

USE OF LABELLED FRESH FORAGES FOR INVESTIGATING NITROGEN METABOLISM IN THE RUMEN

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



By

Reza Tahmasbi

BSc (Ferdowsi University of Mashhad, Iran)

MSc (Ferdowsi University of Mashhad, Iran)

School of Environmental and Rural Science

The University of New England

Armidale, NSW, Australia

Preface

The studies presented in this thesis were completed by the author whilst a postgraduate student in the School of Environmental and Rural Science, Faculty of Art and Science, the University of New England, Armidale, NSW, Australia.

Any assistance received is acknowledged in the text or in the list of acknowledgements. All references cited are included in the bibliography. The work is otherwise original.

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 or qualification.

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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Reza Tahmasbi

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Dedication

I would like to express my great appreciation to my wife, Reihaneh, and our son, Kiarash, for their love, support and patience throughout my thesis program. I would not have been able to accomplish what I have without their help. To them this thesis is dedicated.

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List of Abbreviations

The following abbreviations are used throughout the thesis, except in the chapter where full expressions are used.

AFRC	Agricultural and Food Research Council
AIA	Acid insoluble ash
ATP	Adenosine triphosphate
CP	Crude protein
DAPA	Diaminopimelic acid
DM	Dry matter
DNA & RNA	Deoxyribonucleic acid & Ribonucleic acid
GPT	Gas production technique
h	Hour
M _{ATP}	Energy required by rumen microbes for maintenance
min	Minute (s)
mg	Milligram
MCP	Microbial crude protein
BW ^{0.75}	Metabolic weight
ml & µl	Milliliter (10 ⁻³ L) and microlitre (10 ⁻⁶ L)
mmol/L	Millimol/litre
MPS	Microbial protein synthesis
N	Nitrogen
NAN	Non ammonia nitrogen
NH ₃ -N	Ammonia nitrogen
NPN	Non protein nitrogen
NSC	Non structural carbohydrates
OM	Organic matter
PP/AA	Peptides and amino acids
PD	Purine derivatives
RFC	Readily fermentable carbohydrates
RPM	Revolutions per minute
SC	Structural carbohydrates
SD	Standard deviation
SE or SEM	Standard error or standard error of means
VFA	Volatile fatty acids
WRC	Whole rumen contents
Y _{ATP}	The molar growth yield (g cells per mole ATP)

List of Publications

Some parts of the studies reported in this thesis have been published as follows:

Tahmasbi R, Nolan J.V., Dobos R.C. (2005) Comparison the volatile fatty acid profiles of dry and fresh kikuyu incubated with rumen fluid in vitro. In 'Recent Advances in Animal Nutrition'. Vol 15, p 32A, Armidale, UNE, Australia. (Ed. N.Richardson).

Tahmasbi R, Nolan J.V., Dobos R.C. (2006) Rumen degradability of ¹⁵N-labelled fresh lucerne and ryegrass forages in situ. In 'XII Asian Australian Animal Production'. Busan, South Korea.

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Summary

The studies reported in this thesis were undertaken to investigate the characteristics of the degradation of two species of fresh forages in ruminants, i.e. lucerne (a legume) and ryegrass (a grass). A review of the literature was done to determine current understanding of the degradation of forage protein by plant and microbial proteases in the rumen. Knowledge of degradability of fresh forages was found to be limited and so three studies were undertaken using forages labelled with ^{15}N so that the end-products of forage protein degradation could be identified and their kinetics determined. The ^{15}N -labelled forage was readily produced by growing the plants in pots watered with a solution of $^{15}\text{NH}_4^{15}\text{NO}_3$. Rumen degradability of fresh and frozen-thawed lucerne and ryegrass was evaluated using *in vitro*, *in situ* and *in vivo* techniques.

