

**Potential of intensive rotational grazing for
control of ovine gastrointestinal nematodosis
in a cool temperate environment with summer
dominant rainfall**

by

Alison Frances Colvin

B. Sc. Agr. (Hons) University of Sydney

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

**School of Rural Science and Agriculture
Faculty of Sciences
University of New England
Armidale NSW 2351
Australia**

SEPTEMBER 2006

ATTENTION USERS

This thesis contains various representations that are in colour in the original. This may include maps, charts, graphs, photographs, or other similar diagrams.

If you would like to look at the original you will need to approach the Information Desk.

Colour copies are available on request :-

- On campus users - fill in the Document Request Form available from the Information Desk. You will be required to **PAY** for these pages at the existing charge of \$2.75(gst incl.) per page.
- Off campus users – place a Document Request via any of the mechanisms available to external students. See <http://www.une.edu.au/library/external/index.htm> You will be required to **PAY** for these pages at the existing charge of \$2.75(gst incl.) per page.
- Libraries and other institutions - please contact the Document Delivery Service through an Inter-Library Loan request for a quote.

Charges for other representations that may be included in this thesis are as follows:

- Maps \$3.85(gst incl.) per page
Overheads \$0.55(gst incl.)
Videocassettes \$3.30(gst incl.)
CD-Roms - \$22.00(gst incl.)

Document Services Unit contacts

Inter Library Loans/Document Delivery: 02 6773 3473

External Students' Library Helpline: 02 6773 3124

Russell Nicholson
Lending Services Librarian

Declaration

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



.....

Alison Frances Colvin

Acknowledgements

Firstly I would like to thank my principal supervisor Steve Walkden-Brown for his unmeasurable guidance and support through my candidature, he has an energy and mental capacity which is astounding. Malcolm Knox as my secondary supervisor has provided a wealth of support for my work and I thank him also for his guidance. I would like to thank Jim Scott for his boundless enthusiasm for the Cicerone Project and for his contribution to supervision during my work.

The person who has been most integral to the practical completion of this study is Justin Hoad, to whom I owe a thousand thanks for moving sheep to and fro, fitting into my timetable and for always being a pleasure to work with.

I would like to acknowledge the funding provided by the Australian Sheep Industry Cooperative Research Centre, Australian Wool Innovation and the University of New England. This research and thesis could not have been completed without their financial contributions.

I also thank the Cicerone Board who have provided such an unique environment in which to carry out my studies. In particular I would like to thank Caroline Gaden for providing me with data, Betty Hall for her positive contribution to my work and Colin Lord and Dion Gallagher for their patience in setting up a section of the Cicerone Database in which to store my results.

I have also to thank Michael Raue, John Gorham and Neil Baillie for their invaluable help during experimental work and sample collection without whom, along with the following list of helpers, I would never have compiled such a large amount of data:

Anne Beasley, Emma Doyle, Grahame Chaffey, Lauren O'Connor, Fiona Macarthur, Libuseng Shakane, Steve Walkden-Brown, Sebastian Herry, Jasper Scholten, Mandy Choice, Tanya Norman, Justin Bailey, Carmel Healey, Michael Colvin, Dominic Neimeyer, Troy Kalinowski, Peter Bradley, David Paul, Patrick Sakwa and Greg Stuart.

My colleagues Anne Beasley and Emma Doyle have been a fountain of strength and have been invaluable in their advice and friendship over the last 3 years, I would like to thank them for sharing their journey with me.

I would like to thank my mother Carmel who gave me every opportunity in life to do my best despite hardship, something which can only be repaid through giving my best.

Finally, I would like to thank my husband Michael for his unending love and support which kept me sane and focused during my PhD candidature.

Table of Contents

Declaration.....	i
Acknowledgements	ii
Table of Contents	iii
List of Figures.....	vii
List of Tables.....	xii
List of Abbreviations.....	xiv
List of Publications.....	xv
Abstract.....	xvi
General Introduction.....	1
CHAPTER 1: Review of the literature	5
1.1 Introduction.....	5
1.2 Australian sheep production, rainfall zones and the distribution of the major gastrointestinal nematodes of sheep	5
1.3 Life cycles of the major gastrointestinal nematodes of sheep	7
1.4 Clinical disease and pathological changes.....	8
1.4.1 <i>Haemonchus contortus</i>	9
1.4.2 <i>Trichostrongylus</i> spp.	9
1.4.3 <i>Teladorsagia circumcincta</i> (formerly <i>Ostertagia circumcincta</i>).....	10
1.4.4 Minor species.....	11
1.5 Pathogenesis of ovine gastrointestinal nematodiasis	11
1.5.1 Effect on feed intake.....	12
1.5.2 Effect on feed utilisation and metabolism	12
1.5.3 Effect of nematodes on wool and milk production.....	14
1.6 Epidemiology of ovine gastrointestinal nematodiasis	15
1.6.1 Epidemiology of gastrointestinal nematode infections in sheep on the Northern Tablelands of NSW	16
1.6.2 Environmental influences on epidemiology: Ecology of the free-living stages of parasitic Trichostrongylid nematodes.....	18
1.6.2.1 Development of egg to third stage larvae	18
1.6.2.2 Survival of third stage larvae on pasture.....	28
1.6.3 Pathogen influences on epidemiology	40
1.6.4 Host influences on epidemiology	41
1.6.4.1 Immune response	42
1.6.4.2 Resistance and resilience to gastrointestinal nematode infection	45
1.7 Control of ovine gastrointestinal nematodiasis.....	47
1.7.1 Approaches targeting the parasitic nematode in the host	47
1.7.1.1 Chemotherapy	47
1.7.2 Approaches targeting the host	50
1.7.2.1 Nutrition – parasite interaction	50
1.7.2.2 Vaccines.....	52
1.7.3 Approaches targeting the parasitic nematode in the environment.....	52
1.7.3.1 Grazing management for control of nematode infections.....	52
1.7.3.2 Non-chemical approaches	62
1.8 Conclusions.....	64
CHAPTER 2: General Materials and Methods.....	67
2.1 The Cicerone Project Inc.	67

