

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

**INTERNAL PARASITISM AND  
GROWTH OF FARMED DEER  
FED DIFFERENT FORAGE  
SPECIES**

A thesis in partial fulfilment of the requirements for  
the degree of **DOCTOR OF PHILOSOPHY**  
in Animal Science at Massey University

**SIMONE ORIEL HOSKIN**

**1998**

# DECLARATION

The studies presented in this thesis were completed by the author while a post-graduate student in the Institute of Food, Nutrition and Human Health, College of Sciences, Massey University, Palmerston North, New Zealand. This is all my own work and the views presented are mine alone. Any assistance received is acknowledged in the thesis.

I certify that the substance of this thesis has not been already 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.



Simone O. Hoskin

PhD Candidate



Professor T.N. Barry

Chief Supervisor



Associate Professor P.R. Wilson

Co-supervisor



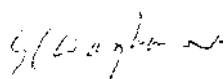
Associate Professor W.A.G. Charleston

Co-supervisor



Professor J. Hodgson

Co-supervisor



Dr. G.C. Waghorn

Co-supervisor

## ABSTRACT

A series of grazing and indoor experiments were conducted to investigate interactions between internal parasitism and different forage species affecting farmed deer growth, carcass production, voluntary feed intake (VFI) and nutrient digestion. These studies have provided information into aspects of internal parasitism in farmed red (*Cervus elaphus*) and hybrid (0.75 red:0.25 elk) deer. These studies have also investigated the potential of forage crops with a different plant morphology to perennial ryegrass/white clover (PRG/WC), such as chicory (*Cichorium intybus*), and those containing condensed tannins (CT) such as sulla (*Hedysarum coronarium*) and birdsfoot trefoil (*Lotus corniculatus*), as natural aids in the control of internal parasites of farmed deer. Use of such forage crops could enhance sustainable management systems for deer production with minimal anthelmintic input. This is consistent with the New Zealand Deer Industry's strategy for clean, green, natural products produced using minimal chemical inputs.

During 1994, a grazing trial was conducted to evaluate the use of sulla (cv Necton), a new forage legume for deer production. Growth and carcass production from weaning to one year of age on sulla was compared with that on chicory (cv Grasslands Puna) and PRG (*Lolium perenne*, cv Nui)/WC (*Trifolium repens* cv Huia) pasture, with all deer receiving three-weekly oral anthelmintic treatment. VFI of deer grazing sulla was greater than for deer grazing chicory in autumn, with pasture being intermediate. Autumn LWG, final liveweight and carcass weight of deer grazing sulla was greater than for deer grazing either pasture or chicory. The proportion of deer reaching 50-65kg carcass weight by one year of age was 100% for sulla and 89% for pasture and chicory. The increased growth and carcass weight of young deer grazing sulla was due to its higher feeding value, particularly during autumn, including increased utilisation of digested nutrients associated with the high CT concentration of sulla (5.1-8.4%).

Concurrently, another grazing trial showed that grazing deer on chicory reduced the development of internal parasitism and hence increased deer growth and carcass production, compared with grazing PRG/WC pasture. Deer on the two forages were either treated with anthelmintic three-weekly to control internal parasites, or anthelmintic was withheld until pre-determined trigger-treatment criteria to minimise the welfare risk to the animal were reached. Chicory and PRG/WC pasture were grazed at the same herbage allowance per animal, but the forages differed in morphology and sward structure, with the broad-leaved chicory sward being taller and more open. Both forages were maintained in the vegetative state, were of high *in-vitro* digestibility and contained

only traces of CT (<0.3%). Untreated deer grazing pasture rapidly developed clinical lungworm infections during the autumn period and required anthelmintic treatment. In contrast, the untreated chicory group required no anthelmintic treatment during the autumn period when grazing chicory, but required treatment 26 days after transfer to pasture during winter. VFI and LWG of untreated deer grazing pasture in autumn was reduced, contributing to lower carcass weights, but anthelmintic treatment had no effect upon the productivity of deer grazing chicory. This experiment also demonstrated limitations of current tools for diagnoses of sub-clinical and clinical internal parasite infections in farmed deer, particularly during the early stages of infection and indicated that further research is needed to investigate the epidemiology, pathogenicity and diagnosis of internal parasite infections. Further research is also needed to partition the effects of plant morphology and plant chemical composition on development of internal parasitism in deer grazing different forage species.

Subsequently, a model for sub-clinical parasite infection in deer was established, in a controlled environment, using individually housed deer fed lucerne hay, upon which further evaluation of forage species could be based, allowing individual animal measurement of factors such as VFI, digestion, growth and aspects of parasitology. The initial model investigated the effect of three sub-clinical dose rates of deer-origin lung (*Dictyocaulus viviparus*) and gastrointestinal (GI) parasite larvae by trickle-infection, relative to an uninfected control group, on deer VFI, liveweight, faecal egg counts (FEC), faecal larval counts (FLC), haematology, serum biochemistry, apparent digestibility, nitrogen (N) retention and digesta N flow at the abomasum and terminal ileum and worm counts at euthanasia. Sub-clinical parasitism reduced liveweight, VFI and serum albumin concentration, elevated serum pepsinogen, gastrin and globulin concentrations and elevated peripheral eosinophil counts, and caused slight haemoconcentration, despite low nematode counts. Reductions in liveweight, N-retention and flow of N at the terminal ileum were shown to be largely due to the reduction in VFI, with no effect of parasite infection on apparent digestibility. This experiment also showed that such studies could be conducted using anthelmintic-treated deer from natural rearing systems as well as artificially-reared deer, thus reducing cost.

Finally, the model was used to investigate the effect of feeding forage legumes containing different concentrations of CT on apparent establishment of lung and GI nematodes, VFI, liveweight, FEC, FLC, haematology and serum biochemistry. Fresh, vegetative lucerne (*Medicago sativa*; 0.1% CT), birdsfoot trefoil (1.9% CT) and sulla (3.5% CT) were compared. This experiment showed a significant negative linear

relationship between dietary CT concentration and apparent establishment of abomasal nematodes, particularly *T. axei*. Deer fed sulla had reduced FLC, higher liveweight gain, carcass weight, dressing-out percentage, serum total protein and albumin concentration and lower serum gastrin concentration, compared with lucerne-fed deer. There were no significant differences in mean VFI between treatment groups during the period of infection, suggesting that the increased liveweight gain of deer fed CT-containing forages was due to an increased efficiency of utilisation of digested nutrients, probably caused by action of CT counteracting protein losses normally associated with parasite infections. It is proposed that the reduced establishment of abomasal nematodes and reduced faecal lungworm larval count in deer fed sulla containing a high concentration of CT may be due to a direct effect of free CT inactivating nematodes in the GI tract.

This study is the first to report and quantify significant reductions in VFI, liveweight gain, N-retention and carcass production in young, farmed deer sub-clinically infected with internal parasites, with most of the reductions being attributable to reduced VFI. Indices for diagnosis of internal parasitism in farmed deer have also been evaluated. Feeding forages containing CT has been shown to reduce apparent establishment of GI nematodes, FLC and increase liveweight gain of parasitised deer. Grazing chicory, a crop of differing plant morphology and sward structure to PRG/WC pasture has also been shown to reduce the development of internal parasitism in farmed deer. The grazing and indoor studies together highlight the potential use of forage crops to increase growth of farmed deer while minimising anthelmintic input. The studies presented in this thesis have great potential significance to the New Zealand Deer Industry. The working model of internal parasitism for deer developed here can now be used to develop further knowledge of deer parasitism and alternative methods of parasite control that are more ecologically sustainable than regular chemical treatment.

## ACKNOWLEDGEMENTS

This thesis could not have been completed without the help of many people:

- Professor Tom Barry (chief supervisor), whose abilities to always see the funny side of things, offer endless encouragement and to get blood (& money) out of stones, are unsurpassed.
- Associate Professor Peter Wilson (supervisor), whose enthusiasm, understanding of human nature, and sense of humor are matchless.
- Associate Professor Tony Charleston (supervisor), whose warmth, patience, and ability to make time when there is no time, are unrivaled.
- Professor John Hodgson (supervisor), whose assistance with the grazing studies was indispensable.
- Dr Gary Waghorn (supervisor), who was able to produce large quantities of specialist forage species out of thin air at short notice, and was the instigator of valuable debate of many issues amongst all supervisors.
- Dr Peter Kemp (as good as a supervisor), for hands-on assistance with the grazing studies.
- Dr Heather Simpson, for consultation regarding physiological aspects of parasitism.
- Geoff Purchas, my No. 1 technician during all experimental work.
- Julie Hanna, for multi-skilled technical assistance in many areas.
- Shirley Calder and Barbara Adlington (Penman), of the Parasitology Laboratory for advice on aspects of parasitology and technical assistance.
- Fliss Jackson, Maggie Zou and Joseph Bateson, of the Nutrition Laboratory for nutritional analyses.
- Dr Abdul Molan, for parasitological expertise and invaluable technical help.
- Charlie Howell, Tim Harvey and Barry Parlane, for experimental logistics.
- Dr Yuxi Wang, Dr Kusmartono, Dr Gono Semiadi, and B.R. Min, for technical and moral support.
- Nicolas Lopez-Villalobos, for statistical and SAS advice.
- Jane Candy and Dr Dave Lawton, for serum pepsinogen and gastrin assays.
- Dr Keith Thompson, staff of the Clinical Pathology Laboratory and Animal Science Physiology Laboratory, for blood sample analyses and advice.
- Simeon Wither, Angela Barry, Marcia Steinezen (and all other visiting students from the Netherlands), Dave Shelton and Bridget Keohane, for technical assistance.

- Alan Alexander and Brigitte Slacek of the Animal Health Services Centre, for collaboration in aspects of parasitology and provision of equipment.
- Brigid Feely, for her encouragement and accurate thesis proof-reading.

Personal financial assistance was provided by:

- AGMARDT Doctoral Scholarship.
- The Massey University Doctoral Scholarship.
- The Massey University Scholar Award.
- The Leonard Condell Farming Trust Scholarship.
- The Johannes A. Anderson Scholarship.

Financial or product assistance for research was provided by:

- AgVax Developments Ltd.
- Aspiring Animal Services.
- Bayer NZ Ltd.
- C.Alma Baker Trust.
- Coopers Animal Health Ltd.
- Dow Elanco NZ Ltd.
- Farmers Mutual Group.
- Gallagher Electronics NZ Ltd
- Game Industry Board.
- Merck Sharp and Dohme NZ.
- The Ravensdown Corporation.
- The New Zealand Veterinary Association Deer Branch Award.
- Wiremakers NZ Ltd.
- Wrightsons Nutrition.

Finally I would like to thank my family, Craig Thompson, my flatmates and working companions Joe and Huntly for their unconditional support during the last four years.



