

EVALUATION OF DUCKWEED AS A PROTEIN SUPPLEMENT FOR RUMINANTS

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



by

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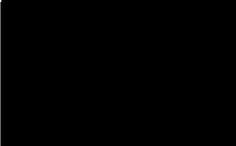
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Preface

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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Damry
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Chapter 1	Introduction.....	1
1.1	General.....	1
1.2	The Thesis Concept.....	3
Chapter 2	Literature Review	4
2.1	Scope of the Review	4
2.2	Part I: Metabolism of N in the Rumen	4
	2.2.1 Introduction.....	4
	2.2.2 Sources of N.....	5
	2.2.3 Degradation of dietary protein.....	9
	2.2.4 Utilisation of N in the Rumen.....	23
2.3	Validity of gas production as an indication of microbial growth efficiency	30
2.4	The use of digesta flow markers	31
2.5	The use of ¹⁵N-labelled feeds.....	32
2.6	Part II: Duckweed.....	33
	2.6.1 General.....	33
	2.6.2 The Growth of Duckweed and Its Control.....	34
	2.6.3 The Nutritional Value of Duckweed.....	36
	2.6.4 Duckweed as a potential protein supplement for ruminants.....	37
2.7	Implications from Literature Review	39
Chapter 3	General Materials and Methods.....	40
3.1	Introduction.....	40
3.2	Experimental Procedures.....	40

3.2.1	Preparation of Cobalt-Ethylene Diamine Tetra Acetic Acid Complex	40
3.2.2	Measurement of Digesta Flow	41
3.2.3	Measurement of Rumen Fluid Kinetics	41
3.2.4	Sampling of Rumen Fluid.....	42
3.2.5	Sampling of Abomasal Digesta	42
3.2.6	Collection of Rumen Fluid for <i>In vitro</i> Experiments.....	43
3.2.7	Isolation of Bacteria.....	43
3.2.8	Measurement of gas production.....	43
3.3	Laboratory Analysis	44
3.3.1	Analysis of Cobalt	44
3.3.2	Determination of AIA	46
3.3.3	Analysis of VFAs.....	46
3.3.4	Analysis of ¹⁵ N.....	48
3.3.5	Analysis of Wool	50
Chapter 4	Degradation and Fermentation of Duckweed in the Rumen	52
4.1	General.....	52
4.2	Experiment 1: Degradability of Duckweed in the Rumen Determined with the <i>In Sacco</i> Technique.....	53
4.2.1	Introduction.....	53
4.2.2	Materials and methods	54
4.2.3	Experimental procedures	56
4.2.4	Results.....	57
4.3	Experiment 2: Determination of N Solubility	61
4.3.1	Experimental procedure	61
4.3.2	Calculation and statistical analysis	62
4.3.3	Results.....	62
4.4	Experiment 3: <i>In Vitro</i> Degradation and Fermentation of Duckweed in the Rumen Fluid	63
4.4.1	Experiment 3.1: Ammonia and Gas Production from Duckweed and Cottonseed Meal when Incubated with Rumen Fluid.....	63
4.4.2	Experiment 3.2: N Degradation from Duckweed and Its Utilisation for Microbial Protein Synthesis	66
4.4.3	Experiment 3.3: Metabolism of Duckweed N in the Rumen Fluid with or without Additional Energy Source	75
4.5	Discussion	85