2.1.1	Terms used throughout the thesis	69
2.1.2	Animals and their husbandry	70
2.2	Farmlet management treatments	70
2.2.1	Farmlet A; High Input (HI).....	70
2.2.2	Farmlet B; Typical management (TYP)	71
2.2.3	Farmlet C; Intensive rotational grazing (IRG).....	71
2.2.3.1	Anthelmintic treatment on the Cicerone Farmlets	72
2.3	Parasitology.....	74
2.3.1	Faecal Worm Egg Counts	74
2.3.1.1	Pooled faecal worm egg counts	75
2.3.2	Pooled Larval Cultures	75
2.3.3	Faecal Egg Count Reduction Test on Farmlet C (IRG treatment).....	76
2.3.3.1	Results of the Faecal Egg Count Reduction Test.....	77
2.4	Haematology	78
2.5	Measurement of anti-nematode IgG	79
2.5.1	ELISA method	82
2.5.1.1	ELISA reagents	83
2.6	Liveweights and Fat Scores	83
2.7	Ultrasound scanning for pregnancy status	84

CHAPTER 3: Experiment 1. Longitudinal study of gastrointestinal nematode infection in sheep on the Cicerone Project Farmlets over two years..... 85

3.1	Introduction.....	85
3.2	Materials and Methods.....	87
3.2.1	Experimental sheep and their husbandry	87
3.2.2	Measurements	87
3.2.3	Statistical Analysis.....	88
3.3	Results.....	89
3.3.1	Implementation of treatments	89
3.3.1.1	Paddock rotations	89
3.3.2	Parasitological variables	90
3.3.2.1	Larval differentiation	90
3.3.2.2	Faecal worm egg count	92
3.3.2.3	Interval between anthelmintic treatments	94
3.3.3	Bodyweight	94
3.3.4	Fat score	96
3.3.5	Wool measurements.....	97
3.3.6	Haematology	98
3.3.6.1	Haematocrit.....	98
3.3.6.2	Eosinophil Count.....	99
3.4	Discussion	100

CHAPTER 4: Experiment 2. Resistance and resilience to infection under a fixed larval challenge

4.1	Introduction.....	107
4.2	Materials and Methods.....	109
4.2.1	Experimental design and challenge protocol	109
4.2.2	Experimental animals	110
4.2.3	Sampling procedures and measurements	110
4.2.4	Statistical Analysis.....	111
4.3	Results.....	111
4.3.1	Faecal worm egg count.....	112

4.3.2	Bodyweight.....	116
4.3.3	Haematology.....	119
4.3.3.1	Haematocrit.....	119
4.3.3.2	Circulating eosinophil count.....	122
4.3.4	Circulating anti-trichostrongylid IgG.....	123
4.4	Discussion.....	124
CHAPTER 5: Experiment 3. Effect of management system on pasture infectivity with infective larvae – Tracer sheep study		131
5.1	Introduction.....	131
5.2	Materials and Methods	133
5.2.1	Experimental design	133
5.2.2	The tracer animals.....	135
5.2.3	Sampling procedures and measurements.....	135
5.2.4	Statistical Analysis.....	135
5.3	Results.....	136
5.3.1	Seasonal comparisons.....	136
5.3.1.1	Total faecal worm egg counts.....	136
5.3.1.2	Larval culture results.....	137
5.3.1.3	Faecal worm egg count by species.....	138
5.3.2	Within season comparisons	139
5.3.2.1	Winter	139
5.3.2.2	Spring.....	141
5.3.2.3	Summer	142
5.3.2.4	Autumn	143
5.4	Discussion.....	144
CHAPTER 6: Experiment 4. Determinants of larval development and survival of <i>Haemonchus contortus</i> on the Cicerone project		149
6.1	Introduction.....	149
6.2	Materials and Methods	150
6.2.1	Experimental design	150
6.2.2	Arrangement of plots	151
6.2.3	Contamination of plots	152
6.2.3.1	Natural contamination.....	152
6.2.3.2	Artificial contamination.....	152
6.2.4	Irrigation	153
6.2.5	Microclimate and climactic data.....	154
6.2.6	Sampling and laboratory procedures	154
6.2.6.1	Sample collection from plots	154
6.2.6.2	Larval recovery from faeces	154
6.2.6.3	Larval recovery from pasture samples	155
6.2.6.4	Laboratory Procedure after Larval Extraction from Pasture.....	156
6.2.6.5	Enumeration of infective larvae recovered from pasture samples.....	157
6.2.6.6	Calculation of larval recovery rate of spiked pasture control samples	158
6.2.6.7	Estimation of Percentage Green in pasture sample.....	159
6.2.6.8	Estimation of Dry Matter	159
6.2.7	Statistical analysis.....	160
6.2.7.1	Climatic variables	160
6.2.7.2	Larval recovery from artificially contaminated plots	160
6.2.7.3	Larval counts from naturally contaminated plots	161
6.3	Results.....	162