## TABLE OF CONTENTS

ABSTRACT.....	i
ACKNOWLEDGEMENTS.....	iv
LIST OF FIGURES.....	vi
LIST OF TABLES.....	viii
LIST OF PLATES.....	xi
LIST OF ABBREVIATIONS.....	xii
CHAPTER 1: Review of Literature.....	1
1.1. THE NEW ZEALAND DEER INDUSTRY: ORIGINS AND HISTORY.....	1
1.1.1. Introduction of Deer to New Zealand.....	1
1.1.2. The History of the New Zealand Deer Industry.....	1
1.2. THE NEW ZEALAND FARMED DEER INDUSTRY.....	2
1.2.1. The New Zealand Farmed Deer Population.....	2
1.2.2. The New Zealand Farmed Venison Industry.....	3
1.2.2.1. New Zealand Farmed Venison vs other Ruminant Meats.....	3
1.2.2.2. Venison Production.....	4
1.2.2.3. Venison Marketing.....	5
1.2.3. Velvet Production.....	6
1.2.4. On-Farm Quality Assurance.....	6
1.3. SEASONALITY IN TEMPERATE DEER SPECIES.....	7
1.3.1. Seasonality in Voluntary Food Intake.....	7
1.3.2. Seasonality in Digestion.....	8
1.3.3. Seasonality in Growth.....	8
1.4. FEED REQUIREMENTS OF TEMPERATE DEER SPECIES AND RELATIONSHIP TO SEASONAL FEED SUPPLY.....	9
1.4.1. Annual Feed Requirements of Temperate Deer.....	9
1.4.2. The Seasonality of Pasture Growth in New Zealand.....	11
1.4.3. Matching Deer Feed Requirements with Feed Supply in New Zealand.....	11
1.5. THE USE OF SPECIAL PURPOSE FORAGES FOR VENISON PRODUCTION .....	12
1.5.1. Forage Crops Evaluated for Venison Production in New Zealand.....	12
1.5.1.1. Summer Forage Crops.....	12
1.5.1.2. Winter Forage Crops.....	14
1.5.2. Potential Forage Crops.....	15

1.6. PRINCIPLES OF FORAGE FEEDING VALUE .....	15
1.6.1. Definition of Feeding Value.....	15
1.6.2. Forage Intake by Grazing Animals.....	16
1.6.2.1. Nutritional and Non-Nutritional Components.....	16
1.6.2.2. Grazing Management.....	18
1.6.3. Nutritive Value of Forages.....	19
1.6.3.1. Forage Chemical and Physical Composition.....	19
1.6.3.2. Digestibility and Site of Digestion.....	21
1.6.3.3. Efficiency of Utilisation of Digested Nutrients.....	24
1.6.4. Differences Between Forages in Feeding Value .....	25
1.6.5. Measuring Voluntary Food Intake in the Grazing Animal .....	25
1.6.5.1. Animal Techniques.....	25
1.6.5.1.1. Chromium Oxide .....	25
1.6.5.1.2. Alkanes.....	26
1.6.5.2. Sward Technique .....	27
1.7. EFFECT OF CONDENSED TANNINS IN TEMPERATE FORAGES ON FEEDING VALUE .....	29
1.7.1. Introduction to Condensed Tannins.....	29
1.7.2. Condensed Tannins and Protein and Carbohydrate Digestion.....	31
1.7.2.1. Protein Digestion .....	31
1.7.2.2. Carbohydrate Digestion.....	32
1.7.3. Condensed Tannins and Animal Production.....	32
1.7.4. The Effect of Condensed Tannins in the Diets of Deer.....	33
1.8. INTERNAL PARASITISM OF TEMPERATE DEER SPECIES.....	36
1.8.1. Lungworm ( <i>Dictyocaulus viviparus</i> ).....	36
1.8.1.1. Epidemiology of Lungworm Infections.....	36
1.8.1.2. Clinical Signs and Pathogenicity of Lungworm Infections .....	39
1.8.1.3. Diagnosis of Lungworm Infections .....	39
1.8.2. Gastrointestinal Parasite Infections .....	39
1.8.2.1. Epidemiology of GI Nematode Infections .....	40
1.8.2.2. Clinical Signs and Pathogenicity of GI Nematode Infections.....	42
1.8.2.3. Diagnosis of GI Nematode Infections.....	44
1.8.3. Susceptibility of Farmed Deer to Internal Parasitism.....	44
1.8.4. Control of Internal Parasites in Farmed Deer.....	45
1.8.4.1. Anthelmintic Efficacy and Use.....	46

1.8.4.2. Grazing Strategies.....	48
1.9. THE USE OF FORAGE CROPS TO CONTROL INTERNAL PARASITES OF RUMINANTS.....	49
1.9.1. Plant Morphology and “Safe Crops”.....	49
1.9.2. Condensed Tannins and Internal Parasites.....	50
1.10. CONCLUSION AND REQUIREMENTS FOR FURTHER RESEARCH.....	52

CHAPTER 2: Growth and carcass production of young farmed deer grazing sulla ( <i>Hedysarum coronarium</i> ), chicory ( <i>Cichorium intybus</i> ) or perennial ryegrass ( <i>Lolium perenne</i> )/white clover ( <i>Trifolium repens</i> ) pasture.....	55
2.1. ABSTRACT.....	56
2.2. INTRODUCTION.....	56
2.3. MATERIALS AND METHODS.....	57
2.3.1. Trial Design.....	57
2.3.2. Forages.....	58
2.3.3. Animals.....	58
2.3.4. Grazing Management.....	59
2.3.5. Forage Sampling and Measurements.....	59
2.3.6. Voluntary Feed Intake.....	59
2.3.7. Slaughter.....	60
2.3.8. Laboratory Analysis.....	60
2.3.9. Calculations and Data Analysis.....	60
2.4. RESULTS.....	61
2.4.1. Herbage Mass and Botanical Composition.....	61
2.4.2. Nutritive Value of Forages.....	65
2.4.3. Voluntary Feed Intake.....	67
2.4.4. Liveweight and Liveweight Gain.....	67
2.4.5. Carcass Production.....	69
2.4.6. Velvet Antler Production.....	69
2.5. DISCUSSION.....	71

CHAPTER 3: Effects of reducing anthelmintic input upon growth and faecal egg and larval counts in young farmed deer grazing chicory ( <i>Cichorium intybus</i> ) and perennial ryegrass ( <i>Lolium perenne</i> )/white clover ( <i>Trifolium repens</i> ) pasture.....	75
3.1. ABSTRACT.....	75

3.2. INTRODUCTION.....	76
3.3. MATERIALS AND METHODS .....	78
3.3.1. Experimental Design.....	78
3.3.2. Forages .....	78
3.3.3. Animals .....	79
3.3.4. Grazing Management.....	79
3.3.5. Forage Sampling and Measurements .....	81
3.3.6. Voluntary Feed Intake.....	81
3.3.7. Slaughter Procedure.....	81
3.3.8. Laboratory Analysis.....	81
3.3.9. Calculations and Data Analysis.....	82
3.4. RESULTS .....	82
3.4.1. Herbage Mass and Botanical Composition .....	82
3.4.2. Nutritive Value and Chemical Composition of the Diet Selected.....	84
3.4.3. Liveweight Gain and Anthelmintic Treatment.....	84
3.4.4. Voluntary Feed Intake.....	88
3.4.5. Carcass Production .....	88
3.4.6. Velvet Antler Production .....	90
3.4.7. Serum Pepsinogen.....	90
3.4.8. Faecal Egg and Larval Counts.....	91
3.5. DISCUSSION .....	94

CHAPTER 4: Development of a model for study of lungworm (*Dictyocaulus* sp.) and gastrointestinal nematode infection in red deer (*Cervus elaphus*)..... 98

4.1. ABSTRACT.....	98
4.2. INTRODUCTION .....	99
4.3. MATERIALS AND METHODS .....	100
4.3.1. Experimental Design.....	101
4.3.2. Animals and Housing.....	101
4.3.3. Infective Larvae .....	102
4.3.4. Voluntary Feed Intake.....	104
4.3.5. Blood Sampling, Serum Biochemistry and Haematology .....	104
4.3.6. Euthanasia and Organ Processing.....	105
4.3.7. Parasitology.....	105
4.3.7.1. Faeces .....	105

4.3.7.2. Lungs.....	106
4.3.7.3. Abomasum.....	106
4.3.7.4. Small and Large Intestines .....	107
4.3.8. Dietary Chemical Analyses .....	107
4.3.9. Statistical Analyses.....	107
4.4. RESULTS .....	108
4.4.1. Diet.....	108
4.4.2. Investigation of Infection Rate .....	108
4.4.2.1. Voluntary feed intake and liveweight .....	108
4.4.2.2. Faecal egg and larval counts.....	111
4.4.2.3. Serum biochemistry .....	114
4.4.2.4. Haematology.....	118
4.4.2.5. Nematode counts .....	122
4.4.3. Rearing Method Comparison .....	127
4.5. DISCUSSION .....	137

CHAPTER 5: Apparent digestibility and nitrogen retention of weaner deer fed lucerne chaff and infected with lungworm (*Dictyoacaulus* sp.) and gastrointestinal nematodes. 147

5.1. ABSTRACT.....	148
5.2. INTRODUCTION.....	148
5.3. MATERIALS AND METHODS .....	149
5.3.1. Experimental Design.....	149
5.3.2. Animals and Housing.....	150
5.3.3. Apparent Digestibility and Nitrogen Balance .....	150
5.3.4. Digesta Flow.....	151
5.3.5. Dietary Chemical Analyses .....	152
5.3.6. Statistical Analyses .....	152
5.4. RESULTS .....	152
5.5. DISCUSSION .....	156

CHAPTER 6: Effect of forage legumes containing different concentrations of condensed tannins on establishment of internal parasites in young red deer. .... 158

6.1. ABSTRACT.....	158
6.2. INTRODUCTION.....	159
6.3. MATERIALS AND METHODS .....	160

6.3.1. Experimental Design.....	160
6.3.2. Animals and Housing.....	161
6.3.3. Forages.....	162
6.3.4. Culture and Administration of Infective Larvae.....	163
6.3.5. Blood Sampling, Serum Biochemistry and Haematology .....	163
6.3.6. Slaughter .....	164
6.3.7. Parasitology .....	164
6.3.8. Dietary Chemical Analyses .....	165
6.3.9. Statistical Analyses .....	166
6.4. RESULTS .....	167
6.4.1. Diet.....	167
6.4.2. Voluntary Feed Intake and Liveweight.....	169
6.4.3. Carcass Data .....	172
6.4.4. Faecal Egg and Larval Counts.....	172
6.4.5. Serum Biochemistry.....	175
6.4.6. Haematology.....	175
6.4.7. Nematode Counts at Slaughter .....	181
6.5. DISCUSSION .....	182
CHAPTER 7: General Discussion .....	190
7.1. INTRODUCTION.....	190
7.2. INTERNAL PARASITISM IN DEER.....	190
7.2.1. Quantifying Sub-clinical Effects of Internal Parasitism in Deer.....	191
7.2.2. The Effects of Internal Parasitism on Growth and Carcass Production.....	191
7.2.3. Diagnosis of Sub-Clinical Parasitism in Deer.....	192
7.3. FORAGES FOR CONTROL OF INTERNAL PARASITES IN DEER .....	196
7.3.1. Effect of Plant Morphology and Sward Structure on Internal Parasites .....	197
7.3.2. Plant Chemical Composition.....	198
7.4. CONCLUSIONS .....	201
LIST OF REFERENCES.....	203
APPENDICES .....	236

## LIST OF FIGURES

<b>Figure 1.1.</b> The New Zealand deer herd ( $\times 10^3$ ) over the last decade.	2.
<b>Figure 1.2.</b> Mean monthly dry matter intake (DMI) of hinds and stags fed indoors for one year.	7.
<b>Figure 1.3.</b> Mean monthly live weight of hinds and stags fed indoors for one year.	9.
<b>Figure 1.4.</b> Life cycle of <i>Dictyocaulus viviparus</i> in red deer.	38.
<b>Figure 1.5.</b> Typical life cycle of trichostrongylid gastrointestinal parasites in red deer.	42.
<b>Figure 3.1.</b> Three-weekly mean (+SE) daily liveweight gain (LWG) of deer grazing PRG/WC pasture (P) or chicory (C) and treated with anthelmintic 3-weekly (T) or trigger-treated (TT).	85.
<b>Figure 3.2.</b> Mean (+SE) faecal egg counts of deer grazing PRG/WC pasture (P) or chicory (C) and treated with anthelmintic 3-weekly (T) or trigger-treated (TT).	92.
<b>Figure 3.3.</b> Mean (+SE) faecal larval counts of deer grazing PRG/WC pasture (P) or chicory (C) and treated with anthelmintic 3-weekly (T) or trigger-treated (TT).	93.
<b>Figure 4.1.</b> Mean (+SE) voluntary feed intake.	109.
<b>Figure 4.2.</b> Mean (+SE) liveweight.	110.
<b>Figure 4.3.</b> Mean (+SE) faecal egg counts.	112.
<b>Figure 4.4.</b> Geometric mean (+SE) faecal lungworm larval count.	113.
<b>Figure 4.5.</b> Mean (+SE) serum total protein, albumin and globulin concentration	115.
<b>Figure 4.6.</b> Mean (+SE) serum pepsinogen concentration.	116.
<b>Figure 4.7.</b> Mean (+SE) serum gastrin concentration.	117.
<b>Figure 4.8.</b> Mean (+SE) total leucocyte (WBC) and erythrocyte (RBC) counts.	119.
<b>Figure 4.9.</b> Mean (+SE) lymphocyte, neutrophil and monocyte counts.	120.
<b>Figure 4.10.</b> Mean (+SE) peripheral eosinophil counts.	121.
<b>Figure 4.11.</b> Mean (+SE) faecal egg counts of artificially (AR) and naturally (NR) reared deer.	129.
<b>Figure 4.12.</b> Geometric mean (+SE) faecal lungworm larval counts of artificially (AR) and naturally (NR) reared deer.	130.