4.5.1	Concentration and production of ammonia-N	85
4.5.2	Fermentation	86
4.5.3	Bacterial-N synthesis	87
4.5.4	Degradation of feed protein	90
Chapter 5 Nitrogen Dynamics in the Rumen of Sheep Determined with		
¹⁵N-Labelled Ammonia or ¹⁵N-Labelled Duckweed		93
5.1	General.....	93
5.2	Experiment 4.1: Intra-ruminal ¹⁵N Administration	94
5.2.1	Introduction.....	94
5.2.2	Materials and methods	95
5.2.3	Results.....	97
5.3	Experiment 4.2: Continuous ¹⁵N Administration (1).....	100
5.3.1	Introduction.....	100
5.3.2	Materials and methods	101
5.3.3	Results.....	102
5.4	Experiment 4.3: Continuous ¹⁵N administration (2)	105
5.4.1	Introduction.....	105
5.4.2	Materials and methods	106
5.4.3	Results.....	108
5.5	Discussion	113
5.5.1	Methodology – isolating a pure sample of bacteria	113
5.5.2	Concentrations of rumen fluid and abomasal metabolites.....	113
5.5.3	Flows of DM and nutrients through the abomasum	114
5.5.4	Rumen fluid NAN.....	115
5.5.5	Rumen fluid ammonia flux and recycling	116
5.5.6	Rumen fluid ammonia enrichments	117
5.5.7	Balance of ¹⁵ N across the rumen (ammonia absorption/urea)	118
5.5.8	Abomasal bacterial flow	119
5.5.9	Duckweed protein escape	120
Chapter 6 The Effects of Duckweed Supplementation on Wool Growth and		
Characteristics in Fine-Wool Merinos.....		121
6.1	General.....	121
6.2	Experiment 5.1: The Effects of Duckweed Supplementation	
	on Wool Growth and Characteristics	122

6.2.1	Introduction.....	122
6.2.2	Materials and Methods.....	123
6.2.3	Results.....	127
6.3	Experiment 5.2: The Effects of Urea, Duckweed or Cottonseed Meal Supplementation on Wool Growth and Characteristics	129
6.3.1	Introduction.....	129
6.3.2	Materials and Methods.....	130
6.3.3	Results.....	132
6.4	Discussion	135
Chapter 7	General Discussion.....	140
7.1	Analysis of ¹⁵N.....	140
7.2	Rumen Degradability of Duckweed	141
7.3	Rumen Utilisation and Abomasum Flow of Duckweed N.....	143
7.4	Duckweed and Ruminant Production.....	145
7.5	Further Studies	147
 Preface ii		
	Acknowledgements.....	iii
	List of Contents.....	Error! Bookmark not defined.
	List of Tables.....	xiii
	List of Figures	xv
	List of Abbreviations.....	xix
	Summary	xx
Chapter 1	Introduction.....	1

1.1	General.....	1
1.2	The Thesis Concept	3
Chapter 2	Literature Review	4
2.1	Scope of the Review	4
2.2	Part I: Metabolism of N in the Rumen.....	4
2.2.1	Introduction.....	4
2.2.2	Sources of N.....	5
2.2.2.1	Ingested feeds.....	5
2.2.2.2	Endogenous body N	6
2.2.2.3	Recycled microbial-N.....	8
2.2.3	Degradation of dietary protein	9
2.2.3.1	Techniques for predicting the degradation of dietary protein	9
2.2.3.2	Source of proteolytic enzymes	16
2.2.3.3	Mechanism of protein degradation.....	18
2.2.3.4	Factors affecting protein degradation.....	20
2.2.4	Utilisation of N in the Rumen	23
2.2.4.1	Ammonia-N.....	23
2.2.4.2	Peptides and amino acids	28
2.3	Part II: Duckweed	33
2.3.1	General	33
2.3.2	The Growth of Duckweed and Its Control.....	34
2.3.3	The Nutritional Value of Duckweed.....	36
2.3.4	Duckweed as a potential protein supplement for ruminants.....	37
2.4	Implications of Literature Review	39