In Experiment 1, chopped ^{15}N -labelled forage samples were incubated with rumen fluid *in vitro* for 12 h and their fermentation and degradability characteristics . Estimates of volatile fatty acids (VFA) and microbial protein production over 12 h indicated that lucerne and ryegrass had different potential OM degradability (41.2 v. 34.5%, respectively) and crude protein (CP) degradability (47.1 and 55.9 %, respectively). Lucerne was, however, degraded more rapidly in the early stages of the incubation than ryegrass and the effective rumen degradability calculated for a rumen outflow rate of 5%/h was therefore higher for lucerne than for ryegrass. Based on VFA production, fresh samples contained more fermentable OM than those that had been stored frozen and then thawed. The addition of 1 g of starch to the incubation flasks containing frozen-thawed forages increased fermentative activity close to the level as for fresh forages although gas and VFA production rates were still lower than those for fresh forages.

In Experiment 2, results obtained using the rumen *in situ* method again showed that there were degradability differences between the two forage species with effective degradabilities being higher in lucerne than ryegrass, i.e. for DM (62.5 v. 57.2%) and for CP (70.6 v. 61.0%), respectively. There were also important effects of sample preparation on estimates of the degradability of DM and CP. The effective CP degradability at a rumen outflow rate of 5%/h was 66.3% and 74.8% for fresh and frozen-thawed lucerne, respectively. The corresponding estimates for fresh and frozen-thawed ryegrass were 59.7% and 62.2%, respectively. Furthermore, true degradability parameters obtained by the *in situ* method were under-estimated because of the growth and attachment of micro-organisms to the plant material as the incubation proceeded. In this experiment, ^{15}N in the forage samples was used as a marker to identify plant N and to distinguish it from un-labelled microbial N. Microbial attachment occurred rapidly with both forage species so that after 1 h, microbial N as a percentage of total N was higher in lucerne than ryegrass (40-60% v. 28-40%, respectively) and by the end of the incubation (48 h) was about 97% for both species. The rumen *in situ* method provided conditions that were close to the real rumen environment than the *in vitro* incubation, because the disappearance of DM was higher and more closely approached an asymptotic value. It was concluded that degradability values, obtained by either *in vitro* or *in situ* methods, for frozen-thawed samples may not reliably represent the true value for fresh samples. Thus, to assess grazed pasture material, fresh plant material should be used whenever feasible, rather than thawed samples as has often been the case in published reports.

In Experiment 3, the ruminal digestion of fresh ryegrass N was studied by allowing sheep on a basal diet of chopped lucerne hay to ingest a single meal of ^{15}N -labelled ryegrass; ^{15}N -ammonia was also given intraruminally to the sheep under the same conditions to enable models of ^{15}N kinetics to be quantified. The subsequent appearance of ^{15}N in secondary N pools (rumen ammonia-N, non-protein NAN, bacteria and soluble protein) was then monitored. Results for rumen ammonia kinetics in the sheep were similar to those reported previously. In addition, N degradation of ingested fresh ryegrass was rapid and extensive (>90%/24 h), supporting the findings in *in vitro* and *in situ* experiments and also the results from the complementary rumen *in situ* estimates made in Experiment 3.

The concentration of peptides and AA in rumen fluid was 100 mg N/L in this study. The N assimilated into rumen bacteria in the sheep originated from both ammonia (34%) and peptides and AA (66%). Application of a published rumen model provided an excellent simulation of the kinetics of labelled ammonia but failed to simulate the flow of ^{15}N from the labelled ryegrass to peptides and AA in rumen fluid and then to the bacterial N. It was necessary to hypothesize that there was a direct route of assimilation of peptides and AA by rumen bacteria that did not pass through the rumen fluid pool of peptides and AA. This could occur if bacteria attached to ryegrass were extracting peptides and AA from plant particles without these materials passing through mixed rumen fluid pools; this suggestion was also supported by the demonstration of extensive attachment of microbial material to forage samples in the rumen *in situ* studies.

Therefore, ^{15}N -labelled plant materials can be used to increase the power of *in vitro* and *in situ* studies. When used with suitable modelling techniques, *in vivo* experiments in which animals ingest the forage under study currently provide the only means of generating reliable information about the kinetics of ruminal digestion of freshly harvested forages in the rumen. Such information is needed to verify *in vitro* and *in situ* methods, particularly when the samples tested have been stored before analysis.