-d period, respectively.

1.1) For sheep in both experiments, the concentration of ruminal fluid ammonia significantly increased ($p<0.01$) with increasing amounts of urea either infused directly into the rumen or included on the feed.

1.2) In both experiments, total volatile fatty acids (VFA) concentrations in ruminal fluid were significantly higher in sheep supplemented with the urea compared to those given oaten chaff without urea ($p<0.01$). The VFA proportions in ruminal fluid were significantly affected when ruminal fluid ammonia was above 200 mgN/l producing a lower ratio of acetogenic (C2+C4) to glucogenic (C3) VFA ($p<0.05$).

1.3) For sheep in both studies, the 24 h *in sacco* digestibility of organic matter in the rumen (OMDR) was significantly higher when supplemental urea was given ($p<0.01$).

1.4) For sheep on the once a day feeding regimen, when concentrations of ruminal fluid ammonia were above 200 mgN/l, the rumen volume was significantly smaller than

the other treatments ($p<0.05$). However, the outflow and the fractional turnover rate were not significantly different amongst sheep on different treatments ($p>0.05$).

1.5) In both studies, the fungal sporangia appearing on oat leaf blades incubated *in sacco* in the rumen for 24 h were significantly higher when urea was included in the diet, indicating that ammonia-N was a growth-limiting nutrient for fungi at levels of ruminal fluid ammonia below 30 mgN/l.

1.6) The density of protozoa in ruminal fluid was highest when ammonia concentrations were adjusted to 30 mgN/l for continuously fed sheep ($4.4x10^5$/ml) and to 168 mgN/l (an average of samples taken just before feeding, 4 and 8 h after feeding) for once-daily feeding ($2.9x10^5$/ml) Thereafter increasing concentrations of ruminal fluid ammonia, caused protozoa densities in ruminal fluid to decline progressively and return to approximately the same density as in the control sheep ($1.8x10^5$/ml in both experiments) when the concentration of ruminal fluid ammonia was increased above 200 mgN/l.

1.7) Urinary excretion of purine derivatives indicated that, when ruminal fluid ammonia was initially increased from 15 to 30 mgN/l, there was an apparent reduction in the microbial biomass flowing out of the rumen. The purine excretion rate then increased with increasing amounts of urea in the diet and appeared to remain constant at ruminal fluid ammonia concentrations between 67 to 179 mgN/l. Further increases appeared to occur when the level of ruminal fluid ammonia increased to above 200 mgN/l. The possibilities are that (1) there is less bacterial cell lysis in the rumen because of the concomitant decrease in the protozoal pool or (2) microbial growth *per se* in the rumen is more efficient with increasing ammonia concentrations.

1.8) For sheep on the once a day feeding regimen, the efficiency of net microbial cell synthesis in the rumen (gN/kg OMDR) calculated from urinary excretion of purine derivatives was 17-47% higher when the level of ruminal fluid ammonia was above 200 mgN/l.

1.9) It can be concluded that the requirement for ammonia for optimum bacterial growth is above 200 mgN/l which is much higher than that previous recognised on roughage based diets.

2) The Effects of Increasing Ruminal Fluid Ammonia on the Microbial Ecosystem in Fauna-free and Refaunated Lambs (Chapter 5).

A study was made of the effects of an incremental increase in ingested urea (0, 7, 14, 21 g/d), and resulting changes in concentrations of ruminal fluid ammonia, on the rumen ecosystem, feed digestion and net microbial cell synthesis in the rumen of lambs given 29.17 g/h of oaten chaff plus additives. Studies were made in fauna-free (Period 1) and refaunated (Period 2) lambs.

2.1) Concentrations of ammonia and ammonia pool sizes in ruminal fluid were lower ($p<0.01$) in fauna-free lambs. These differences were associated with a 47 % increase in microbial nitrogen assimilation in fauna-free lambs. The concentrations and pool sizes of ammonia increased ($p<0.01$) with increasing urea intake in all lambs. The concentrations ranged from 3-252 mgN/l.