Chapter 3	General Materials and Methods.....	40
3.1	Introduction.....	40
3.2	Experimental Procedures	40
3.2.1	Preparation of Cobalt-Ethylene Diamine Tetra Acetic Acid Complex	40
3.2.2	Measurement of Digesta Flow	41
3.2.3	Measurement of Rumen Fluid Kinetics	41
3.2.4	Sampling of Rumen Fluid.....	42
3.2.5	Sampling of Abomasal Digesta.....	42
3.2.6	Collection of Rumen Fluid for <i>In vitro</i> Experiments.....	43
3.2.7	Isolation of Bacteria.....	43
3.2.8	Measurement of gas production.....	43
3.3	Laboratory Analysis.....	44
3.3.1	Analysis of Cobalt.....	44
3.3.1.1	Sample preparation and digestion	44
3.3.1.2	Standards	45
3.3.2	Determination of AIA	46
3.3.3	Analysis of VFAs.....	46
3.3.3.1	Total N.....	46
3.3.3.2	Ammonia N	47
3.3.3.3	Bacterial-N	47
3.3.3.4	Non-ammonia N	48
3.3.4	Analysis of ¹⁵ N.....	48
3.3.4.1	Preparation of N samples	48
3.3.4.2	Oxidation of samples and estimation of ¹⁵ N abundance	49
3.3.4.3	Calculation of ¹⁵ N abundance and enrichment.....	49
3.3.4.4	Checks of the precision and accuracy of the ¹⁵ N analysis methods	50
3.3.5	Analysis of Wool	50
3.3.5.1	Rate of fibre elongation.....	50
3.3.5.2	Diameter of wool fibre	51
3.3.5.3	Wool volume	51
Chapter 4	Degradation and Fermentation of Duckweed in the Rumen	52
4.1	General.....	52
4.2	Experiment 1: Degradability of Duckweed in the Rumen Determined with the <i>In Sacco</i> Technique.....	53
4.2.1	Introduction.....	53
4.2.2	Materials and methods	54
4.2.2.1	Animals and their diets.....	54
4.2.2.2	Bags and procedure of incubation	54
4.2.2.3	Laboratory analysis	55
4.2.2.4	Calculation and statistical analysis.....	55
4.2.3	Experimental procedures.....	56

4.2.3.1	Experiment 1.1: The effects of DM quantity of fresh duckweed on its degradability in the rumen.....	56
4.2.3.2	Experiment 1.2: The effects of drying on the degradability of duckweed DM and N in the rumen	56
4.2.4	Results	57
4.2.4.1	Experiment 1.1: The effects of quantity of fresh duckweed on its degradability in the rumen	57
4.2.4.2	Experiment 1.2: Rumens DM and N degradability of dried and fresh duckweed	59
4.3	Experiment 2: Determination of N Solubility.....	61
4.3.1	Experimental procedure	61
4.3.2	Calculation and statistical analysis	62
4.3.3	Results	62
4.4	Experiment 3: <i>In Vitro</i> Degradation and Fermentation of Duckweed in the Rumens Fluid	63
4.4.1	Experiment 3.1: Ammonia and Gas Production from Duckweed and Cottonseed Meal when Incubated with Rumens Fluid	63
4.4.1.1	Introduction	63
4.4.1.2	Experimental procedures	64
4.4.1.3	Calculation and statistical analysis.....	64
4.4.1.4	Results	65
4.4.2	Experiment 3.2: N Degradation from Duckweed and Its Utilisation for Microbial Protein Synthesis	66
4.4.2.1	Introduction	66
4.4.2.2	Experimental procedures	67
4.4.2.3	Calculation and statistical analysis.....	70
4.4.2.4	Results	70
4.4.3	Experiment 3.3: Metabolism of Duckweed N in the Rumens Fluid with or without Additional Energy Source.....	75
4.4.3.1	Introduction	75
4.4.3.2	Experimental procedures	76
4.4.3.3	Calculation and statistical analyses	77
4.4.3.4	Results	77
4.5	Discussion.....	85
4.5.1	Concentration and production of ammonia-N	85
4.5.2	Fermentation	86
4.5.3	Bacterial-N synthesis	87
4.5.4	Degradation of feed N.....	90
Chapter 5	Nitrogen Dynamics in the Rumens of Sheep Determined with ¹⁵N-Labelled Ammonia or ¹⁵N-Labelled Duckweed	93
5.1	General.....	93
5.2	Experiment 4.1: Intra-ruminal ¹⁵ N Administration.....	94
5.2.1	Introduction.....	94
5.2.2	Materials and methods	95