6.3.1	Rainfall and air temperature	162
6.3.2	Ground Temperature	164
6.3.2.1	Effect of watering treatment on ground temperatures	166
6.3.3	Parasitology – Third stage (infective) larvae	167
6.3.3.1	Infective larvae recovered from pasture washings - Summer	167
6.3.3.2	Infective larvae recovered from faecal culture	170
6.4	Discussion	173
CHAPTER 7: Experiment 5. Cost of gastrointestinal nematode infection on sheep production performance on the Cicerone Project Farmlets		180
7.1	Introduction	180
7.2	Materials and Methods	182
7.2.1	Experimental design	182
7.2.2	Animal selection and sampling	182
7.2.2.1	Worm-Free (WF) sheep	182
7.2.2.2	Conventionally managed (CM) sheep	183
7.2.3	Measurement schedule	184
7.2.4	Along Staple Fibre Diameter Measurement	184
7.2.5	Estimation of economic costs	185
7.2.6	Statistical analysis	185
7.3	Results	186
7.3.1	Larval differentiation	186
7.3.2	Faecal worm egg count	186
7.3.3	Bodyweight	189
7.3.4	Fat Score	189
7.3.5	Ultrasound scanned pregnancy status	190
7.3.6	Greasy fleece weight	190
7.3.7	Yield of clean wool	190
7.3.8	Derived mean fibre diameter along the staple	191
7.3.9	Mean fibre diameter (cored mid-side sample, commercial lab)	192
7.3.10	Staple strength	192
7.3.11	Staple length	192
7.3.12	Summary of the effect of gastrointestinal nematodiasis on animal production	193
7.3.13	Economic costs of production differences	193
7.4	Discussion	194
CHAPTER 8: General Discussion		199
8.1	How does IRG work?	200
8.2	Species differences in the effect of IRG	201
8.3	Possible differences in anthelmintic resistance between management systems	202
8.4	Comparison of the HI and TYP management systems	203
8.5	Production differences between management systems	204
8.6	Review of the experimental approach	205
8.7	Implications for the application of intensive rotational grazing for worm control	206
8.8	Main Conclusions	207
References		209

List of Figures

Figure 1-1: Distributions of sheep enterprises across Australia (UNE lecture notes ANPR211, 2005).	6
Figure 1-2: Proportion of sheep production per Australian state (Australian Wool Innovation website, accessed in June 2006, www.wool.com.au).	6
Figure 1-3: Rainfall distribution across the continent of Australia showing the different rainfall zones (Crowther 1995).	7
Figure 1-4: Seasonal patterns of nematode infection in summer and winter rainfall areas: (left) <i>Haemonchus contortus</i> in spring-born lambs grazing contaminated pastures from birth to 18 months of age. Average rainfall and screen temperatures at Armidale, NSW; (right) <i>Teladorsagia</i> spp. (<i>Ostertagia</i>) and <i>Trichostrongylus</i> spp. infections in the Western District of Victoria. Average rainfall and screen temperatures at Hamilton, Victoria (Donald <i>et al.</i> 1978b).	17
Figure 1-5: Survival of <i>Trichostrongylus colubriformis</i> unembryonated (left) and embryonated (right) eggs at varying temperatures tested over six time periods (Data from Anderson <i>et al.</i> 1966).	22
Figure 1-6: Representation of the rate of development (—) and survival (-----) of the eggs of three major nematode species at varying temperatures. The values for these figures are taken from various authors cited in section 1.5.2.1.1.	25
Figure 1-7: Number of days <i>Haemonchus contortus</i> and <i>Trichostrongylus colubriformis</i> infective larvae were recovered from vegetation after L3 had been placed on pasture, monthly for 1967--1969 (<i>H. contortus</i>) and 1965--1966 (<i>T. colubriformis</i>) (Levine <i>et al.</i> 1974).	30
Figure 1-8: An application of the model of Barger <i>et al.</i> (1972) showing the effect of temperature on survival of <i>Haemonchus contortus</i> larvae on pasture as a percentage of larvae surviving from an original population. (Created by L. Kahn using data from Barger <i>et al.</i> 1972).	33
Figure 1-9: Graphic representation of the data collected by Boag and Thomas (1985) on the survival time of the third stage larvae of 3 major nematode species.	37
Figure 1-10: Self-cure reaction to <i>Haemonchus contortus</i> infection involving IgE mediated response (Tizard 1996).	43
Figure 1-11: Geometric mean faecal egg counts of set-stocked or rotationally grazed goats in Tonga from October 1991 to October 1992. Anthelmintic treatments for set-stocked goats shown by arrows. (Barger <i>et al.</i> 1994).	56
Figure 1-12: Average geometric mean egg counts per gram of faeces for sheep in each year of the experiment (Barger and Southcott 1978). The control group of sheep were weaned onto	

