EVALUATION OF DUCKWEED AS A PROTEIN SUPPLEMENT FOR RUMINANTS

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by

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Preface

The studies presented in this thesis were completed by the author whilst a postgraduate student in the School of Rural Science and Agriculture, Faculty of Sciences, 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.

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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List of Abbreviations

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

AIA	=	acid insoluble ash
ATP	=	adenosine triphosphate
CP	=	crude protein
d	=	day (s)
DM	=	dry matter
g	=	gram (s)
h	=	hour (s)
ha	=	hectare (s)
kg	=	kilogram (s)
l	=	litre (s)
ME	=	metabolisable energy
min	=	minute (s)
MJ	=	megajoule (s)
N	=	nitrogen
NAN	=	non-ammonia-nitrogen
NPN	=	non-protein nitrogen
OM	=	organic matter
SD	=	standard deviation
SEM	=	standard error of mean

Summary

The general purpose of the experiments reported in this thesis was to investigate whether duckweed, a protein-rich aquatic plant of the family *Lemnaceae*, is a potentially valuable dietary amino acid source for ruminants, especially in tropical and sub-tropical regions. As all dietary proteins ingested by ruminants are subjected to major modification in the rumen, the general features of degradation and synthesis of protein in the rumen are presented and discussed in Chapter 2. Background information on duckweed is also reviewed and included in this chapter.

Determination of rumen degradability of a potential protein supplement is generally the initial approach taken in assessing its value for ruminants. This view was adopted in this thesis, and the rumen degradability of fresh and oven-dried duckweed, in comparison with that of cottonseed meal (chosen to represent an 'escape' protein supplement of about 60 % degradability) was evaluated using various techniques (Chapter 4). Results obtained *in sacco* showed that the effective N degradability at a rumen outflow rate of 5 %/h was 36 and 40 % for fresh and dried duckweed, respectively. The corresponding estimate for cottonseed meal was 67 %. This *in sacco* experiment suggested that about 62 % of duckweed protein would normally escape rumen fermentation.

The rumen degradability and bacterial utilisation of duckweed N was also investigated *in vitro* (Chapter 4). A solubility test showed that oven-drying reduced the content of buffer-soluble N in duckweed. When finely ground feed samples were incubated with strained rumen fluid, the *net* ammonia production was found to be highest for dried duckweed, lower for fresh duckweed and lower again for cottonseed meal: the higher net production for duckweed could have been due either to a higher rate of protein degradation or a lower rate of bacterial utilisation of ammonia, or both. However, the cumulative gas production during incubation of either dried or fresh duckweed was lower than for cottonseed meal: this result was consistent with the *in sacco* results, both tests indicating that duckweed is less fermentable in the rumen than cottonseed meal, and therefore its protein is probably also less

degradable and more likely to 'escape' fermentation in the rumen than the protein in cottonseed meal.

Much clearer information on *in vitro* N metabolism was obtained in Experiment 3.3. In these tests, an energy source was added to the incubation system used in Experiment 3.2 and bacterial synthesis from ammonia-N was quantified by adding ^{15}N-ammonia at the start of incubation. Net ammonia concentration was markedly reduced by the addition of energy because ammonia was assimilated at a faster rate by bacteria to support their enhanced protein synthesis. The amount of bacterial-N synthesised from feed N did not differ between cottonseed meal and dried duckweed. However, the proportion of the bacterial-N derived from ammonia-N was lower for duckweed than for cottonseed meal, which implied that a higher proportion of bacterial-N was synthesised from duckweed NAN than from cottonseed meal NAN.

New findings of relevance to forage protein metabolism in the rumen in general, as well as to duckweed protein in particular, are reported in Chapter 5. The metabolism of duckweed N in the rumen of sheep was studied by intra-ruminal administration of ^{15}N-labelled duckweed or ^{15}N-ammonia (the ^{15}N-labelled duckweed was readily produced by growing the plant on a synthetic growth medium containing ^{15}N-ammonia; see Appendix 2). The two forms of ^{15}N tracer were administered under similar conditions on different occasions and the appearance of ^{15}N in secondary N pools (such as rumen ammonia-N, NAN and bacterial-N) was then followed.

The enrichment of rumen ammonia-N was always lower when ^{15}N-duckweed rather than ^{15}N-ammonia was made available in the rumen. This indicated, firstly, that duckweed N was not completely fermented to ammonia in the rumen. Secondly, the fraction of bacterial-N synthesised from rumen ammonia (*i.e* the ratio of bacterial-N enrichment to that of rumen ammonia-N when ^{15}N-ammonia was made available to the rumen (which was 50 – 70 %) was in general agreement with published values. However, the same ratio was always higher when ^{15}N-duckweed rather than when ^{15}N-ammonia was supplied and this was a direct *in vivo* indicator of

the significant contribution made by duckweed NAN to bacterial-N synthesis in the rumen.

In the sheep studied in Experiment 4.3, about 30 % of the ^{15}N in duckweed administered into the rumen passed through the abomasum as non-microbial-NAN, although reasons are advanced as to why the true value for duckweed protein 'escape' may have been under-estimated. The majority of the rumen-degraded ^{15}N in duckweed was converted to bacterial-N and about half of the ^{15}N in bacteria was assimilated as NAN. The likelihood that direct incorporation by microorganisms of feed protein degradation intermediates such as peptides rather than ammonia may increase their efficiency of protein synthesis is discussed.

In the last experiment, duckweed was included in hay-based diet for Merino sheep (described in Chapter 6) to investigate duckweed's acceptance as a feedstuff and to determine its potential as an 'escape' protein supplement, on wool growth and wool fibre characteristics. The sheep readily ingested the duckweed, either fresh or dried, after a short period of adaptation, and no clinical ill-effects were apparent when it was 10 % of the animals' DM intake. In Experiment 5.1, the response of sheep on a maintenance diet to a duckweed-protein supplement in terms of wool growth and wool fibre characteristics was determined. Results showed that these wool measures did not differ between supplemented (up to 100 g/d dried duckweed) and non-supplemented animals. The relatively small increase (estimated to be 1-5 g/d) in total protein leaving the stomachs of the duckweed-supplemented sheep was probably too small to generate a significant response in wool growth or fibre characteristics. In Experiment 5.2, wool production responses were again investigated when these sheep were given iso-nitrogenous amounts of duckweed, urea or cottonseed meal. Relative to urea alone, duckweed and cottonseed meal both stimulated wool growth. The responses to duckweed and cottonseed meal were similar and were probably due to additional amino acids made available for intestinal absorption by both meals. Both were therefore equally valuable sources of 'escape' protein for ruminant animals.