5.2.2.1	Experimental animals	95
5.2.2.2	Diet and feeding	95
5.2.2.3	Experimental procedures	95
5.2.2.4	Laboratory analysis	96
5.2.2.5	Data analysis and calculation	97
5.2.3	Results	97
5.3	Experiment 4.2: Continuous ^{15}N Administration (1)	100
5.3.1	Introduction	100
5.3.2	Materials and methods	101
5.3.2.1	Experimental animals	101
5.3.2.2	Diets and feeding	101
5.3.2.3	Experimental procedures	101
5.3.2.4	Laboratory analysis	102
5.3.2.5	Data analysis and calculation	102
5.3.3	Results	102
5.4	Experiment 4.3: Continuous ^{15}N Administration (2)	105
5.4.1	Introduction	105
5.4.2	Materials and methods	106
5.4.2.1	Experimental animals, diets and procedures	106
5.4.2.2	Analysis and calculation	107
5.4.3	Results	108
5.5	Discussion	113
5.5.1	Methodology – isolating a pure sample of bacteria	113
5.5.2	Concentrations of rumen fluid and abomasal metabolites	113
5.5.3	Flows of DM and nutrients through the abomasum	114
5.5.4	Rumen fluid NAN	115
5.5.5	Rumen fluid ammonia flux and recycling	116
5.5.6	Rumen fluid ammonia enrichments	117
5.5.7	Balance of ^{15}N across the rumen (ammonia absorption/urea)	118
5.5.8	Abomasal bacterial flow	119
5.5.9	Duckweed protein escape	120

Chapter 6 The Effects of Duckweed Supplementation on Wool Growth and Characteristics in Fine-Wool Merinos 121

6.1	General	121
6.2	Experiment 5.1: The Effects of Duckweed Supplementation on Wool Growth and Characteristics	122
6.2.1	Introduction	122
6.2.2	Materials and Methods	123
6.2.2.1	Experimental animals	123
6.2.2.2	Duckweed	123
6.2.2.3	Experimental procedures	124
6.2.2.4	Laboratory analysis and calculation	126
6.2.2.5	Statistical analysis	126
6.2.3	Results	127
6.2.3.1	The edibility of duckweed	127

6.2.3.2	Feed intakes	127
6.2.3.3	Live-weight change	127
6.2.3.4	Wool yield, fibre elongation and fibre diameter	128
6.3	Experiment 5.2: The Effects of Urea, Duckweed or Cottonseed Meal Supplementation on Wool Growth and Characteristics.....	129
6.3.1	Introduction	129
6.3.2	Materials and Methods.....	130
6.3.2.1	Experimental animals	130
6.3.2.2	Duckweed	130
6.3.2.3	Experimental procedures	130
6.3.2.4	Laboratory analysis and calculation	132
6.3.2.5	Statistical analysis	132
6.3.3	Results	132
6.3.3.1	Feed intakes	132
6.3.3.2	Rates of wool fibre elongation and fibre diameter	132
6.3.3.3	Rumen ammonia concentration and pH	133
6.4	Discussion.....	135
Chapter 7	General Discussion.....	140
7.1	Analysis of ¹⁵ N.....	140
7.2	Rumen Degradability of Duckweed	141
7.3	Rumen Utilisation and Abomasum Flow of Duckweed N	143
7.4	Duckweed and Ruminant Production	145
7.5	Further Studies	147
	Bibliography.....	149
	Appendices	163