the same paddock each year with no cattle grazing in the interim; SC 6 treatment involved alternately grazing paddocks with cattle and sheep for 6 months and SC 12 involved alternate grazing for 12 months of cattle and sheep.....	59
Figure 2-1: Rainfall (black), evaporation (grey), minimum (solid line) and maximum (dashed line) temperatures for the experimental period November 2003 to October 2005 (Burr 2006).....	68
Figure 2-2: Cicerone Project map of farmlets showing paddock distribution across the farm. Farmlet A – High Input (pink), Farmlet B – Typical (green) and Farmlet C – Intensive Rotational Grazing (blue), periphery paddocks (white).....	69
Figure 2-3: Total pasture dry matter divided into green dry matter (black) and dead dry matter (white).	72
Figure 2-4: Linear regression of haematocrit determined by Cell Dyn® on haematocrit determined by the microhaematocrit method. Samples (n=10) are from day 35 of the summer fixed challenge in Experiment 2. (Chapter 4).	79
Figure 2-5: Example of a standard curve from ELISAs with a cubic log-linear curve fitted.....	81
Figure 2-6: Serial dilution of four samples with high antibody titre demonstrating high repeatability of final concentration over a range of dilutions. Concentration values are adjusted for dilution.	82
Figure 2-7: Best position for assessment of fat on sheep and lambs (Holst and White 2001). ...	84
Figure 3-1: Mean rest period (black) and back-transformed mean graze period (white) with 95% CI by season and management treatment. Columns not sharing a common letter within management treatment differ significantly (P<0.05).	89
Figure 3-2: Mean raw a) Ewe b) Hogget c) Lamb total worm egg counts (white), <i>Haemonchus contortus</i> worm egg counts (grey) and <i>Trichostrongylus</i> spp. worm egg counts (black) over the experimental period with anthelmintic treatments indicated by arrows; moxidectin (white), short acting (black), weaning (W), mating (J) and quarantine (Q).....	90
Figure 3-3: Backtransformed mean (with 95% CI) proportion of <i>Haemonchus contortus</i> (white) and <i>Trichostrongylus</i> spp. (black) by management treatment. Means not sharing a common letter within nematode species differ significantly (P<0.05).	92
Figure 3-4: Mean (\pm SEM) proportion of positive faecal worm egg counts (WEC) showing the management treatment by class interaction for total WEC, <i>H. contortus</i> WEC and <i>Trichostrongylus</i> spp. Means within management treatments not sharing a common letter differ significantly (P<0.05).....	93
Figure 3-5: Backtransformed least square means with 95% confidence intervals for faecal worm egg count showing significant interaction between the effects of year, management	

treatment and class. Means within years not sharing a common letter differ significantly (P<0.05).....	94
Figure 3-6: Monthly mean bodyweight per management treatment (HI-- , TYP-----, IRG—) and class over the experimental period; a) Actual bodyweights; b) Bodyweights with initial bodyweight fitted as a covariate. Vertical dotted lines indicate the transition between classes.....	95
Figure 3-7: Daily bodyweight gain and losses for lambs over the experimental period for each management treatment (HI-- , TYP----- , IRG—).....	96
Figure 3-8: Mean fat score per farmling by class over the experimental period (HI -- , TYP -----,IRG—).	97
Figure 3-9: Least squared mean (\pm SEM) fleece weight by management treatment and class. Means within class not sharing letters are significantly different (P<0.05).....	98
Figure 3-10: Backtransformed least squared mean haematocrit (with 95% CI) showing the interaction between management treatment and class. Means within management treatments not sharing a common letter differ significantly (P<0.05).	99
Figure 3-11: Backtransformed least square mean eosinophils with 95% CI showing interaction between management treatment (HI: white, TYP: grey, IRG: black), class and year.	100
Figure 4-1: Timing of experimental events and sample collection for the fixed larval challenge.	110
Figure 4-2: Fixed challenge data (LSM \pm SEM) by management system (HI-- , TYP----- , IRG—) and season. a) Total faecal worm egg count (eggs/g faeces); b) <i>H. contortus</i> faecal worm egg count; c) <i>T. colubriformis</i> faecal worm egg count.	112
Figure 4-3: Linear regressions of predicted faecal worm egg count against bodyweight (left) and haematocrit (right) for the spring fixed challenge.....	113
Figure 4-4: Linear regression of predicted faecal worm egg count against haematocrit for the summer fixed challenge.	114
Figure 4-5: Linear regression of predicted faecal worm egg count against haematocrit for the autumn fixed challenge.	115
Figure 4-6: Linear regressions of predicted faecal worm egg count against bodyweight (top left), haematocrit (top right) and eosinophil count (bottom) for the winter fixed challenge.	116
Figure 4-7: Bodyweight least squared means (\pm SEM) by management treatment (HI -- , TYP -----, IRG—) a) with day 0 bodyweight fitted as a covariate; b) Actual recorded bodyweight.	118
Figure 4-8: Haematology least squared means by management system (HI-- , TYP-----, IRG—) for a) Haematocrit (%); b) Circulating eosinophils (cells $\times 10^6$ /ml); Peripheral IgG antibody level (Arbitrary units).....	120

Figure 4-9: Linear regression of predicted haematocrit against bodyweight for the spring fixed challenge.....	121
Figure 5-1: Timing of experimental events and sample collection for each tracer study.....	134
Figure 5-2: Backtransformed mean faecal worm egg count with 95% confidence intervals for tracers on days 28 and 35 by class, management treatment and season.	140
Figure 6-1: Graphical representation of a block containing 5 replicates of each treatment for destructive sampling (20 plots). Plots were 80cm x 80cm and separated from each other by metal inserts.....	151
Figure 6-2: Layout of Block B from the Spring experiment without (left) and with (right) shade cloth.....	151
Figure 6-3: Timing of events for preparation of passage sheep.	153
Figure 6-4: Rainfall (black column), evaporation (white column), maximum air (—), maximum ground (.....), minimum air (—) and minimum ground (.....) temperatures for each experimental period a) spring, b) summer and c) autumn. Ground temperature data are from the experimental site itself while other climatic data is from Armidale, 20km away (Burr 2006).....	163
Figure 6-5: Rainfall (black column), evaporation (white column), maximum air (—), maximum ground (.....), minimum air (—) and minimum ground (.....) temperatures from day 0 to day 7 for each experimental period a) spring, b) summer and c) autumn. Ground temperature data are from the experimental site itself while other climatic data is from Armidale, 20km away (Burr 2006).	164
Figure 6-6: Regressions for maximum and minimum ground and air temperatures by season and for seasonal data combined.	165
Figure 6-7: Summer experiment – Artificial contamination. Average <i>Haemonchus contortus</i> infective larvae recovered (adjusted for spiked control recovery) per 10 000 eggs deposited as the artificial infection for watered and un-watered plots.	168
Figure 6-8: Survival of <i>Haemonchus contortus</i> infective larvae on pasture over the experimental period represented as a proportion of day 7 larval recovery with a fitted exponential curve.	169
Figure 6-9: Summer experiment – naturally contaminated plots. Average <i>Haemonchus contortus</i> infective larvae recovered (adjusted for spiked control recovery) per plot (80 x 80cm) from the natural infection for watered and un-watered treatments.	170
Figure 7-1: Experiment 5 data by management system from August 2004 to July 2005 for ‘worm-free’-WF (.....) and conventionally managed-CM (—) sheep; Arithmetic mean faecal worm egg count (for CM sheep only) expressed as a) <i>Haemonchus contortus</i> eggs/g, with anthelmintic treatments for CM sheep indicated by arrows: moxidectin (white), short-	