<b>Figure 4.13.</b> Mean (+SE) serum total protein, albumin and globulin concentration of artificially (AR) and naturally (NR) reared deer.	132.
<b>Figure 4.14.</b> Mean (+SE) serum pepsinogen concentration of artificially (AR) and naturally (NR) reared deer.	133.
<b>Figure 4.15.</b> Mean (+SE) serum gastrin concentration of artificially (AR) and naturally (NR) reared deer.	134.
<b>Figure 4.16.</b> Mean (+SE) total leucocyte (WBC) and erythrocyte (RBC) counts of artificially (AR) and naturally (NR) reared deer.	135.
<b>Figure 4.17.</b> Mean (+SE) lymphocyte, neutrophil and monocyte counts of artificially (AR) and naturally (NR) reared deer.	136.
<b>Figure 4.18.</b> Mean daily VFI vs daily liveweight gain for each group following start of infection.	138.
<b>Figure 4.19.</b> Group mean pre-euthanasia serum pepsinogen concentration vs group mean GI counts.	141.
<b>Figure 4.20.</b> Pre-euthanasia individual serum pepsinogen concentration vs GI nematode count.	142.
<b>Figure 4.21.</b> Pre-euthanasia peripheral eoinophil count vs GI nematode count.	143.
<b>Figure 4.22.</b> Pre-euthanasia peripheral eosinophil count vs lungworm count.	144.
<b>Figure 4.23.</b> Pre-euthanasia individual serum gastrin concentration vs GI nematode count.	145.
<b>Figure 5.1.</b> Nitrogen intake vs nitrogen retention.	154.
<b>Figure 6.1.</b> Mean (+SE) voluntary feed intake of deer fed lucerne (L), birdsfoot trefoil (B) or sulla (S).	170.
<b>Figure 6.2.</b> Mean (+SE) liveweight of deer fed lucerne (L), birdsfoot trefoil (B) or sulla (S).	171.
<b>Figure 6.3.</b> Mean (+SE) faecal egg counts of deer fed lucerne (L), birdsfoot trefoil (B) or sulla (S).	173.
<b>Figure 6.4.</b> Mean (+SE) faecal lungworm larval counts of deer fed lucerne (L), birdsfoot trefoil (B) or sulla (S).	174.
<b>Figure 6.5.</b> Mean (+SE) serum total protein, albumin and globulin concentration of deer fed lucerne (L), birdsfoot trefoil (B) or sulla (S).	176.
<b>Figure 6.6.</b> Mean (+SE) serum pepsinogen concentration of deer fed lucerne (L), birdsfoot trefoil (B) or sulla (S).	177.



<b>Figure 6.7.</b> Mean (+SE) serum gastrin concentration of deer fed lucerne (L), birdsfoot trefoil (B) or sulla (S).	178.
<b>Figure 6.8.</b> Mean (+SE) peripheral eosinophil counts of deer fed lucerne (L), birdsfoot trefoil (B) or sulla (S).	179.
<b>Figure 6.9.</b> Mean (+SE) blood haemoglobin concentration of deer fed lucerne (L), birdsfoot trefoil (B) or sulla (S).	180.
<b>Figure 6.10.</b> Group mean number of abomasal worms at slaughter as a function of forage condensed tannin concentration.	183.
<b>Figure 6.11.</b> Group mean number of abomasal <i>T.axei</i> at slaughter as a function of forage condensed tannin concentration.	183.
<b>Figure 6.12.</b> Group mean pre-slaughter faecal larval count as a function of forage condensed tannin concentration.	184.
<b>Figure 6.13.</b> Lungworm recovered at slaughter vs pre-slaughter faecal larval count.	188.
<b>Figure 6.14.</b> GI nematodes recovered at slaughter vs pre-slaughter faecal egg count.	188.
<b>Figure 7.1.</b> Gastrointestinal nematode count at slaughter vs lungworm count at slaughter for Chapters 4 & 6 combined.	195.

## LIST OF TABLES

<b>Table 1.1.</b> New Zealand farmed deer slaughter statistics.	3.
<b>Table 1.2.</b> The composition of uncooked lean meat.	4.
<b>Table 1.3.</b> Seasonal ME requirement and target liveweight of red deer.	10.
<b>Table 1.4.</b> Daily liveweight gain (g/day) for young red deer stags grazing perennial ryegrass/white clover pasture (PRG/WC), red clover (R), chicory (C) or birdsfoot trefoil (B) in summer, autumn and spring and PRG/WC in winter.	13.
<b>Table 1.5.</b> Effect of species, plant component and maturity on the chemical composition of pasture (% dry matter).	21.
<b>Table 1.6.</b> Concentrations (g/kgDM) of extractable (ECT), protein-bound (PCT), fibre-bound (FCT) and total condensed tannins (TCT) in a range of whole plants.	30.
<b>Table 1.7.</b> Helminth parasites identified from temperate deer species worldwide.	37.
<b>Table 1.8.</b> Percentage of weaner deer shedding faecal parasite eggs and lungworm larvae, and mean and range of geometric means (larvae/g) of positive faecal larval counts 1992-1994.	43.

<b>Table 1.9.</b> Mean number of annual anthelmintic treatments by farm and age/sex group.	48.
<b>Table 2.1.</b> Seasonal pre- and post-grazing herbage mass (kgDM/ha $\pm$ SE) of perennial ryegrass/white clover pasture, chicory or sulla grazed deer.	62.
<b>Table 2.2.</b> Seasonal botanical composition (%DM $\pm$ SE) of perennial ryegrass/white clover pasture, chicory or sulla on offer.	64.
<b>Table 2.3.</b> Chemical composition (mean $\pm$ SE) of forage on offer and diet selected by deer grazing either perennial ryegrass/white clover pasture, chicory or sulla.	66.
<b>Table 2.4.</b> Condensed tannin concentration (%DM $\pm$ range) of diet selected by deer grazing either perennial ryegrass/white clover pasture, chicory or sulla.	67.
<b>Table 2.5.</b> Voluntary feed intake (gOM/d), liveweight (kg) and liveweight gain (g/d) of deer grazed on perennial ryegrass/white clover pasture, chicory or sulla.	68.
<b>Table 2.6.</b> Carcass production of red and hybrid stags grazed on perennial ryegrass/white clover pasture, chicory or sulla.	70.
<b>Table 2.7.</b> Velvet antler production from red and hybrid yearling stags grazing either perennial ryegrass/white clover pasture, chicory or sulla.	71.
<b>Table 3.1.</b> Seasonal pre- and post-grazing herbage mass (kgDM/ha $\pm$ SE) of perennial ryegrass/white clover pasture or chicory.	83.
<b>Table 3.2.</b> Seasonal chemical composition (%DM $\pm$ SE) of diet selected by deer grazing either perennial ryegrass/white clover pasture or chicory.	84.
<b>Table 3.3.</b> Seasonal voluntary feed intake (gOM/d), liveweight (kg) and liveweight gain (g/d) of deer grazed on perennial ryegrass/white clover pasture or chicory and treated with anthelmintic 3-weekly or trigger-treated.	87.
<b>Table 3.4.</b> Carcass measurements of stags and hinds, treated (T) or trigger-treated (TT) with anthelmintic and grazed on perennial ryegrass/white clover pasture or chicory.	89.
<b>Table 3.5.</b> Serum pepsinogen concentration (mU tyrosine/l $\pm$ SE) from deer grazing either perennial ryegrass/white clover pasture or chicory and treated with anthelmintic 3-weekly (T) or trigger-treated (TT).	90.
<b>Table 4.1.</b> Experimental design.	101.
<b>Table 4.2.</b> Sequence of experimental events.	101.
<b>Table 4.3.</b> Mean ( $\pm$ SE) number of lung and gastrointestinal nematodes recovered from deer experimentally infected at three different levels of infection with nematode larvae and control deer.	123.

<b>Table 4.4.</b> Species of nematodes identified from deer artificially infected with nematode larvae, expressed as a proportion (%) of the total number of identifiable nematodes counted.	126.
<b>Table 4.5.</b> Liveweight, VFI and numbers of lung and gastrointestinal nematodes (mean $\pm$ SE) recovered from artificially and naturally reared deer infected with nematode larvae.	127.
<b>Table 5.1.</b> Experimental design.	149.
<b>Table 5.2.</b> Chemical composition (%DM $\pm$ SE) of chaffed lucerne hay fed.	153.
<b>Table 5.3.</b> Apparent digestibility (%) of DM, OM, gross energy, ADF, NDF, cellulose, hemicellulose and lignin in deer fed lucerne chaff and infected with zero (C), low (LT), medium (MT) and high (HT) levels of nematode larvae.	153.
<b>Table 5.4.</b> Apparent N digestibility, N-retention and flow of N measured at the abomasum and terminal ileum, in g/d and expressed as a percentage of N intake, in deer fed lucerne chaff and infected with zero (C), low (LT), medium (MT) and high (HT) levels of nematode larvae.	155.
<b>Table 6.1.</b> Experimental design.	160.
<b>Table 6.2.</b> Sequence of experimental events.	161.
<b>Table 6.3.</b> Dry matter (%) and chemical composition (%DM $\pm$ SE) of fresh forage fed to weaner red deer housed indoors and fed chicory (adjustment period), lucerne, birdsfoot trefoil or sulla.	168.
<b>Table 6.4.</b> Group mean ( $\pm$ SE) VFI and LWG of deer fed lucerne, birdsfoot trefoil or sulla during infection with internal parasites (weeks 1-7), carcass weight (CW; with and without initial liveweight as a covariate) and carcass dressing out percentage (DR%) at slaughter.	172.
<b>Table 6.5.</b> Lung and gastrointestinal nematodes (mean $\pm$ SE) recovered from weaner red deer fed lucerne, birdsfoot trefoil or sulla.	182.
<b>Table 7.1.</b> Growth (g/d) of red (R) and hybrid (H) stags to one year of age grazing perennial ryegrass/white clover pasture, red clover, chicory and sulla, and feeding value of forages relative to perennial ryegrass/white clover pasture.	197.

## LIST OF PLATES

<b>Plate 2.1.</b> Perennial ryegrass/white clover pasture grazed in early spring.	62.
<b>Plate 2.2.</b> Chicory grazed in early spring.	63.
<b>Plate 2.3.</b> Sulla grazed in early spring.	63.
<b>Plate 3.1.</b> Method of paddock bisection using a five-strand electric fence. This shows winter grazing with TT groups on the left and T groups on the right.	80.
<b>Plate 4.1.</b> Artificial rearing of deer calves using ewe milk replacer.	111.
<b>Plate 4.2.</b> Spicules of <i>Spiculopteria assymetrica</i> from the abomasum.	118.
<b>Plate 4.3.</b> Spicules of <i>Skryabinagia kolchida</i> from the abomasum.	124.
<b>Plate 4.4.</b> Spicules of <i>Ostertagia leptospicularis</i> (a morph of <i>S.kolchida</i> ) from the abomasum.	124.
<b>Plate 4.5.</b> Spicules of <i>Ostertagia circumcincta</i> from the abomasum.	125.
<b>Plate 4.6.</b> Spicules of <i>Cooperia punctata</i> from the small intestine	125.