List of Tables

Table 1	<i>In vivo</i> determination of the percentage of rumen bacterial-N, protozoal-N and mixed microbial-N synthesised from ammonia in animals with different experimental diets, different N intakes and obtained in experiments using different routes of ¹⁵ N administration.....	25
Table 2	Range of nutrient concentrations in waters for the growth of <i>Lemnaceae</i> (Landolt, 1996).....	36
Table 3.	<i>In sacco</i> degradation parameters for DM of fresh duckweed and cottonseed meal and their effective degradabilities at assumed rumen outflow rates of 2, 5 and 8 %/h.....	58
Table 4.	Degradation parameters of DM and N for dried and fresh duckweed and cottonseed meal and their effective degradabilities at assumed rumen outflow rates of 2, 5 and 8 %/h.	61
Table 5.	Chemical composition (g/l) of McDougall's buffer solution.....	62
Table 6	N contents (% air-dry) and solubility (% , mean \pm SD) in McDougall's buffer solution of dried duckweed, fresh duckweed and cottonseed meal	63
Table 7.	Means (\pm SEM) of total VFA concentration (mmol/flask), production (mmol/g feed DM), molar proportion of individual VFA (%) and calculated ATP production (mmol/g DM) at the end of a 3-h incubation of rumen fluid (RF) and RF to which either cottonseed meal (CSM), dried (DDW) or fresh duckweed (FDW) had been added.....	72
Table 8.	Means (\pm SEM) of N degradation parameters for cottonseed meal (CSM), dried duckweed (DDW) and fresh duckweed (FDW) after incubation for 3 h with strained rumen fluid.	75
Table 9.	Total VFA concentration (mmol/flask) and individual VFA percentages (%) at 3 and 6 h of incubation of rumen fluid (RF), RF added with cottonseed meal (RF+CSM) or dried duckweed (RF+DDW) in the presence (+E) or absence (-E) of additional energy sources.	79
Table 10.	Intake of DM and flows of DM and water out of the rumen and through the abomasum of sheep given a diet of oaten chaff (400 g/d as fed) and lucerne chaff (300 g/d as fed).....	108
Table 11.	Intake of N and kinetics of various N-containing fractions in the rumen and abomasum of sheep given a diet of oaten chaff (400 g/d) and lucerne chaff (300 g/d) and either ¹⁵ N-ammonia or ¹⁵ N-labelled duckweed.....	110
Table 12.	Amounts of ¹⁵ N flowing in various N materials through the abomasum (mmol/d) during a continuous provision into the rumen of ¹⁵ N-ammonia or ¹⁵ N-labelled duckweed.	110
Table 13	Dietary treatments, live weights and wool fibre diameters of the treatment groups at the start of the experiment on 27 th January 1998 (mean \pm SEM, n = 9)	125

Table 14.	Least-square means for intakes (g/d) of OM and CP based on analysis of ingredients, and estimated MEI (MJ (Mcal)/d) and the amounts (g/d) of digestible protein leaving the stomach during the experimental phase.	128
Table 15.	Least-square means (\pm SEM) for yield of greasy wool (mid-side patch), rate of wool fibre elongation (dye-band), fibre diameter and fibre volume production in the experimental phase (adjusted for same characteristics in the pre-experimental phase).....	129
Table 16.	Dietary treatments, mean \pm SEM (n = 7) of live weight and wool fibre diameter of sheep in treatment groups at the start of pre-experimental period (19 th October 1998).	131
Table 17.	DM (% air-dry), OM and CP (% DM) contents of dietary ingredients.....	131
Table 18.	Table 19 Least-square means for intakes of DM, OM and CP and estimated ME (MEI) and digestible protein leaving the stomach (DPLS) during the experimental phase.	133
Table 20.	Least-square means \pm SEM for rate of wool fibre elongation, fibre diameter and volume in the experimental phase (adjusted for the corresponding wool characteristics in the pre-experimental period).....	133
Table 21	Stock solutions of individual macronutrients and their concentrations.....	183
Table 22	Stock solutions of micronutrients (g/l) and Fe-EDTA (g/250ml)	183
Table 23	Composition of individual nutrient stock solution (ml/l) in the original medium used to grow duckweed	184
Table 24	Exponential equation for the growth of duckweed on different nutrient concentration and time (d) required for the frond to double in number.....	188
Table 25	Exponential equations for the growth of duckweed on a nutrient solution of '0.25 strength' (25 ml N/ l) and the time (d) required for the fronds to double in number.....	189
Table 26	Results of ¹⁵ N abundance analyses performed on various occasions of an IAEA ¹⁵ N standard (standard No. 311A; theoretical ¹⁵ N abundance 2.0500 %)	192
Table 27	Representative results of ¹⁵ N abundance analyses for standards.....	193
Table 28	Recovery of Co standards analysed on an ICP-OES.....	196
Table 29	A representative calculation of the amount and abomasum flow of true digesta (fresh and DM) in sheep given a mixed diet of (g/d) 400 oaten chaff and 300 lucerne chaff	197