acting (black), quarantine (Q) and mating (J); or b) *Trichostrongylus* spp. eggs/g; c) LSM (\pm SEM) bodyweight of WF and CM sheep; d) LSM (\pm SEM) fat score; e) LSM (\pm SEM) fibre diameter with initial fibre diameter fitted as a covariate.188

Figure 7-2: Experiment 5. Interaction plots (LSM \pm SEM) showing the effects of management system and worm control treatment on wool yield (left) and staple length (right); HI (■), TYP (▼) and IRG (○).193

List of Tables

Table 1-1-1: The effect of temperature on the rate of development of the free-living stages of <i>Haemonchus contortus</i> in moist faeces (Rose 1963):	20
Table 2-1: Summary of the Cicerone Project management system treatments.	70
Table 2-2: Total days of supplementary feed per sheep class under each management system over the experimental period from November 2003 to October 2005. The main type of supplement given was lupins and with some maize given on the HI management system.	72
Table 2-2-3: Anthelmintic treatments during the experimental period by sheep class and management treatment; moxidectin (MOX), albendazole (ABZ), levamisole (LEV), naphthalophos (NAP). Where LEV was given without other anthelmintics it was administered at double the recommended dose rate under veterinary advice (Dr E. Hall, personal communication). Asterisks denote fixed treatments imposed for reasons other than perceived risk of GIN.	73
Table 2-4: Reduction of worm egg count with lower confidence interval for each anthelmintic treatment used in the faecal egg count reduction test on Farmlet C.	78
Table 2-5: Standard curve dilutions for ELISA plates	80
Table 2-6: Fat scoring system based on 12th rib at the GR site (Holst and White 2001).	84
Table 3-1: Overall raw proportions of parasitic nematode species found under each management treatment.	91
Table 5-1: Backtransformed least square mean tracer faecal worm egg counts by management treatment and season with 95% confidence intervals. Different letters within season denote a significant difference ($P < 0.05$).	137
Table 5-2: Backtransformed mean proportions of the 3 major parasitic nematode species recovered from tracer faecal cultures. Different letters within season indicate a significant difference ($P < 0.05$).	138
Table 5-3: Backtransformed faecal worm egg counts with 95% confidence intervals (95% CI) for the three major nematode species by season. Different letters within season indicate a significant difference ($P < 0.05$).	139
Table 5-4: Mean proportions of the three major parasitic nematodes collected from bulked tracer faecal cultures by season and management treatment. Numbers in brackets represent actual numbers counted when less than 100 larvae were counted.	141
Table 5-5: Backtransformed mean faecal worm egg counts of tracers with 95% confidence intervals (95% CI) apportioned into the three major nematode parasites using the	

proportions of each parasite counted in faecal cultures by management treatment and class (E- ewes, H- hoggets, L- Lambs) within season.	145
Table 6-1: Faecal worm egg counts and larval differentiation results by season for the sheep grazing paddocks immediately prior to day 0.	152
Table 6-2: Average weight of faeces, average number of eggs per gram of faeces and average number of eggs deposited on plots.	153
Table 6-3: Mean recovery rates and coefficient of variation (%CV) for the pasture controls (entire recovery procedure) and the sediment controls (post-washing steps only) by season.	159
Table 6-4: Average weight of faeces collected from plots by treatment for each season. UA – unwatered, artificial contamination; WA – Watered, artificial contamination; UN – Unwatered, natural contamination; WN – watered , natural infection.	170
Table 6-5: Average number of <i>Haemonchus contortus</i> infective larvae per 10 000 eggs deposited recovered from faecal culture on artificially contaminated plots, by season and irrigation treatment, with the number of plots positive for infective larvae.	171
Table 6-6: Average number of <i>Haemonchus contortus</i> larvae per plot from the natural infection recovered from cultured faeces by season and irrigation treatment with number of plots that were positive for infective larvae, with number of plots positive for infective larvae.	172
Table 6-7: Average number of larvae per plot recovered from cultured faeces from naturally contaminated plots for nematodes other than <i>Haemonchus contortus</i> (Tr- <i>Trichostrongylus</i> spp., Te- <i>Teladorsagia circumcincta</i> , Oes- <i>Oesophagostomum</i> spp.) by season and irrigation treatment, with number of plots that were positive for infective larvae of these genera.	173
Table 7-1: Experiment 5. Anthelmintic treatments given to conventionally managed sheep on each management system from July 2004 to July 2005. Moxidectin (MOX), albendazole (ABZ), levamisole (LEV), naphthalophos (NAP). Asterisks denotes quarantine drench given at shearing prior to the start of this experiment.	183
Table 7-2: Raw proportions of the three major parasitic nematodes found in faecal cultures of conventionally managed sheep under each management system from September 2004 to July 2005.	186
Table 7-3: Summary of least squared means \pm SEM and percentage difference in production variables in worm-free and conventionally managed (CM) sheep on each management system. dMFD – derived mean fibre diameter, cMFD – cored mean fibre diameter.	194