## LIST OF ABBREVIATIONS AND CODES

\$	New Zealand dollars	<i>et al.</i> ,	and others
%	percentage	Exp.	experiment
°C	degrees Celsius	Eqn.	equation
/ (g)	per (per gram)	FEC	faecal egg count /g faeces
AA	amino acids	FCT	fibre-bound CT
ADF	acid detergent fibre	Fig.	figure
ANOVA	analysis of variance	FLC	faecal lungworm larval count /g faeces
BCT	bound CT	FO	faecal output
B	birdsfoot trefoil	FrCT	free CT
BW	body weight	FV	feeding value
<i>C.</i>	<i>Cooperia</i>	g	gram
C	chicory	GE	gross energy
Cm	centimetre	GI	gastro-intestinal
CP	crude protein	GIB	Game Industry Board
Cr	chromium	GR	soft tissue depth over 12th rib at a point 16cm from carcass midline
CT	condensed tannin	GT	grazing time
CW	carcass weight	h	head (animal)
<i>D.</i>	<i>Dictyocaulus</i>	<i>H.</i>	<i>Haemonchus</i>
D	digestibility	hr (s)	hour (s)
d	day	Ha	hectare
DLWG	daily liveweight gain	Hb	haemoglobin (g/l)
DM	dry matter	HI	high
DMI	dry matter intake	HT	hydrolysable tannins
DSP	deer slaughter premises	IB	intake per bite
EAA	essential amino acids	iu (IU)	international units
ECT	extractable CT	K <sub>f</sub>	efficiency of utilization of ME for fattening
epg	eggs per gram faeces	K <sub>g</sub>	efficiency of utilization of ME for growth

K <sub>l</sub>	efficiency of utilization of ME for lactation	NS	not statistically significant at p<0.05
K <sub>m</sub>	efficiency of utilization of ME for maintenance	NV	nutritive value
kg	kilograms	NZ	New Zealand
KJ	kilojoule	<i>O.</i>	<i>Ostertagia</i>
L	litres	<i>Oe.</i>	<i>Oesophagostomum</i>
L <sub>3</sub>	third stage larvae (infective stage)	OM	organic matter
lpg	larvae per gram faeces	OMD	organic matter digestibility
Ltd	Limited	OMI	organic matter intake
L	lucerne ( <i>Medicago sativa</i> )	P	probability statistic
LW	liveweight	pers. comm.	personal communication
LWG	liveweight gain	PCT	protein-bound CT
m	metres	PCV	packed cell volume (l/l)
ME	metabolizable energy	PEG	polyethylene glycol
MED (med)	medium	PRG	perennial ryegrass
ME <sub>g</sub>	ME for growth	R	red clover
MEI	ME intake	RB	rate of biting
ME <sub>m</sub>	ME for maintenance	RBC	red blood cell count (10 <sup>12</sup> /l)
mg	milligram	RFC	readily fermentable carbohydrate
MJ	megajoule	rpm	revolutions per minute
ml	millilitres	SAA	sulphur AA
mm	millimetres	SE	standard error
MRT	rumen mean retention time	SI	small intestine
mU	milli-international enzyme unit	t	tonne
MW	molecular weight	<i>T.</i>	<i>Trichostrongylus</i>
N	nitrogen	TCT	total CT
NAN	non-ammonia nitrogen	STP	serum total protein (g/l)
nd	not determined	µg	microgram
NDF	neutral detergent fibre	UK	United Kingdom

μl	microlitre	VFI	voluntary feed intake
USA	United States of America	WBC	white blood cell count (10 <sup>9</sup> /l)
VFA	volatile fatty acids	WC	white clover

# CHAPTER 1.

## Review of Literature

### 1.1. THE NEW ZEALAND DEER INDUSTRY: ORIGINS AND HISTORY

#### 1.1.1. Introduction of Deer to New Zealand

Eight species of deer were introduced to New Zealand (NZ) between 1861 and 1910 and successfully established in the wild (Challies, 1985). Red deer (majority *Cervus elaphus scoticus*) were the most commonly introduced species, originating from a variety of English parks and the Scottish highlands. The other introduced deer were fallow (*Dama dama*), North American wapiti (or elk; *Cervus elaphus canadensis*), moose (*Alces alces andersoni*), sambar (*Cervus u. unicolor*), rusa (*Cervus timorensis rusa*), sika (*Cervus nippon*) and white-tailed (*Odocoileus virginianus borealis*). Although moose have not been reliably reported since 1952 (Tustin, 1974) all other introduced deer species still exist in the wilds of NZ. In 1983 the rare Pere Davids deer (*Elaphurus davidianis*) and in 1985 the rare Mesopotamian fallow deer were introduced to NZ, but are confined to farms (Otway, 1992a, b).

The North American wapiti were released in Fiordland (south-west NZ). Drew and Hogg (1990) and Fennessy and Pearce (1990) reported that cross-breeding between the North American wapiti and red deer has occurred in the wild in this area, producing a high proportion of the feral Fiordland herd, now known as NZ wapiti (NZW). NZW have on average 56% of pure wapiti genes (Drew and Hogg, 1990).

Deer numbers increased rapidly due to the ideal environmental conditions and in the 1920's-30's wild deer became pests of economic importance, damaging forests and high-country watersheds and contributing to erosion. Large-scale deer control operations began in 1931 and in 1956 deer were classified as noxious pests (Challies, 1985).

#### 1.1.2. The History of the New Zealand Deer Industry

Skins were the main saleable products obtained from wild deer until the early 1960's. The NZ venison industry originated about 1959 when substantial markets for wild venison of all cuts were established in Europe, notably West Germany. Deer were killed in the wild, collected largely by helicopter and transported to processing companies for processing and export as frozen product. There were few controls on the handling of



carcasses until the introduction of the Game (packing and export) Regulations 1967, which was replaced by the stricter Game Regulations (1975) which still apply today.

Venison produced from the wild peaked at about 4300 tonnes/annum in 1972 representing 130,000 carcasses and then rapidly declined, due to the depletion of feral deer (Spiers, 1987). This reduction in a lucrative form of game meat production stimulated the commencement of commercial deer farming in NZ, to produce a regular supply of deer products under controlled conditions (Barry and Wilson, 1994).

The farming of deer behind fences became legal in NZ in 1969 through the Noxious Animals in Captivity Regulations and the Deer Farming Regulations and the first commercial deer farm was set up in 1970 (Yerex, 1982). After a slow start, interest in deer farming increased rapidly from the mid-1970's, and by 1983 there were about 240,000 deer on farms (Challies, 1985).

## 1.2. THE NEW ZEALAND FARMED DEER INDUSTRY

### 1.2.1. The New Zealand Farmed Deer Population

Figure 1.1. The New Zealand deer herd ( $\times 10^3$ ) over the last decade. (GIB, 1997)

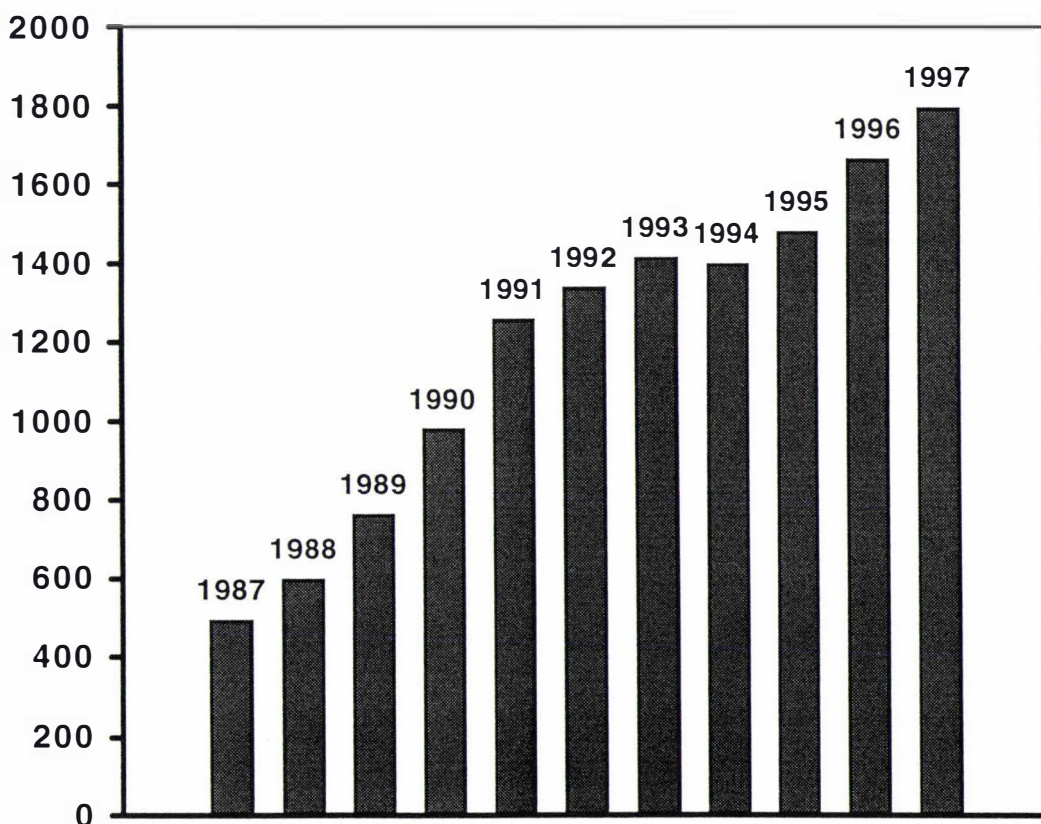


Figure 1.1. shows the NZ farmed deer population markedly increased from about 0.5 million deer in 1987 to about 1.4 million deer in 1992. The population remained between 1.4 and 1.5 million from 1992 to 1995, but has increased more rapidly during the past two years. In 1993, approximately 85% of the recorded farmed deer population were pure red deer, 10% were wapiti or wapiti-red hybrids, and 5% were fallow (Guild, 1993).

### 1.2.2. The New Zealand Farmed Venison Industry

Table 1.1. shows the NZ farmed deer slaughter statistics from 1990 to 1997.

**Table 1.1. New Zealand farmed deer slaughter statistics.**

	Annual Season (1 October year one- 30 September year two)					
	90/91	92/93	93/94	94/95	95/96	96/97
<b>All Deer</b> (000's)	203	418	351	408	309	313
<b>Carcass Weight</b> (tonnes)	10,200	21,800	18,000	20,300	16,400	18,500
<b>Average kg/hd</b>	22	52	51	50	53	59

Game Industry Board (pers.comm.).

Export earnings for the deer industry peaked during the year to September 1996, reaching NZ\$221.8 million, of which venison contributed NZ\$141.9 million. For the year ending September 1997 export earnings were NZ\$201.3 million, of which venison contributed NZ\$135.9 million (GIB, 1997).

#### 1.2.2.1. New Zealand Farmed Venison vs other Ruminant Meats

In comparison with other farmed ruminants, deer have a superior carcass weight to liveweight ratio (dressing out percentage, DR%) with DR% of mature, pasture-fed stags (59%) being higher than for young sheep or cattle (40-50%, Drew, 1985). Carcass muscle groups which make up the high-priced cuts (hind-leg and saddle) are proportionately 8-23% heavier in deer than in cattle, while muscles around the rib-cage and shoulder are less well developed in the deer (Berg and Butterfield, 1976). However, the major advantage of venison is that young deer carcasses contain 50-80% less fat than sheep and cattle (Drew, 1985). Deer have a greater potential to produce lean meat than sheep or cattle and one kilogram (kg) of carcass gain in young stags comprises 0.23kg fat compared with 0.41kg fat/kg gain in ram lambs (Fennessy *et al.*, 1982).

Table 1.2. shows the composition of venison in comparison to beef and lamb, with venison being high in protein and iron, but low in fat and energy. Thus venison is the ideal meat for today's health conscious consumer.

**Table 1.2. The composition of uncooked lean meat.**

(values are expressed per 100g fresh meat)

	<b>Energy</b>	<b>Moisture</b>	<b>Protein</b>	<b>Fat</b>	<b>Ash</b>	<b>Iron</b>
	(kJ)	(g)	(g)	(g)	(g)	(mg)
<b>Beef loin</b>	534	72.6	22.0	4.3	1.0	2.3
<b>Lamb loin</b>	627	71.9	20.8	7.4	1.0	1.3
<b>Venison loin</b>	545	70.8	24.7	3.3	1.4	3.8

Adapted from Seman and McKenzie-Parnell (1989)

#### 1.2.2.2. Venison Production

Deer appear to differ markedly from traditional domesticated species in that body fat (and some protein) is largely mobilised during the autumn rut and there is little capacity for replenishment during the winter, irrespective of feeding level (Drew and Suttie, 1982, see section 1.3.). Older stags can lose up to 25% of their body weight during the rut (Stevenson *et al.*, 1992). However, during spring and summer, fat deposition occurs in a similar way to that in sheep and cattle and overfatness can be a problem in red deer if the animals are slaughtered in summer at more than two years of age.

An objective of any meat production system is the production of a desired carcass weight in the shortest possible period of time (Barry and Wilson, 1994). Most NZ farmers specialising in venison production produce stags for slaughter at an age of 12-24 months (Barry and Wilson, 1994) or even longer (15-27 months; Drew, 1985). However, it is economically preferable to produce carcass weights of 50-65kg at one year of age or less (during August-November), coinciding with peak market demands and high sale price (see section 1.2.2.3.).

Because of the strong seasonality of voluntary feed intake (VFI) and liveweight gain (LWG; see section 1.3.) in stags and to a lesser extent in hinds, optimum carcass weights (minimum standard 50kg carcass or 92kg LW) for efficient slaughter systems are not easily achievable in red deer before 12 months of age. Options for achieving the minimum 50kg carcass weight at 10-12 months include the use of large deer species such

as Canadian or NZ wapiti (see section 1.1.1.). These deer species crossed with red deer to produce F1 hybrids can be used directly for venison production, or more commonly the F1 stags can be used as terminal sires over red hinds to produce F2 offspring (0.75 red: 0.25 wapiti deer) for venison production (Drew and Hogg, 1990; Fennessy and Pearce, 1990; Pearce, 1992). In productive terms, wapiti or their hybrids are larger, faster growing, and leaner at the same age than pure red deer (Drew and Hogg, 1990).