2.2) Significant differences of organic matter digestibility *in sacco* over 24 h between fauna-free and refaunated animals occurred only in lambs fed the control diet. Organic matter digestibility in fauna-free lambs tended to increase with increasing concentrations of ruminal fluid ammonia.

2.3) In both fauna-free and refaunated lambs, with increasing concentrations of ruminal fluid ammonia, rumen volume increased with the first increment in ruminal fluid ammonia but decreased thereafter. Rumen volume in fauna-free lambs was larger but the fractional turnover rate of ruminal fluid was lower than in refaunated lambs ($p<0.01$).

2.4) Molar proportions (%) of individual volatile fatty acids (VFA) were inconsistently changed with increasing urea intake. Molar proportions of acetic,

isobutyric, isovaleric and valeric acids in fauna-free lambs were higher than in refaunated lambs but those of propionic and butyric acids were lower ($p<0.05$ or $p<0.01$). Total VFA concentrations in ruminal fluid were significantly lower in fauna-free than refaunated lambs ($p<0.01$) but total VFA pool sizes did not differ ($p>0.05$).

2.5) The pH of ruminal fluid was higher ($p<0.01$) by 0.1 unit in fauna-free than in refaunated lambs, irrespective of urea intake.

2.6) The density of fungal sporangia appearing on oat leaf blades incubated in the rumen of fauna-free lambs was slightly higher than in refaunated lambs ($p>0.05$). The density increased initially with the concentration of ruminal fluid ammonia, but decreased thereafter with increasing concentrations of ruminal fluid ammonia in both fauna-free and refaunated lambs ($p<0.01$).

2.7) The density of bacteria in the fluid phase (cell DM/ml) of fauna-free lambs was almost twice that of refaunated lambs ($p<0.01$) and increased with increasing concentrations of ruminal fluid ammonia in both fauna-free and refaunated lambs ($p<0.01$).

2.8) The purine:total-N ratio of organisms in the rumen was not influenced by concentrations of ruminal fluid ammonia or faunation status ($p>0.05$).

2.9) The efficiency of net microbial cell synthesis that leaves the rumen (estimated from purine excretion) increased with increasing urea intake in all lambs ($p<0.01$). It was 49 % higher in lambs on the 21 g/d urea intake than in lambs on the control diet and was increased by a further 34 % in the fauna-free lambs, suggesting that the microbial milieu in the fauna-free rumen is considerably more efficient and therefore requires substantially higher ammonia-N than the rumen with protozoa present as a part of the milieu.

2.10) An estimation of the outflow of bacteria in the fluid phase moving out of the rumen by multiplying the cell dry matter/ml ruminal fluid by the flow rate of ruminal

fluid, indicates that the microbial cells from the fluid in the rumen may represent more than 65% of the total microbial biomass leaving the rumen.

2.11) It is concluded that the efficiency of net microbial cell synthesis in the rumen can be considerably increased by increasing ruminal fluid ammonia concentrations. This appears to be associated with a substantial reduction in the turnover of microbial cells within the rumen in particular when protozoa is removed, while the specific growth rate (indicated by purine:total-N ratio of organisms) of rumen microbes is not changed.

3) A Comparison of Ammonia and Preformed Protein as a Source of Nitrogen for Microbial Growth in the Rumen of Sheep Given Oaten Chaff (Chapter 6).

Two experiments were carried out to examine the efficiency of net microbial cell synthesis in the rumen and its response to increasing ammonia concentrations brought about by increasing urea and/or degradable dietary protein in the diet. In the first study, 2 levels of urea (7.5 and 21 g/d) and casein (45 and 135 g/d), resulting in similar ammonia concentrations of 100 and 200 mgN/l respectively, were added to the rumen of wethers given 31.25 g/h of oaten chaff plus additives. In the second study, casein (25 g/d) and 3 levels of urea (7.2, 14.4 and 21.6 g/d) and the mixture of the urea and casein were added to the rumen of wethers fed 33.33 g/h of oaten chaff plus additives.