List of Abbreviations

- BZ:** Benzimidazole/ albendazole anthelmintic compound
- CM:** Sheep conventionally managed for worms on each management system
- cMFD:** Mean fibre diameter estimated from a cored mid-side sample (commercial laboratory)
- dMFD:** Derived mean fibre diameter along the staple
- ELISA:**
- FECRT:** Faecal egg count reduction test
- GIN:** Gastrointestinal nematodosis
- HCT:** haematocrit
- HeWEC:** *Haemonchus contortus* faecal worm egg count
- HI:** Farmlet A, High input management system
- IgG:** Anti-trichostrongylid Immunoglobulin G
- IRG:** Farmlet C, intensive rotational grazing system
- LEV:** Levamisole anthelmintic compound
- MOX:** moxidectin anthelmintic compound
- NAP:** Napthalophos anthelmintic compound
- OstWEC:** *Teladorsagia circumcincta* faecal worm egg count
- PBST:** Phosphate buffered saline plus Tween detergent
- QC:** Quality control
- TcWEC:** *Trichostrongylus colubriformis* faecal worm egg count
- TWEC:** *Trichostrongylus* spp. faecal worm egg count
- TYP:** Farmlet B, conventional/typical management system
- WEC:** Faecal worm egg count
- WF:** 'Worm free' sheep

List of Publications

- Colvin AF, Walkden-Brown SW (2006) Assessing the cost of parasitic nematodes on 3 management systems. In 'ASP & ARC/NHMRC Research Network for Parasitology Annual Conference'. Gold Coast p. 83
- Colvin AF, Walkden-Brown SW, Knox MR (2006a) Sheep management system markedly effects worm infections in the New England. In 'Wool meets Meat -Tools for a modern sheep enterprise, Proceedings of the 2006 Australian Sheep Industry CRC Conference'. Orange. (Eds PB Cronje and DK Maxwell) pp. 225-226. (Australian Sheep Industry CRC)
- Colvin AF, Walkden-Brown SW, Knox MR, Scott JM (2006b) Intensive rotational grazing assists control of gastrointestinal nematodosis of sheep in a cool temperate environment with summer-dominant rainfall. *Veterinary Parasitology* Submitted.
- Healey AF, Hall E, Gaden CA, Scott JM, Walkden-Brown SW (2004a) Intensive rotational grazing reduces nematode faecal egg counts in sheep on the Cicerone Project. In 'Animal Production in Australia'. Melbourne pp. 85-88. (CSIRO Publishing)
- Healey AF, Walkden-Brown SW (2004) Intensive rotational grazing: A potential tool for worm control on the Northern Tablelands. In 'Australian Society of Animal Production One-day Research Symposium'. Armidale
- Healey AF, Walkden-Brown SW (2005) Dissecting the effects of intensive rotational grazing on gastrointestinal nematodiasis. In '20th International Conference of the World Association for the Advancement of Veterinary Parasitology'. Christchurch p. 214
- Healey AF, Walkden-Brown SW, Knox MR (2004b) Intensive rotational grazing; prospects for worm control on the New England Tablelands. In 'Proceedings of the 2004 Annual Postgraduate Conference of the Australian Sheep Industry CRC'. Coffs Harbour
- Healey AF, Walkden-Brown SW, Knox MR (2005a) Superior performance of worm-free sheep over conventionally managed sheep in the New England region is dependant upon the production system. In 'Proceedings of the 2005 Annual Postgraduate Conference of the Australian Sheep Industry CRC'. Noosa
- Healey AF, Walkden-Brown SW, Scott JM (2005b) Dissecting the effects of intensive rotational grazing on worm egg counts in sheep on the Cicerone Project. In 'The Cicerone Farms Under the Microscope -Symposium 2005'. Armidale. (Ed. JM Scott). (Cicerone Project Inc.)
- Scott JM, Gaden CA, Shakhane L, Healey AF (2004) Holding a measuring stick up to the Cicerone farmlets: how are they shaping up? In 'Proceedings of the 19th Grasslands Society of NSW Conference'. Gunnedah pp. 63-68

Abstract

To date there have been no reports of practical rotational grazing systems for the control of ovine gastrointestinal nematodosis in cool temperate climates, despite their success in the humid tropics. However there is anecdotal evidence that the intensive rotational grazing systems (such as “cell grazing”) that are gaining in popularity in these regions, offer significant control. Intensive rotational grazing involves the use of large groups of animals at high stock densities moving through a series of 20 to 40 paddocks at a rate dependant on the amount of feed on offer and pasture growth rate (not based on rigid time periods). The grazing period generally ranges from 2-3 days with rest periods of 40-90 days, resulting in paddocks being rested for 90-95% of the year. The work contained in this thesis was conducted to investigate the merits of these claims. The unifying hypothesis was that intensive rotational grazing reduces faecal worm egg counts in sheep by interrupting the nematode lifecycle in its free-living stages and that the greatest effect will be on the blood-sucking parasite *Haemonchus contortus*. The work was conducted on the Cicerone Project, a producer-led project comparing three different sheep management systems in the New England Region of Northern NSW. The three management systems were typical (TYP – moderate input, limited rotational grazing, graze periods average 53 ± 0.1 days and rest periods average 78 ± 10 days), high input (HI, high input, limited rotational grazing, graze periods average 40 ± 0.1 days, rest periods average 65 ± 8 days) and intensive rotational grazing (IRG, moderate input, short graze periods average 3 ± 0.1 days, long rest periods average 108 ± 4 days).