Another option is growing pasture species that produce high dry matter (DM) and have a high feeding value (FV; see section 1.6.) during summer-autumn or winter, when conventional perennial ryegrass/white clover (PRG/WC) pastures are unable to provide the quantity and quality of feed required for good deer growth rates (see section 1.5.).

### **1.2.2.3. Venison Marketing**

NZ farmed venison comes under the classification of 'game' as opposed to 'meat' and is therefore slaughtered and processed at specialist Deer Slaughter Premises (DSP's) which do not handle other ruminant or animal species. NZ venison is mainly vacuum packed when transported as a chilled product (Drew, 1992).

With the formation of the NZ Game Industry Board (GIB) in 1984, the NZ deer industry has been strongly export market-led. Key aspects of the market-led approach have been to produce and market venison which is consistently tender and of low fat content and at the carcass weight range and times dictated by the market (Barry and Wilson, 1994). The industry has a strategic market plan to coordinate all sectors in order to maintain profitability which has been implemented and financed by the GIB, through the collection of compulsory levies on sales of venison and velvet (see section 1.2.3.).

The GIB has developed an appellation marketing strategy for NZ farm-raised venison (Syme, 1993). The ZEAL™ quality mark is used to identify chilled and frozen NZ venison destined for the existing, high volume, wide product mix European market. The premium CERVENA® brand is used for chilled 'NZ natural tender venison' to develop high value new markets including the USA, Canada, Australia and NZ. CERVENA® specifications dictate that only the venison from carcasses of 50-65kg from farmed animals under three years of age can carry the CERVENA® brand. These brands differentiate NZ farm-raised venison from NZ feral-venison and from farmed venison produced by other countries which have variable quality, and allow premium chilled CERVENA® venison to be available at all times of the year. CERVENA® is promoted

in the high value restaurant and hotel trade, with ZEAL™ also available in some European supermarkets.

Venison exporters offer attractive schedule prices based on carcass quality, weight and leanness (as expressed by the GR, or tissue depth measurement over the 13th rib) and these vary according to season, but are always greatest for carcasses in the 50-65kg weight range from animals under three years of age (CERVENA® specifications). The price schedule is highest from September to November as existing markets for chilled venison are driven by demand corresponding to the northern hemisphere autumn, although an all-round supply of chilled product into new markets (i.e. USA) is being developed.

### **1.2.3. Velvet Production**

Velvet antler of stags is grown annually and harvested at an early stage of growth (55-65 days from casting) predominantly for export to South Korea. Removal of antlers from stags also prevents injury to other deer, humans or fences, and ensures safety during transportation. In NZ the velvet season runs from October through until early February for harvest of regrowth. Velvet is harvested under a strict NZMAF code of conduct by veterinarians or certified farmers trained by veterinarians in the use of analgesic drugs. The shape and weight of velvet antler are important criteria of quality, and this is determined by the age and breed of stag, the stage of growth at harvest, nutrition and the care with which the velvet is removed and frozen (Muir and Sykes, 1988).

### **1.2.4. On-Farm Quality Assurance**

A national 'pasture-to-plate' quality assurance scheme (GIB, 1996) is currently being implemented whereby farmers who have gained accreditation will produce animals to specification, transport them using accredited transporters to an accredited DSP, having sold the deer as part of a managed supply programme for export. This makes the industry completely accountable for the product produced and allows the consumer complete assurance of quality, which is guaranteed by the brand. The GIB marketing strategy, presenting NZ deer products as being from a clean, green environment, focuses on natural production systems fulfilling requirements for animal production and wellbeing, and is requiring a re-evaluation of chemical use on farms. In the future it can be expected that farmers will need to accurately record chemical usage according to specifications

requiring minimal chemical use, before venison products can be marketed under the quality-assured CERVENA® name.

### 1.3. SEASONALITY IN TEMPERATE DEER SPECIES

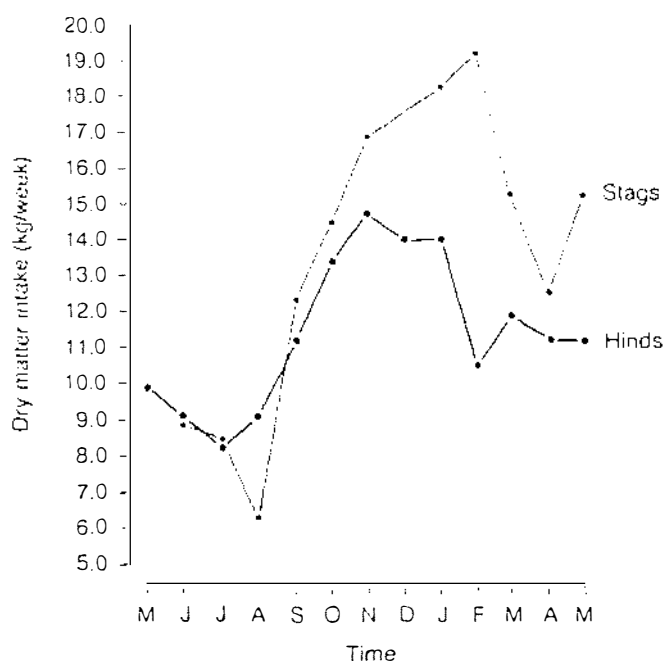
Temperate deer species that evolved in cold temperate regions (ie. red, fallow deer and wapiti) exhibit strong seasonality patterns characterised by pronounced annual cycles of reproduction, voluntary food intake (VFI), growth and rumen digestion. Deer have evolved these endogenous cycles, which are cued or timed to photoperiod by the hormone melatonin.

#### 1.3.1. Seasonality in Voluntary Food Intake

Figure 1.2. shows the seasonality of dry matter intake (DMI) in red deer stags and hinds. Voluntary feed intake (VFI) by red deer (Fennessy *et al.*, 1981; Kay and Staines, 1981; Suttie *et al.*, 1987; Suttie and Corson, 1991) and Canadian wapiti (Watkins and Hudson, 1984) shows marked seasonal trends, being high in the summer and low in the winter, resulting in corresponding fluctuations in body weight and liveweight gain (Adam, 1991). The amplitude of this cycle is greater for stags than hinds, calves and castrates (Kay, 1979), and greater in adult stags than young stags (Pollock, 1975).

**Figure 1.2. Mean monthly dry matter intake (DMI) of hinds and stags fed indoors for one year.**

(Suttie *et al.*, 1987)



The seasonality of VFI is not only observed in the field, but has been found when deer were fed indoors with lucerne hay (Domingue *et al.*, 1991a; Freudenberger *et al.*, 1994a), low quality grass or good quality chopped or pelleted grass (Milne *et al.*, 1978) or grass hay (Sibbald and Milne, 1993).

### **1.3.2. Seasonality in Digestion**

Contrary to what may be expected, red deer can maintain digestibility as VFI increases from winter to summer (Milne *et al.*, 1978; Barry *et al.*, 1991; Domingue *et al.*, 1991a). Lower rumen fractional outflow rate of liquid and particulate matter (ie. increased rumen mean retention time, MRT), higher rumen capacity and pool size, and increased rumen ammonia production in summer compared to winter (Milne, 1980; Barry *et al.*, 1991; Domingue *et al.*, 1991a, b; Freudenberger *et al.*, 1994a) may all be contributing factors to apparent digestibility remaining constant as VFI is increased in summer. Red deer have also been found to have higher nitrogen (N) retention in summer than winter (Domingue *et al.*, 1991b; Freudenberger *et al.*, 1994a). This shows that in red deer there are seasonal cycles in digestive function and in body protein deposition.

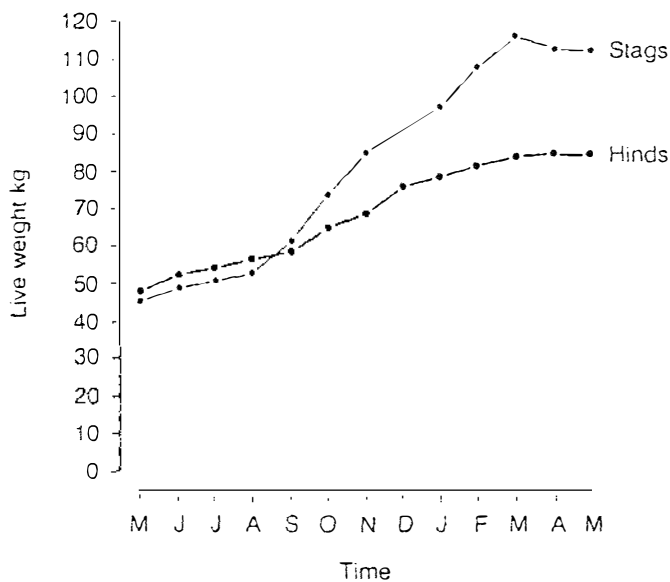
### **1.3.3. Seasonality in Growth**

It is believed that two cycles, one of growth potential and the other of food intake, explain the growth seasonality we see in deer (Suttie and Corson, 1991). However, although the seasonal cycles of growth are functionally and intimately related with those of VFI, they are not strictly identical (Freudenberger *et al.*, 1994a).

Figure 1.3. shows hinds and stags both exhibit seasonality in growth, but as with VFI a lower amplitude of seasonality is observed in hinds than in stags when offered identical diets (Suttie *et al.*, 1987). The growth of young red deer slows greatly during their first winter, even when ample food is provided and then accelerates in spring (Suttie *et al.*, 1983; Adam and Moir, 1985).

**Figure 1.3. Mean monthly liveweight of hinds and stags fed indoors for one year.**

(Suttie *et al.*, 1987)



#### **1.4. FEED REQUIREMENTS OF TEMPERATE DEER SPECIES AND RELATIONSHIP TO SEASONAL FEED SUPPLY**

Temperate deer have evolved a seasonal system of timing their feed requirements and calving pattern in response to variations in available plant resources resulting from a long harsh winter and a short summer in their natural habitat (Barry and Wilson, 1994). While this has enabled red, fallow and wapiti deer to survive conditions at far northern latitudes, it has caused problems in the use of these species for deer farming in milder NZ conditions, where forage production commences in early spring instead of early summer. In NZ calving occurs from mid-November to late-December, which is after the flush of PRG/WC pasture growth in spring (September). In addition, the point of peak lactation of hinds, and high calf growth occurs in the January-February period, when pasture quality can be very poor and pasture growth rates slowed by low rainfall and high summer temperatures (Kemp, 1996).

##### **1.4.1. Annual Feed Requirements of Temperate Deer**

Table 1.3. shows the seasonal ME requirement and target liveweight of red deer. Fennessy *et al.*, (1981) estimated energy requirements for maintenance ( $ME_m$ ) of red deer stags based on a relationship between LWG and metabolisable energy intake (MEI) of



stags fed indoors ( $ME_m = 0.57MJ ME/kg^{0.75}/day$ ), and for stags fed outdoors in winter ( $ME_m = 0.85MJ ME/kg^{0.75}/day$ ). The same authors estimated the  $ME_m$  of stags grazing outdoors at 30, 50, 20 and 10% above that of stags indoors during autumn, winter, spring and summer respectively (ie. 0.74, 0.85, 0.68 and  $0.63MJ ME/kg^{0.75}/day$ ). They estimated ME requirements for growth ( $ME_g = 37MJ ME/kg LWG$ ) and for suckling calves (53MJ ME/kg LWG). Suttie *et al.*, (1987) estimated the  $ME_m$  and  $ME_g$  of red deer hinds penned indoors being  $0.52MJ ME/kg^{0.75}/day$  and 53MJ ME/kg respectively. However, such estimates are fraught with errors introduced by the assumed partitioning of ME intake between maintenance and growth, and must be interpreted with caution.

There are no published evaluations of critical protein requirements by farmed red deer (Adam, 1991) and at present it is assumed that deer requirements (per kgDMI) are similar to those of sheep.

**Table 1.3. Seasonal ME requirement and target liveweight of red deer**

Adapted from Fennessy and Milligan (1987).