List of Figures

Figure 1	Pathways of N assimilation by microorganisms at low or high ammonia concentration (Brown <i>et al.</i> , 1974).....	27
Figure 2	Apparatus used for <i>in vitro</i> incubation of duckweed with rumen fluid. Incubation bottles (volume 1 litre) were held in a shaking water bath and tubes passing through the bungs in each bottle were connected to 500 ml measuring cylinders to enable gas production to be determined by a liquid displacement technique.	44
Figure 3.	<i>In sacco</i> DM degradability (%) of fresh duckweed (determined with quantities of 1.0 and 1.7 g per bag) and cottonseed meal (4.8 g DM per bag) were incubated for different periods in the rumen (mean \pm SD, n = 2). The lines represent the fitted curves (Orskov and McDonald, 1979).....	58
Figure 4.	<i>In sacco</i> DM degradability (%) for cottonseed meal (■), dried (●) and fresh (▲) duckweed when incubated for different periods in the rumen (mean \pm SD, n = 2). The lines represent the fitted curves (Orskov and McDonald, 1979).....	60
Figure 5.	<i>In sacco</i> N degradability (%) for cottonseed meal (■), dried (●) and fresh (▲) duckweed when incubated for different periods in the rumen (mean \pm SD, n = 2). The lines represent the fitted curves (Orskov and McDonald, 1979).....	60
Figure 6.	Net ammonia production (mg N/g feed N) for cottonseed meal (■), dried (●) and fresh (▲) duckweed at different times (min) during incubation with strained rumen fluid.....	65
Figure .7	Cumulative gas production (ml/g feed DM) for cottonseed meal (■), dried (●) and fresh (▲) duckweed at different times (min) during incubation with strained rumen fluid.....	66
Figure 8.	A diagram depicting the transactions of N during <i>in vitro</i> incubation of feeds (duckweed and cottonseed meal) with rumen fluid (Hristov and Broderick, 1994). The ammonia-N pool was initially labelled with ¹⁵ N-ammonia and the enrichment decreased over time as the labelled ammonia-N was diluted by incoming unlabelled ammonia-N released from feed N. Bacterial-N became labelled with ¹⁵ N as the cells grew and assimilated the ¹⁵ N-ammonia. The NAN pool consisted of undegraded feed N, the NAN that originated from rumen fluid and bacterial-NAN; and the ¹⁵ N found in this pool was therefore due to the enrichment of bacterial-N.	69
Figure 9.	Proportion (% total-N) of ammonia-N (□) and NAN (■) at the end of a 3-h incubation of rumen fluid (RF) and RF to which had been added cottonseed meal (CSM), dried duckweed (DDW) or fresh duckweed (FDW).....	71
Figure 10.	Means (\pm SEM) of enrichment (mol ¹⁵ N/100 mol total-N) of NAN (■) and bacterial-N (□) for cottonseed meal (CSM), dried duckweed	