Experiment 1 comprised a 2-year longitudinal study of faecal worm egg count (WEC) and performance in lambs, hoggets and ewes of the three management groups. It revealed lower *Haemonchus contortus* WEC and a markedly reduced number of anthelmintic treatments in sheep on the IRG treatment (Chapter 3). The subsequent experiments were designed to tease out the mechanisms behind this phenomenon. A fixed larval challenge study (Experiment 2, Chapter 4) showed that IRG sheep exhibited resistance to infection that was no better, and in two seasons much worse, than sheep on the HI and CON treatments ruling out improved host resistance as the factor mediating the effects of IRG. In contrast a tracer experiment investigating levels of pasture contamination with infective larvae (Experiment 3, Chapter 5) and a study into the free-living ecology of *Haemonchus contortus* on the Cicerone project (Experiment 4, Chapter 6) demonstrated reduced pasture infectivity for all classes of stock on the IRG treatment for the 3 seasons of the year (winter, spring and summer) when the short graze periods and long rest periods were maintained. The tracer studies and fixed larval challenge both show that the dynamics of GIN epidemiology can change rapidly with changes in the rotations on the IRG

system (longer graze periods of up to 30 days in April 2005) which reinforces the hypothesis that GIN is reduced on IRG through interruption of the free-living stages of the parasitic lifecycle. The ideal graze and rest periods for worm control fluctuate with weather conditions, during warm, wet, summer months graze periods need to be around 3 days and rest periods around 60-80 days. In winter, graze periods can be lengthened to 7 days and rest periods lengthened to 100 days or more. The lower proportions of *H. contortus* contributing to WEC on the IRG treatment in Experiment 1 (Chapter 3) were also observed in the tracer experiment (Chapter 5) confirming a differential effect of IRG on *H. contortus* relative to *Trichostrongylus* spp. and *Teladorsagia circumcincta*. Experiment 4 (Chapter 6) demonstrated that the half-life of *H. contortus* infective larvae on pasture was 19 days in summer meaning that rest periods on IRG in that season were sufficient for most of the deposited larvae to have died off between grazings. There was also very limited development of eggs to L₃ for *Haemonchus* in spring and autumn but there were recoveries of *Trichostrongylus* spp. and *Teladorsagia circumcincta* infective larvae from faecal culture in all seasons on IRG. Experiment 5 (Chapter 7) investigated the production and economic impacts of worm infection on the different management systems and further confirmed the high level of control of GIN achieved on the IRG system, with no production losses attributable to nematodes on IRG whilst bodyweights, fat scores and fleece weights were higher in 'worm-free' sheep on the HI and TYP management systems. Levels of GIN were very similar on the TYP and HI treatments with no consistent differences between them across the different experiments.

The main conclusions were that intensive rotational grazing markedly reduces faecal worm egg counts in sheep and the level of anthelmintic intervention required. It does so by breaking the nematode lifecycle in two ways: i) short graze periods (2 to 4.5 days) prevent autoinfection from the current graze period, and ii) long rest periods (80 to 140 days) ensure most of larvae that developed from the last grazing incident have died before sheep return to graze. The improved control of GIN on IRG was not associated with improved performance when compared to HI and TYP managed sheep. The implementation of IRG on the Cicerone Project requires fine-tuning to obtain the full benefits of better nematode control with improved productivity. Intensive rotational grazing was most effective against *Haemonchus contortus* due to its susceptibility to desiccation and cold in its egg and larval stages. The effect of IRG on *Trichostrongylus* spp. and *Teladorsagia circumcincta* was less pronounced as these worm species have the ability to survive as eggs in drier and colder conditions than *H. contortus*. Therefore, the unifying hypothesis was accepted.

General Introduction

Gastrointestinal nematodosis (GIN) is the principal disease of sheep in Australia and world wide. In the most recent published estimate of its cost to the Australian sheep industry GIN was estimated at \$222 million dollars per year and it caused the greatest economic loss of all sheep diseases (McLeod 1995). Chemical anthelmintic treatments have been the principal method of worm control over the last 40 years, however, resistance has developed to all major classes of anthelmintic compounds, with the possible exception of Napthalophos, an organophosphate, and is rapidly worsening. The development of new anthelmintic compounds is constrained by the relatively small size of the worldwide market for sheep anthelmintics and the lack of significant anthelmintic resistance problems in the much bigger cattle market. Hence, alternative control methods are required to form part of an integrated approach to gastrointestinal nematode control. Grazing management is an obvious alternative to anthelmintic treatment and has been used with mixed results to date. Successful grazing management strategies include dilution strategies such as mixed grazing of susceptible sheep classes with non-susceptible animals (eg: cattle or older dry sheep) and preventative strategies such as grazing cattle alternately with sheep to 'clean' the pasture of infective larval nematodes. Our understanding of the ecology of the free-living stages of parasitic nematodes also suggests that rotational grazing systems could assist in the control of GIN in sheep by interrupting the nematode lifecycle (Donald 1967). However, up to the late 1980s, there was little success in developing and implementing practical rotational grazing systems that reduced GIN. Early studies on rotational grazing in cool temperate environments involved grazing periods of 7 days and rest periods between grazing events ranging from 3 to 7 weeks (Morgan 1933; Morgan and Oldham 1934; Roe *et al.* 1959; Gibson and Everett 1968). These rotations, however, were ideal for the proliferation of parasitic nematodes allowing both autoinfection from the current grazing period in summer months, and re-grazing at the peak of L₃ availability. *Haemonchus contortus* will develop from egg to L₃ in 3-5 days at 25-26°C but will take 15-30 days at 10-11°C (Rose 1963). Season therefore determines the length of safe grazing periods that prevent autoinfection. The time of peak L₃ on pasture in the Sydney Basin, NSW is generally around 35 days after deposition with smaller peaks at days 14 and 28 (Donald 1967). This author concluded that the spelling period for a paddock should be no less than 8 weeks to enable a significant reduction in pasture infectivity. This may also vary with season as L₃ on pasture survive longer in cooler conditions than warm or hot conditions (Dinaburg 1944a; Thomas and Boag 1972; Southcott *et al.* 1976; Besier and Dunsmore 1993a).