Deer age	Target liveweight	Daily ME requirement (MJ ME/head/day)				Annual total ME requirement (MJ ME/head)
		autumn	winter	spring	summer	
Years	(kg)	65d	100d	100d	100d	365d
<b>STAGS</b>						
0.25-1.25	48	16.0	20.9	27.0	26.5	8300
1.25-2.25	105	24.5	28.0	31.5	30.0	10500
2.25-3.25	140	23.5	33.0	38.0	36.2	12200
3.25-4.25	175	19.5	33.0	38.5	38.2	12200
4.25-5.25	190	18.5	34.5	43.5	39.0	12900
>5.25	200	19.0	26.0	42.5	38.0	12900
<b>HINDS</b>						
0.25-1.25	44	15.0	17.5	22.0	21.0	7000
1.25-2.25	83	20.5	23.5	23.5	45.0	10500
2.25-3.25	94	22.5	24.0	47.5	47.5	11000
>3.25	100	23.5	22.5	24.5	47.5	10900

Note: Metabolisable energy requirements have been calculated from the equations given below.

(I) For growing animals, adult stags and non-lactating hinds

$$ME = S\{0.57 LW^{0.75}\} + 37 DLWG$$

where,

ME is metabolisable energy requirement in MJ ME/day

S is the seasonal coefficient; 1.30 for autumn, 1.50 for winter, 1.20 for spring and 1.10 for summer

LW is the liveweight in kg

DLWG is the daily liveweight gain in kg/day

(II) For lactating hinds and their calves at foot

$$ME = S\{0.57 LW^{0.75} \text{ hind}\} + 37 DLWG \text{ hind} + 65 DLWG \text{ calf}$$

where,

DLWG is the daily liveweight gain in kg/day for the hind or calf as indicated

#### **1.4.2. The Seasonality of Pasture Growth in New Zealand**

The seasonality of pasture growth in NZ varies according to geographic region and season. However, under typical NZ pastoral deer farming conditions utilising conventional ryegrass/white clover pasture, a feed surplus usually occurs in early and mid spring on deer farms, whilst a feed deficit occurs in mid to late summer and again in winter.

#### **1.4.3. Matching Deer Feed Requirements with Feed Supply in New Zealand**

The endogenous cycles of temperate deer can be adjusted by changing the photoperiod (Suttie *et al.*, 1992) and by administration of the hormone melatonin (Asher *et al.*, 1988). However, the NZ deer industry has not adopted the commercial use of melatonin to advance the onset of the breeding season (Wilson *et al.*, 1991) to coincide calving with peak pasture production, because of consumer concerns about the use of hormones and the risk to marketing image.

Hybridisation of red deer with other species such as Pere Davids deer (Fennessy and Mackintosh, 1992) and sambar deer (Semiadi, 1995) that have earlier seasonal cycles of reproduction (and hence calving), VFI and growth, offer a potential way of matching deer feed demand and supply in NZ. Although this needs to be thoroughly investigated.

In NZ, the choice of deer farming system affects the relationship between deer feed requirements and feed supply. A breeding operation requires a large feed input during summer when hinds are lactating and is more suited to a summer-wet region; whereas in summer-dry areas, finishing of weaners for venison means peak feed demands coincide better with pasture production in autumn, winter and spring.

The timing of events in a given deer farming operation can also help alleviate pasture supply problems. Strategies such as early weaning (late February) and slaughtering yearling venison stags in late spring can be used to lower feed requirements during a dry summer period (Wilson, pers. comm.).

Another option is to grow specialist pasture species or crops for deer that produce high dry matter (DM) yields of high nutritive value during the summer-autumn or winter deficit periods (see section 1.5.).

## **1.5. THE USE OF SPECIAL PURPOSE FORAGES FOR VENISON PRODUCTION**

Grazing weaner red deer stags at high pasture surface height (10-8cm) and herbage mass (2100-1600kgDM/ha) with PRG/WC pasture can result in 75% attaining 50kg carcass weight by one year of age, provided winter growth rates of 140g/day can be maintained (Ataja *et al.*, 1992b). Introduction of annual ryegrass can increase the number of stags achieving target slaughter weights by 19%, due to small but consistent increases in liveweight gain during winter and spring (Ataja *et al.*, 1992b). However, forage crops which grow during spring, summer and autumn offer potential increases in calf growth rate during summer prior to weaning, during autumn post-weaning and during spring just prior to slaughter.

Combining the use of forage crops during summer, autumn and spring with 10cm PRG/WC pasture during winter, offers the maximum potential growth rates of young deer from birth to one year of age.

### **1.5.1. Forage Crops Evaluated for Venison Production in New Zealand**

#### **1.5.1.1. Summer Forage Crops**

The herb chicory (*Cichorium intybus*; C) and legumes red clover (*Trifolium pratense*; R) and birdsfoot trefoil (*Lotus corniculatus*; B) have been evaluated as specialist forage crops for deer production. These forages have deep root systems for water extraction and have high spring-summer-autumn DM production, even in drier areas. If managed correctly these crops should persist for 3-5 years, and are best grown on no more than 25% of the total farm area, to avoid creating feed shortage problems in winter.

These crops are largely dormant during winter and it is not advisable to graze them at this time due to high plant death caused by pugging damage. Forages such as C, R and B are slow to establish and require specialist management for control of weeds and

to prevent plants from becoming excessively reproductive in summer which markedly reduces their feeding value (FV, see section 1.6.).

Table 1.4. shows the superiority of growth rates of weaner red deer stags grazing perennial forage crops in summer, autumn and spring, relative to PRG/WC pasture, where all deer grazed pasture during winter in these experiments. This superiority was despite attempting to maintain post-grazing pasture mass above 1500kg DM/ha (Kemp, 1996). The main advantages of these crops for increasing growth was during summer and autumn, when PRG/WC pasture is of lowest feeding value. Table 1.4. summarises experiments undertaken in different years, of which B has only been evaluated in one year.

**Table 1.4. Daily liveweight gain (g/day) for young red deer stags grazing perennial ryegrass/white clover pasture (PRG/WC), red clover (R), chicory (C) or birdsfoot trefoil (B) in summer, autumn and spring and PRG/WC in winter**

(Data from Massey University Deer Research Unit)

	PRG/WC	R	C	B
<b>Season</b>		<b>Lactation</b>		
<b>Summer</b>	345	410	394	456
		<b>Post-weaning Growth</b>		
<b>Autumn</b>	177	250	241	235
<b>Winter</b>	94	99	53	63
<b>Spring</b>	284	356	295	283

Adapted from Kemp (1996)

Sources: Ataja *et al.*, (1992a); Niezen *et al.*, (1993a); Semiadi *et al.*, (1993); Soetrisno *et al.*, (1994); Adu *et al.*, (1996); Kusmartono *et al.*, (1996a); Min *et al.*, (1997).

‘Grasslands Puna’ chicory (C) has a low N and fibre content (Hoskin *et al.*, 1995), but has a high mineral (Crush and Evans, 1990) and energy content (Hoskin *et al.*, 1995). Hunt and Hay (1990) found C to be one of the most preferred forage species by red deer, while PRG was one of the least preferred species.

When fed to castrate fistulated deer indoors, C had a higher DM, organic matter (OM) and energy digestibility, but lower fibre digestibility than PRG (Hoskin *et al.*, 1995). Indoor and field studies of feeding and rumination activity in deer fed C or PRG showed that those fed C spent a similar time eating, but spent markedly less time ruminating (Hoskin *et al.*, 1995; Kusmartono *et al.*, 1996a). Kusmartono *et al.*, (1996b) found that C is broken down faster in the rumen of deer, with less rumination being required than for

PRG, and that some deer fed C can break down swallowed chicory to below the critical particle size for outflow from the rumen without any rumination. The faster clearance of DM from the rumen largely explains the high voluntary feed intake (VFI) of deer grazing C (Kusmartono *et al.*, 1996b).

Chicory has been shown to have higher feeding value than PRG/WC pasture for both lactating hinds and calves as well as weaner deer (Kusmartono *et al.*, 1996a). Weaner deer fed C achieve higher carcass weights at one year of age than those grazing PRG/WC, allowing 100% of stags to reach 50-65kg carcass weight by one year of age. This effect is greatest for hybrid stags (0.25 wapiti) due to their higher growth potential. Grazing C also advanced the date of first-cut velvet antler and increased the weight of velvet harvested in weaner stags, compared to stags grazing pasture (Kusmartono *et al.*, 1996a).

Red clover (R) has a high N content and a lower fibre concentration compared with PRG (Freudenberger *et al.*, 1994b) and is highly preferred by red deer (Hunt and Hay, 1990; Semiadi *et al.*, 1995). The use of R has increased the growth of deer calves during lactation (Niezen *et al.*, 1993a) and during post-weaning growth to one year of age where all stags grazing R reached the minimum slaughter weight compared to 75% of those grazing PRG/WC pasture (Semiadi *et al.*, 1993; Soetrisno *et al.*, 1994). Semiadi *et al.*, (1993) also found all weaner stags grazing R produced velvet prior to one year of age, relative to 75% of those grazing PRG/WC.

Birdsfoot trefoil (B) was highly preferred by red deer stags and hinds in a study by Scott (1989), but is considered a new crop for deer production and is currently under evaluation for weaner venison production at Massey University. Initial research has shown high deer calf growth rates and weaning weights of hinds and calves grazing B, compared with those grazing PRG/WC (Edu *et al.*, 1996). Birdsfoot trefoil contains condensed tannins (20-30g/kg DM) which may offer advantages in growth rates of young deer due to an increased supply of undegraded protein to the small intestine (see section 1.7.) and possible effects on internal parasites (see section 1.9.).

#### **1.5.1.2. Winter Forage Crops**

Little research has been conducted on short-term winter forage crops for deer, however deer will readily consume most brassica crops. Greenfeed oats have been used with some success, but deer only utilise a small proportion of the crop and cattle are required to clean up what the deer leave. Since the growth potential of young deer is

slow during winter, but high during spring, summer and autumn, it is more difficult to increase deer growth rates over the winter period, regardless of amount and quality of feed supplied.

### 1.5.2. Potential Forage Crops

It has been well documented that when given a choice, red deer select legumes over grasses (Scott, 1989; Bootsma *et al.*, 1990; Hunt and Hay, 1990; Semiadi *et al.*, 1995). Lucerne (*Medicago sativa*), sulla (*Hedysarium coronarum*), lotus major (*Lotus pedunculatus*) and sainfoin (*Onobrychis viciifolia*) are several legumes preferred by deer (Scott, 1989; Hunt and Hay, 1990) that are yet to be evaluated for use in NZ farmed deer production systems.

Sulla, birdsfoot trefoil, lotus major and sainfoin are of particular interest due to their condensed tannin content (see section 1.7.). Sulla is of interest for deer production due to its potential for extremely high summer growth rates, high annual dry matter production (18.0t/Ha), and unlike other forage legumes, in milder areas of NZ it also exhibits moderate winter growth (Krishna *et al.*, 1990). Sulla has a tall, erect growth habit that is thought to be particularly suitable for deer grazing. However, difficulties in establishing sulla and the poor persistence of existing varieties (1-2 years) may limit its commercial use at this stage.

## 1.6. PRINCIPLES OF FORAGE FEEDING VALUE

### 1.6.1. Definition of Feeding Value

Feeding value (FV) is defined as the animal production response to grazing a specific forage under a given set of environmental circumstances and is a function of voluntary feed intake (VFI, see section 1.6.2.) and nutritive value (NV, see section 1.6.3.) per unit of intake (Ulyatt, 1973), see Equation 1.1.

Eqn. 1.1.

$$FV = f(VFI \times NV)$$

FV is typically measured as LWG for growing animals or milk production for lactating animals. With most domesticated ruminants, FV is assumed to be largely a

function of the feed only, but in the case of temperate deer the growth performance achieved will be a function of the stage of the animal's seasonal cycle as well as diet (see section 1.3.).

## **1.6.2. Forage Intake by Grazing Animals**

The amount of feed consumed by grazing ruminants has been estimated by Ulyatt (1984) to account for 50-70% of the variation between pastures in their capacity to sustain animal production, and Hamilton and Blaxter (1981) found the growth of young deer is relative to level of feed intake. VFI of ruminants has been shown to vary as a function of characteristics of the feed, the animal and its environment.

### **1.6.2.1. Nutritional and Non-Nutritional Components**

When animals are grazing pasture, intake is determined by the level of opportunity for animals to harvest pasture. Poppi *et al.*, (1987) has shown that when pasture is offered to an animal in increasing quantities, intake increases curvilinearly to reach a plateau. In the ascending part of the curve, the ability of the animal to harvest pasture is affected by non-nutritional factors such as environmental conditions (temperature, day length), grazing behaviour of the animal and the physical structure of the sward canopy. At the plateau section of the curve, factors such as palatability, digestibility, crude protein content, digesta passage, gut distension, concentration of metabolic products and the efficiency of utilisation of digested nutrients are important. However, these nutritional factors appear to be important in controlling intake only if forage availability is unlimited.