3.1) In both experiments, concentrations of ruminal fluid ammonia were increased with increasing nitrogenous supplements ($p<0.01$). The pH in ruminal fluid and 24 h organic matter digestibility *in sacco* were not different ($p>0.05$) irrespective of levels or sources of nitrogenous supplements.

3.2) In both experiments, molar proportions of isoacids (isobutyric and isovaleric acids) and valeric acid increased with increasing ingested casein but decreased with increasing urea intake ($p<0.01$). The molar proportion of propionic acids was significantly greater only when the ammonia concentration was derived from urea and

in excess of 200 mgN/l ($p<0.01$). However, total volatile fatty acids did not differ among the treatments regardless of levels or sources of nitrogen intake.

3.3) In the second study, peptides, essential and non-essential amino acids accumulated in ruminal fluid of the sheep when casein was added and concentrations of both peptides and amino acids tended to increase with increasing ruminal fluid ammonia concentrations. This suggests that proteolytic activity of rumen microbes and transportation of peptides and amino acids across the microbial membrane may be regulated by the metabolite mechanism (intracellular amino acids and $^{+}NH_4$, respectively).

3.4) In the second study, both densities of total viable and cellulolytic bacteria in ruminal fluid increased with increasing ammonia concentrations but that of small *Entodinium sp.* decreased, irrespective of nitrogen sources. On the other hand, the densities in ruminal fluid of large *Entodinium* and *Holotrich sp.* were greater when casein was fed regardless of ammonia concentrations. The density of fungal sporangia growth on oat leaf blades was affected by ingested urea and casein ($p<0.01$) and decreased with increasing ammonia concentrations in the absence of casein but appeared to remain constant in the presence of casein in the diet.

3.5) In the second study, the purine:total-N ratio of fluid-associated and particle-associated microbes in the rumen was not affected by either levels or sources of ingested nitrogen.

3.6) In both experiments, relative to control sheep, urinary excretion of purine derivatives was significantly increased with increasing ruminal fluid ammonia concentrations. The calculated efficiency of net microbial cell synthesis in the rumen was 15-28% higher ($p<0.01$), when ammonia concentrations increased from 100 to above 200 mgN/l, irrespective of nitrogen sources.

3.7) In conclusion, supplementation of preformed protein had no effect on rumen digestion and efficiency of net microbial cell synthesis. This could not be accounted for by its effect on ruminal fluid ammonia. In addition, nitrogenous supplements had no

effect on the specific growth rate (indicated by purine:total-N ratio) of rumen microbes. The increased efficiency of net microbial cell synthesis with increasing ammonia levels may be due to a reduction in turnover of microbial cells within the rumen.

4) General conclusion

Protozoa appeared to have an important role in fibre digestion in the rumen only when the concentration of ruminal fluid ammonia was below 40 mgN/l. Increased efficiency of net microbial cell synthesis in the rumen by increasing the availability of ruminal fluid ammonia appeared to be associated with a reduction in protozoal pool in ruminal fluid while the specific growth rate (indicated by purine:total-N ratio) of bacteria remained unchanged. This indicates that increasing the efficiency of net microbial cell synthesis is due to reduced turnover of microbial cells within the rumen.

Since dry matter digested in the rumen is either fermented to VFA or used in the synthesis of microbial cells, an increasing microbial growth efficiency will have large effects on the ratio of microbial protein to VFA available for digestion and absorption respectively. For a forage that is low in crude protein, therefore, the ratios of protein:energy and acetogenic:glucogenic substrates available for digestion and absorption from the digestive tract of ruminants may be optimised by adjusting the concentration of ruminal fluid ammonia to or above 200 mgN/l.