	(DDW) and fresh duckweed (FDW) at the end of a 3-h incubation with strained rumen fluid.....	73
Figure 11.	Means (\pm SEM) of amounts of bacterial-N synthesised from feed N (mol N/100 mol feed N) at the end of a 3-h incubation of cottonseed meal (CSM), dried duckweed (DDW) and fresh duckweed (FDW) with strained rumen fluid.....	74
Figure 12	Amounts (mol N/100 mol feed N; means \pm SEM) of ammonia-N (\square) and total-N (\blacksquare) released from cottonseed meal (CSM), dried duckweed (DDW) and fresh duckweed (FDW) after incubation for 3 h with rumen fluid.	74
Figure 13.	Gas production (ml/flask) for rumen fluid (RF), RF added with cottonseed meal (RF+CSM) or duckweed (RF+DDW) during the incubation in the presence (filled symbols) or absence (unfilled symbols) of additional energy. (B represents flask + rumen fluid, but no added substrate; B+E represents B+added energy substrate; CSM = cottonseed meal; DW = dried duckweed.)	78
Figure 14.	Net ammonia-N release (means \pm SEM; % total-N) for cottonseed meal and duckweed at 3 h (left) and 6 h (right) of incubation with strained rumen fluid with (\blacksquare) or without (\square) additional energy.	80
Figure 15.	Enrichment of ammonia-N (means \pm SEM; mol ^{15}N /100 mol total-N) for cottonseed meal and duckweed at 3 h (left) and at 6 h (right) of incubation with strained rumen fluid with (\blacksquare) or without (\square) additional energy source.....	81
Figure 16.	Enrichment of NAN (means \pm SEM; mol ^{15}N /100 mol total-N) for cottonseed meal and duckweed at 3 h (left) and at 6 h (right) of incubation with strained rumen fluid with (\blacksquare) or without (\square) additional energy source.....	81
Figure 17.	Enrichment of bacterial-N (means \pm SEM; mol ^{15}N /100 mol total-N) for cottonseed meal and duckweed at 3 h (left) and at 6 h (right) of incubation with strained rumen fluid with (\blacksquare) or without (\square) an additional energy source.....	82
Figure 18.	Percentage of NAN that was bacterial-N for cottonseed meal and duckweed after 3 h (left) and after 6 h (right) of incubation with strained rumen fluid, with (\blacksquare) or without (\square) an additional energy source.....	83
Figure 19.	Bacterial-N synthesis (mg N/100 mg feed N) for cottonseed meal and duckweed at 3 h (left) and at 6 h (right) of incubation with strained rumen fluid, with (\blacksquare) or without (\square) an additional energy source.....	83
Figure 20.	Percentage of bacterial-N synthesised from ammonia-N for cottonseed meal and duckweed at 3 h (left) and at 6 h (right) of incubation with strained rumen fluid with (\blacksquare) or without (\square) an additional energy source.....	84
Figure 21.	Production of ammonia (mg N/100 mg feed N, mean \pm SEM) for cottonseed meal and duckweed after 3 h (left) and after 6 h (right) of incubation with strained rumen fluid, with (\blacksquare) or without (\square) an additional energy source.....	85