The primary nutritional factor limiting *ad-lib* intake is normally thought to be the physical capacity of the rumen, but Bines and Davey (1970) found this did not hold true for diets of high digestibility. Relationships between intake, rumen fill and digesta passage rates as such do not prove the existence of a physical restriction of intake, and in certain situations (during lactation or cold stress) ruminants can greatly increase roughage intake by increasing digesta passage rates (Ketelaars and Tolkamp, 1992). A more recent theory points to the significance of the efficiency of utilisation of digested nutrients in relation to the physiological state of the animal in regulating feed intake (Ketelaars and Tolkamp, 1992).

Animal genotype, maturity and physiological state will affect an animal's demand for nutrients, digestive capacity and eating capability, all of which influence VFI. Young, fast-growing animals consume more herbage per unit liveweight than mature animals.

Consequently, animal production is likely to be more sensitive to variations in sward conditions in high-performance than in low-performance animals (Hodgson, 1990).

Different classes of stock and different ruminant species exhibit differences in diet selection, ingestive behaviour and herbage intake. However, different classes of stock respond in similar ways to changes in sward conditions (Hodgson, 1990).

For the grazing animal, Hodgson (1990) defined the daily amount of herbage eaten as the product of time spent grazing and the rate of herbage intake during grazing. Hodgson (1982) presented the estimation of herbage intake through Equation 1.2.

**Eqn. 1.2.**

$$I = GT \times RB \times IB$$

Where:

I = daily intake of herbage by a grazing animal (mgOM/kg LW/day)

RB = the rate of biting during the grazing period (bites/min)

IB = herbage intake per bite or bite size (mgOM/kg LW)

GT = the time spent grazing (min/day)

Herbage mass and sward height are the two major components influencing grazing behaviour (RB, IB & GT) with temperate pastures (Hodgson, 1985; Poppi *et al.*, 1987) but factors such as leaf:stem ratio can have greater effects when grazing forage crops such as erect, stalky legumes like lucerne. Mitchell *et al.*, (1991) found the ingestive behaviour of deer and sheep was very similar in relation to height and bulk density, with height having the greatest effect on intake.

Intake per bite (IB) is the most sensitive animal response to variations in characteristics of the sward canopy and increases linearly with increasing sward height or herbage mass (Laca *et al.*, 1992). Herbage intake increases curvilinearly with IB (Leaver, 1985). Spalinger *et al.*, (1988) found for black-tailed deer consuming forbs or shrubs that intake rate was highly correlated with bite size, as well as plant fibrousness (neutral detergent fibre concentration).

Biting rate (RB) increases in direct response to changes in sward conditions such as decreases in sward height or herbage mass and has been found to be combined with a decrease in the ratio of manipulatory to harvesting bites in sheep (Laca *et al.*, 1992). Thouless (1990) found social behaviour to effect RB in deer where red deer hinds had a



reduced RB when they were closer to dominant individuals, but no change in RB was evident when they were close to subordinates.

Grazing time (GT) increases when animals are grazed on shorter swards as the animal tries to prevent a decrease in VFI associated with decreasing IB.

#### **1.6.2.2. Grazing Management**

Grazing management involves the manipulation of pasture availability to animals by adjusting grazing frequency and intensity in order to achieve maximum pasture and animal production. However, in achieving maximum animal production, pasture production is often compromised.

Wickstrom *et al.*, (1984) found that herbage intake in large wild herbivores including elk and mule deer is dependant on herbage availability, which in a paddock situation is the amount of pasture available per animal per day or pasture allowance (Rattray and Clark, 1984). To maximise intake, attention must be paid to both pre-grazing pasture mass and the target residual pasture mass. Analyses by McCall *et al.*, (1986) showed that for ewes grazing to a common residual (400kg greenDM/Ha) intake varied between 1.32 and 0.75 kg greenDM/h/day where pre-grazing mass varied between 2500 and 1000 kg greenDM/Ha.

During grazing, pasture mass (and height) declines at a decreasing exponential rate through time (Sheath, 1983). Sheep respond to a reduction in sward height by increasing GT and RB. Any rise in RB or GT of sheep grazing predominantly grass swards below 6cm height is not enough to compensate for the decline in IB leading to lowered intake (Hodgson, 1985) and hence lowered production. Heydon *et al.*, (1993) observed higher intakes by red deer hinds on a 5cm than a 3cm pasture sward, and Ataja *et al.*, (1992a) showed that for weaner red deer, liveweight gains were significantly higher on a 10cm than on a 5cm PRG/WC pasture sward. Hamilton *et al.*, (1995) found liveweight gain and final liveweight of yearling red stags to be significantly lower on a 4cm sward than on a 6, 8 or 10cm sward, but there were no significant differences between the performance of deer on the 6, 8 and 10cm swards. However, Hamilton *et al.*, (1995) concluded that their results suggested that to achieve maximum output per hectare, optimum sward height may be closer to 8cm.

Low stocking rates give increased herbage mass and hence increased herbage intake, but over time the proportion of green leaf declines and the proportion of stem and dead tissue material increases significantly. High stocking rates ensure the maintenance of

a high proportion of leaf, however they can result in lowered digestibility of the diet selected due to the short, dense sward reducing the opportunity for selective grazing (Hodgson, 1990).

Herbage intake relationships derived from continuously stocked swards with little change in sward characteristics over time, and rotationally grazed swards involving rapid changes over time, indicated responses in IB over similar ranges of variation of sward height, but the magnitude of the response was substantially greater under the rotational grazing management (Hodgson, 1981).

### **1.6.3. Nutritive Value of Forages**

NV is a function of the physical and chemical composition of forages, digestibility, rate and site of digestion and the efficiency of utilisation of absorbed nutrients (Ulyatt *et al.*, 1976).

#### **1.6.3.1. Forage Chemical and Physical Composition**

Table 1.5. shows the effects of species, plant component and maturity on the chemical composition of pasture species.

The chemical composition of pasture plant material varies widely between species and their component parts such as leaf, stem, flower and seed. The composition of each component within a species can change markedly as the plant matures, and composition also varies with growing conditions. Legumes tend to have less cell wall constituents than grasses at the same stage of maturity, but they have greater proportions of storage and soluble carbohydrate, protein and lipid (Black, 1990).

As pasture species mature, a greater proportion of the plant is made up of the stem fraction relative to the leaf fraction, especially as the plant becomes reproductive. Stems contain a greater amount of total cell wall material, particularly lignin, than leaves. The leaf component of grasses and legumes is eaten in greater quantities and sustains a higher rate of animal performance than the stem fraction (Minson, 1981). The higher VFI of leaf compared to stem of similar digestibility was associated with a longer time spent in the rumen for stem fraction compared to leaf fraction (Minson, 1982).

The water content of a forage has a major effect on animal preference and DMI. Kenney *et al.*, (1984) showed that the rate of intake of fresh forage increased as its water content increased, but the rate of DMI declined once the DM content of the forage fell below 40%. Black *et al.*, (1987) found that a 1% increase in DM of forages containing

10, 20, 35 or 95% DM was estimated to increase preference by 10, 7, 3 and 0.3% respectively (Black *et al.*, 1987). Tinworth (1998) showed the VFI of chicory by red deer increased by 17% as fresh chicory was wilted from 11% to 24% DM.

The chemical composition and NV of pasture plants can be improved by fertiliser application to the soil (Ozanne *et al.*, 1976; Rees and Minson, 1978). Weather conditions can also affect plant composition. For example, low light intensity increases the non-protein N, water and ash content and reduces the soluble carbohydrate content of plant cells (Deinum, 1966).

**Table 1.5. Effect of species, plant component and maturity on the chemical composition of pasture (% dry matter)**

(Black, 1990)

Plant Material	CWC	Lignin	Pectin	Soluble CHO	True Protein	NPN	Lipid	Ash
<b>Whole Plant: Vegetative</b>								
White clover	38.8	8.2	7.1	7.9	22.0	0.62	9.3	10.3
Perennial ryegrass	44.8	6.8	1.4	12.6	17.4	0.49	6.6	12.7
<b>Plant Components</b>								
White clover: flowering								
Green leaf	21.8	6.1	10.6	6.3	29.5	0.99	5.6	9.9
Stem	39.9	18.9	7.8	6.8	13.8	0.87	3.2	9.3
Flower	45.4	16.4	9.7	3.8	19.9	0.99	2.7	8.0
<b>Stage of Maturity</b>								
<i>Danthonia linkii</i> : vegetative								
Green leaf	58.1	3.2	2.5	8.1	11.1	0.20	2.7	9.4
Stem	69.1	5.3	2.2	10.9	5.2	0.24	1.0	6.5
<i>Danthonia linkii</i> : flowering								
Green leaf	69.4	6.0	1.1	2.3	11.2	0.15	2.8	9.1
Dead leaf	74.0	8.4	0.9	0.7	3.8	0.11	2.3	9.3
Stem	84.6	9.6	0.7	2.3	1.8	0.09	0.8	5.1
Flower/seed	59.4	5.2	0.1	0.15	8.0	0.20	2.0	4.8

CWC = cell wall constituents; CHO = carbohydrate; NPN = non-protein nitrogen

### 1.6.3.2. Digestibility and Site of Digestion

Apparent digestibility has long been used as an index of NV of forages. There is a linear relationship between the VFI of herbage and its digestibility (Hodgson, 1977). Apparent digestibility (D) is defined in Equation 1.3.

Eqn. 1.3.

$$D = \frac{I - F}{I} \times 100$$

Where:

I = Intake of feed DM or component such as organic matter (OM), energy, neutral detergent fibre (NDF) etc. (g)

F = corresponding output in faeces (g)

Apparent digestibility refers only to the difference between intake and undigested residue and an unknown amount of endogenous loss, expressed as a percentage of intake and cannot distinguish the proportion of nutrients absorbed at different sections of the digestive tract. Poppi *et al.*, (1987) considered the rate of digestion and rate of passage as important parameters in quantifying the extent of digestion at specific digestion sites in the gastrointestinal (GI) tract.

Rate of digestion refers to the proportion of feed digested per unit time. The rate of digestion is affected by the physical and chemical composition of the forage, microbial mass, rumen pH, nutrients limiting the growth of the rumen microbial population and by the outflow rate of digesta and microbes from the rumen. Digestion rate declines with increasing fibre content or an increase in the structural:soluble carbohydrate ratio. The rate of digestion of cell wall constituents has been observed to vary by as much as eight-fold across plant materials, although variations of three to four-fold are more common within a species as it matures (Smith *et al.*, 1972).

Rate of passage is a measure of how long digesta is retained in the gut and is dependent on the processes of comminution, microbial fermentation, digestion and absorption (Mertens and Ely, 1983). Flow of material from the rumen is determined by the frequency and amplitude of rumen contractions and the size and density of particles within the rumen (Kennedy and Murphy, 1988). The major factors altering the rate of passage for forages are chewing during eating and ruminating, and microbial digestion (Weston, 1984). Hendrickson *et al.*, (1981) and Moseley (1981) found the material flowing fastest from the rumen was the material broken down to small particles during eating. Warner (1981) found increasing the amount of fibre in a diet generally results in a slower rate of passage from the rumen. It is still not clear from animal studies whether

passage rate and VFI are limited more by the time to break down large particles or by the hindrance of small particle passage from the rumen (Wilson and Kennedy, 1996).

The fractional outflow rate of solutes from the rumen is much faster for deer than for other farmed ruminants, and in deer is affected greatly by season (Domingue *et al.*, 1991a, see section 1.3.2.). The ability to decrease the rumen fractional outflow rate of particulate matter in summer enables deer to maintain apparent digestibility in summer when VFI rapidly increases.

There is no deer-specific information available about the site of digestion of particular nutrients. The major sites of digestion in ruminants are the rumen/reticulum (microbial fermentation), the small intestine (SI, animal enzymes) and the large intestine (microbial fermentation). The rumen accounts for 55-65% of total apparent OM digestion, the SI 25-30% and the large intestine 5-15% (Waghorn and Barry, 1987). Using white clover and short-rotation ryegrass, Ulyatt and Macrae (1974) found 90% of the digestible carbohydrate was digested in the rumen. Digestion and supply of nutrients at the SI is from microbial OM leaving the rumen and a variable proportion of the diet that escapes rumen degradation. As the undegraded dietary component is quantitatively small, nutrient supply to the SI is greatly dependent on rumen digestion and the efficient capture of degraded N by microbial cell synthesis (Storm and Orskov, 1984).