GENERAL INTRODUCTION

Donald (1967) thought that such long rest periods were inefficient in terms of optimal pasture utilisation. This is supported to some extent by Robertson and Fraser (1933) using much longer rest periods than those suggested by Donald. Their study in north Scotland achieved control of GIN through what they termed 'progressional grazing' and was especially effective in reducing the incidence of *H. contortus*. The rotation employed for 'progressional grazing' was 10 days of grazing followed by 100 days of rest. The authors concluded that 10 days was a sufficiently safe period of time for grazing before eggs reached the infective stage. However, over-mature grass undermined the success of 'progressional grazing' with sheep failing to maintain body weight despite lower parasite burdens.

Some 60 years after Robertson and Fraser (1933) an effective rapid rotational grazing system was devised by Barger *et al.* (1994) for small ruminants in the humid tropics based on the findings of Banks *et al.* (1990) in Fiji on the rates of larval survival and mortality in hot, humid environments. The system comprised a grazing period of 3.5 days and a rest period of 31.5 days and has been used with success throughout the tropics in both sheep and goats (Barger *et al.* 1994; Chandrawathani *et al.* 1995; Sani *et al.* 1996; Gray *et al.* 2000). However, Banks *et al.* (1990) and Barger *et al.* (1994) suggest that rapid rotational grazing would not be economically viable in cooler climates, presumably because the rigid application of timing of graze and rest periods would be unsuitable given the seasonal variability of temperature and rainfall which are the major drivers of development and survival of the free-living stages of parasitic nematodes in these environments.

In the early 1990s in the temperate regions of Australia, intensive rotational grazing systems such as 'cell grazing' and 'holistic grazing' were introduced on the basis of improved pasture and animal performance. These systems claimed to increase pasture biodiversity, ground cover, and the ratio of palatable to non-palatable plant species, and improve soil structure (Earl and Jones 1996; McCosker 2000; Sparke 2000). They involve the use of large groups of animals at high stock densities moving through a series of 20 to 40 paddocks at a rate dependant on the amount of feed on offer and pasture growth rate (not based on rigid time periods). The grazing period generally ranges from 1-3 days with rest periods of 40-90 days, resulting in paddocks being rested for 90-95% of the year (Earl and Jones 1996). This type of grazing management has become increasingly used throughout Australia with its highest prevalence being in the New England region on the Northern Tablelands of NSW (Reeve and Thompson 2005). The consequences of such intensive grazing systems on GIN in sheep in cool temperate climates have

GENERAL INTRODUCTION

not been documented despite considerable anecdotal evidence of marked reductions in faecal worm egg counts (WEC) and the number of anthelmintic treatments required.

The Cicerone project is a farmer-led project comparing three sheep management systems in the New England region of Northern NSW. The 3 management systems are Typical (TYP), High Input (HI), and Intensive Rotational Grazing (IRG) and are detailed later in the thesis. There was evidence that sheep on the IRG management system, had lower faecal worm egg counts than those run with slower rotational grazing management on the TYP and HI systems. A retrospective analysis of the routine faecal worm egg counts under the three management systems strongly supported the proposition that intensive rotational grazing reduced GIN (Healey *et al.* 2004). This doctoral study was designed to confirm this finding in a controlled, balanced study and to:

- determine on which classes of stock it was operative
- determine which times of the year it is operative
- determine which parasite species are affected by it
- uncover the underlying mechanisms by which its was working

The study also aimed to investigate what differences, if any there were in GIN between the TYP and HI management systems on the Cicerone project.

This thesis details the epidemiology of GIN on the Cicerone Project farmlets dissecting the host, environment and pathogen effects on the disease under the 3 different management systems. A two-year longitudinal study (Experiment 1) aims to confirm the effect of the three management systems on GIN and animal production and provide insight into possible mechanisms. A fixed larval challenge study (Experiment 2) investigates host effects on GIN while the pasture larval contamination is investigated in a tracer sheep experiment (Experiment 3). The development and survival of free-living stages of *Haemonchus contortus* on the Cicerone project is investigated in a plot study (Experiment 4). Finally, the cost of GIN on animal performance is investigated by comparison of 'worm-free' sheep with those managed for worm control within each management system in Experiment 5. Grazing systems in general are not designed for worm control, so an holistic approach has been adopted with animal performance measured in this doctoral study whilst a fellow doctoral student, Libuseng Shakhane, has investigated the pasture aspects.

The general hypothesis under test in this thesis is that intensive rotational grazing reduces faecal worm egg count in sheep through interruption of the nematode lifecycle in its free-living stages.

GENERAL INTRODUCTION

Subsidiary hypotheses are that the reductions in WEC associated with IRG will be greatest for *Haemonchus contortus* due to the greater susceptibility of its free-living stages to desiccation and cold, and that WEC will be higher in the high input grazing system than the typical grazing system.