Figure 22. Degradation of N (%; mean \pm SEM) for cottonseed meal and duckweed after 3 h (left) and after 6 h (right) of incubation with strained rumen fluid, with (■) or without (□) an additional energy source.....	85
Figure 23. The rumen concentration of Co (ln mg/l) with time (h) for Sheep 1 (▲) and Sheep 2 (■) following an intra-ruminal injection of Co-EDTA.	98
Figure 24. The rumen ammonia concentration (mmol N/l) with time (h) for Sheep 1 (▲) and Sheep 2 (■) after administration of ¹⁵ N-labelled materials.	98
Figure 25. Enrichments (y axis; mol ¹⁵ N/100 mol total-N) in rumen fluid ammonia (○), rumen fluid NAN (□) and bacterial-N (△) versus time (x axis; h) after intra-ruminal administration of 2 mmol ¹⁵ N as ¹⁵ N-labelled ammonia (Sheep 1, left) or ¹⁵ N-labelled dried duckweed (Sheep 2, right). The lines represent fitted curves with two exponential functions.....	99
Figure 26. Enrichments (y axis; mol ¹⁵ N/100 mol total N) versus time (x axis; h) in rumen fluid ammonia (○), rumen fluid NAN (□) rumen bacterial-N (△), reconstituted abomasum ammonia (x) and abomasum NAN (◇) of sheep during a 48 h continuous infusion of 2 μ mol ¹⁵ N/min as ¹⁵ N-labelled ammonia.....	104
Figure 27. Enrichments (y axis; mol ¹⁵ N/100 mol total N) versus time (x axis; h) in rumen fluid ammonia (○), rumen fluid NAN (□) rumen bacterial-N (△), reconstituted abomasum ammonia (x) and abomasum NAN (◇) of sheep during a 24 h period of ingestion of 2 μ mol ¹⁵ N/min as ¹⁵ N-labelled duckweed.	104
Figure 28. Enrichments (y axis; mol ¹⁵ N/100 mol total) versus time (x axis; h) in rumen fluid ammonia (○), rumen fluid NAN (□) rumen bacterial-N (△), abomasum ammonia (x) and abomasum NAN (◇) during a 72 h administration of 2 μ mol ¹⁵ N/min as ¹⁵ N-ammonia (top) or ¹⁵ N-duckweed (bottom) to Sheep C.	111
Figure 29. Enrichments (y axis; mol ¹⁵ N/100 mol total) versus time (x axis; h) in rumen fluid ammonia (○), rumen fluid NAN (□) rumen bacterial-N (△), reconstituted abomasum ammonia (x) and abomasum NAN (◇) when 2 μ mol ¹⁵ N/min as ¹⁵ N-ammonia (top) or ¹⁵ N-duckweed (bottom) was continually supplied to the rumen of Sheep D.	112
Figure 30. Live weights of animals (kg) offered the four experimental diets on different dates during the experiment.....	128
Figure 31. Rumen ammonia concentration (means \pm SEM; mg N/l) of animals on the three different diets at 0.5, 4.5 and 7.5 h after feeding (119.1 \pm 5.20 mg N/l).....	134
Figure 32. Rumen fluid pH (means \pm SEM) of animals on the three different diets at 0.5, 4.5 and 7.5 h after feeding.	134
Figure 33. Changes in the number of duckweed fronds for the 'big leaves' variant when grown on a medium at nutrient concentration of either '1.0 strength' (◆), '0.5 strength' (■) or '0.25 strength' (▲)	186

Figure 34	Changes in the number of duckweed fronds for the 'small leaves' variant when grown on a medium at nutrient concentration of either '1.0 strength' (◆), '0.5 strength' (■) or '0.25 strength' (▲).	187
Figure 35	Changes in the number of duckweed leaves for the 'big leaves' when grown on the '0.25 strength' medium having a N concentration of about 25 mg/l provided as KNO ₃ (◆), CO(NH ₂) ₂ (■) or NH ₄ Cl (▲)	187
Figure 36	Changes in the number of duckweed leaves for the 'small leaves' when grown on the '0.25 strength' medium having a N concentration of about 25 mg/l provided as KNO ₃ (◆), urea (■) or NH ₄ Cl (▲).....	188
Figure 37	Relationship between calculated and measured ¹⁵ N abundance of a series of standard differing in ¹⁵ N abundance.	194
Figure 38	The enrichment of various N-containing materials from the fluid (□) and particle (◇) fractions of the abomasum digesta.....	195

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.