The particular site of OM digestion determines the form of metabolisable energy (ME) available, either as volatile fatty acids (VFA) from the reticulo-rumen, or post-ruminal microbial and undegraded dietary constituents. The partition of OM digestion differs with pasture species and VFI. A greater intake of perennial ryegrass increased the proportion of OM digested in the rumen relative to that digested in the SI, but there was no change in the partitioning of OM digestibility in sheep fed white clover (Ulyatt and MacRae, 1974).

Ulyatt *et al.*, (1975) found 70% of fresh forage protein was degraded in the rumen of sheep and 50% of the protein entering the duodenum was derived from microbial protein. Large differences observed in N intake between grass and legume diets were largely eliminated in the duodenal NAN supply due to rumen microbial transactions in a study by Cruickshank *et al.*, (1992). When fed fresh forage diets, a loss of as much as 30 to 50% of ingested N can occur through the ruminant stomach in the form of ammonia absorbed from the rumen (Ulyatt and MacRae, 1974; Ulyatt *et al.*, 1975; Cruickshank *et al.*, 1985). This represents a major inefficiency in N use in ruminants fed temperate fresh forages of high digestibility.

### 1.6.3.3. Efficiency of Utilisation of Digested Nutrients

The most commonly used index for utilisation of digested nutrients is the efficiency of utilisation of metabolizable energy (ME) ( $k$ ) defined in Equation 1.4.

Eqn. 1.4.

$$k = \frac{\Delta E r}{\Delta M E i}$$

Where:

$k$  = efficiency of utilisation of ME for maintenance ( $k_m$ ), growth ( $k_g$ ), fattening ( $k_f$ ) or lactation ( $k_l$ )

$Er$  = incremental change in energy retained

$MEi$  = incremental change in metabolisable energy intake

Between feeds, the efficiency of ME utilisation for liveweight gain ( $k_g$ ) shows a wide variation that has been linked to differences in feed metabolisability ( $q$ , metabolisable energy as a fraction of gross energy) and  $N$  (Blaxter, 1989). Ketalaars and Tolkamp (1992) have shown the relationship between  $q$ , which is proportional to digestibility, and  $N$  and  $k_g$ .

Ulyatt (1970) and Rattray and Joyce (1974) both recorded higher  $k_g$  for white clover compared to ryegrass fed to young sheep. Minson (1981) reported that the higher efficiency of utilisation of legumes was due to the production of a lower acetic:propionic volatile fatty acid ratio of legumes compared to grasses.  $k_f$  and  $k_g$  are negatively related to the fibre content of the diet.  $k_m$  is higher than  $k_g$  and is not as greatly influenced by diet as  $k_g$ .

The efficiency of utilisation of absorbed amino acids depends on the amount absorbed in relation to the energy available to the animal, and on the match between the proportions of amino acid absorbed and those required for all body functions. A deficiency in protein supply is most likely to occur in ruminants grazing grass-based pastures when potential animal growth rates are high and when body protein loss is substantial during infestation with internal parasites (see section 1.8.2.2.).

#### 1.6.4. Differences Between Forages in Feeding Value

In general, fresh forages consumed by ruminants can be categorised as grasses, legumes, herbs and browse (Langer, 1990).

Temperate legumes have a higher FV than grasses and support higher levels of animal production (Ulyatt, 1973; Ulyatt, 1981). Legumes are eaten in greater quantities than grasses of similar energy digestibility (Minson, 1982; Rattray and Clark, 1984). Ulyatt (1970) showed a higher efficiency of feed utilisation and liveweight gain of sheep fed white clover compared to perennial ryegrass.

The herb chicory has been found to have a higher FV for deer than PRG/WC pasture (Kusmartono, 1996a).

#### 1.6.5. Measuring Voluntary Food Intake in the Grazing Animal

The VFI of grazing ruminants can be measured indirectly, by the use of external indigestible markers or internal markers such as alkanes in individual animals, or by using pasture sampling for groups of animals.

##### 1.6.5.1. Animal Techniques

###### 1.6.5.1.1. Chromium Oxide

Continuous uniform release of the indigestible marker chromium sesquioxide ( $\text{Cr}_2\text{O}_3$ ) for 20-30 days provided by intraruminal controlled release capsules (CRC) is currently the most commonly used method. Chromium CRC are used to estimate faecal output (FO; kgOM/day) following Equation 1.5. below (Parker *et al.*, 1989).

Eqn. 1.5.

$$F O = \frac{X}{Y}$$

Where:

X =  $\text{Cr}_2\text{O}_3$  release rate from the capsule (mg/day)

Y =  $\text{Cr}_2\text{O}_3$  concentration in the faeces (mg/gOM)

VFI (kgOM/day) is then estimated by Equation 1.6. using *in-vitro* OM digestibility (D) of the diet selected and FO. The diet selected is obtained either by hand-plucking a



sample of forage by imitating animal grazing behaviour and herbage selection following close animal observation, or via oesophageal fistulae.

**Eqn. 1.6.**

$$VFI = \frac{FO}{1 - D}$$

An assumption with this method is that the administration or ruminal presence of CRC does not influence intake, and that animals with CRC have intakes equal to those without. Despite problems with an earlier design of CRC (Parker, 1990; Parker *et al.*, 1991) the modern capsules appear to provide unbiased estimates of herbage intake (Parker *et al.*, 1991). However, shortcomings of CRC include large deviations between observed and manufacturer-specified release rates with both cattle and sheep (Buntinx *et al.*, 1992), variation in release rates according to feed (Parker 1990) and an animal by CRC interaction in release rate (Pond *et al.*, 1990). This emphasises the need to measure chromium oxide release rate in each study, with the manufacturers information being used as a general guide only.

This method is prone to errors arising principally from inaccuracies in the digestibility estimate. This is most commonly due to the difficulty in obtaining an accurate sample of the diet selected.

#### **1.6.5.1.2. Alkanes**

Alkanes (n-alkanes: saturated, long-chain hydrocarbons, C<sub>25</sub>-C<sub>35</sub>) of plant cuticular wax, in combination with orally-administered synthetic alkanes (even-chain) can be used to estimate herbage intake in individual animals (Mayes *et al.*, 1986). Alkanes are not completely recoverable in faeces, but this does not matter if the estimate is based on a pair of alkanes (one natural, one dosed) adjacent in chain length, since their recoveries will be similar. Alkane-based estimates of intake agree well with known intakes and faecal alkane concentration can also be used to estimate the composition of the diet selected of individual grazing animals in terms of plant species or plant parts (Dove, 1993). The calculation of intake using alkanes is derived from Equation 1.7.

Eqn. 1.7.

$$I = \frac{\frac{F_i}{F_j} D_j}{H_i - \frac{F_i}{F_j} H_j}$$

Where:

I = daily herbage intake (kgDM/day)

F<sub>i</sub> = faecal concentration of the odd-chain plant alkane (mg/kgDM)

F<sub>j</sub> = faecal concentration of the even-chain dosed alkane (mg/kgDM)

D<sub>j</sub> = dose rate of the synthetic, even-chain alkane (mg/day)

H<sub>i</sub> = herbage concentration of the odd-chain plant alkanes (mg/kgDM)

H<sub>j</sub> = herbage concentration of the even-chain plant alkanes (mg/kgDM)

From the equation above it can be seen that when using a pair of alkanes to estimate intake, only the faecal ratio of the natural to dosed alkanes is needed. The n-alkane methods for estimating herbage intake requires daily dosing of animals with the marker n-alkane. At present n-alkanes can only be administered in pellets of impregnated paper, or in capsules containing a mixture of alkane and cellulose fibre or gelatin, but research has started on the development of an intra-ruminal controlled alkane release device (IRCRD; Dove *et al.*, 1991).

The alkane approach results in accurate estimates of herbage intake, with the major advantage that the method automatically takes into account the differing herbage digestibilities occurring in individual animals, regardless of their level of intake, supplement intake or parasite burden (Dove, 1993). However, widespread adoption of this method will require the ready availability of an IRCRD and good gas chromatograph facilities.

#### 1.6.5.2. Sward Technique

Mean apparent dry matter intake (DMI) or pasture disappearance for groups of animals can be estimated from the difference between pre- and post-grazing herbage mass (kgDM/ha) using the Equation 1.8.

Eqn. 1.8.

$$DMI(kg / head / d) = \frac{DM1 - DM2}{N}$$

Where:

DM1 = pre-grazing herbage mass (kg)

DM2 = post-grazing herbage mass (kg)

N = number of animal grazing days

Since pasture will be growing during the grazing period, a correction factor needs to be applied to account for this herbage accumulation if grazing periods are longer than three to four days (Walters and Evans, 1979). Ulyatt *et al.*, (1974) reported a 30 to 40% lower value for VFI estimated using the sward technique compared to that estimated using animal methods.

## **1.7. EFFECT OF CONDENSED TANNINS IN TEMPERATE FORAGES ON FEEDING VALUE**

### **1.7.1. Introduction to Condensed Tannins**

Tannins are naturally occurring plant phenolic secondary compounds, classified as condensed tannins (CT) or hydrolysable tannins (HT). HT are rare in temperate region forages. CT are polymeric flavanols or leucoanthocyanidins. Table 1.6. lists the concentration of CT in some temperate forage plants.

It has been suggested that that plants evolved CT as a defense against invasion by bacteria and fungi, due to their ability to complex with protein and polysaccharides (Swain, 1979). Feeny (1976) proposed CT in plants have evolved as chemical defense agents through their astringent taste and by inactivating digestive enzymes of their predators such as insects and herbivores.

CT are present in the leaves and stems of a number of specialised forage legumes, but in some plants such as white and red clover they occur only in the flower petals (Barry, 1989).

CT concentrations in plants can be influenced by a number of factors such as season, soil conditions, stage of plant maturity and leaf to stem ratio, with highest levels found in summer (Cope *et al.*, 1971), under low fertility, acid soil conditions (Barry and Forss, 1983), and when the plant is mature with a high leaf to stem ratio (Douglas *et al.*, 1993).

Laboratory CT analyses (Terrill *et al.*, 1992a) measure total CT (TCT) which includes extractable CT (ECT), protein-bound CT (PCT) and fibre-bound CT (FCT) of freeze-dried forage and digesta samples. In this review CT refers to TCT unless otherwise stated.

**Table 1.6. Concentrations (g/kg DM) of extractable (ECT), protein-bound (PCT), fibre-bound (FCT) and total condensed tannins (TCT) in a range of whole plants.**

<b>FORAGE</b>	<b>ECT</b>	<b>PCT</b>	<b>FCT</b>	<b>TCT</b>	<b>Reference</b>
<b>Legumes</b>					
Hairy canary clover	121.0	65.0	1.0	187.0	324
Hairy canary clover	45.3	25.5	1.35	72.2	101
Prostrate canary clover	100.0	23.0	3.0	126.0	324
Prostrate canary clover	32.2	20.9	1.45	54.5	101
Canary clover	83.0	54.0	6.0	143.0	324
Lotus major	59.4	12.9	1.7	73.9	101
Lotus major	58.0-97.6	-	-	-	192
Sulla	33.0	9.0	3.0	45.0	324
Sulla	27.5	8.05	0.65	36.2	101
Crownvetch	16.0	13.0	2.0	31.0	324
Crownvetch	13.6	5.7	0.55	19.8	101
Birdsfoot trefoil	7.0	13.0	1.0	21.0	324
Birdsfoot trefoil	14.3	5.95	1.0	21.3	101
Birdsfoot trefoil	1.3-39.0	-	-	-	192
Narrow leaved birdsfoot trefoil	2.0	3.0	1.0	6.0	324
Russel lupin	1.15	0.5	0.3	1.95	101
Sainfoin	29.0	-	-	-	35
<b>Herbs</b>					
Sheep's burnet	1.0	1.4	1.0	3.4	324
Sheep's burnet	0.35	1.45	0.35	2.1	101
Chicory	1.4	2.6	0.2	4.2	324
Dock	11.3-22.9	-	-	-	348
<b>Grass</b>					
Yorkshire fog	1.1	0.3	0.4	1.8	324
Yorkshire fog	0.9	0.5	0.3	1.65	101

## 1.7.2. Condensed Tannins and Protein and Carbohydrate Digestion

### 1.7.2.1. Protein Digestion

CT can react and form complexes with both carbohydrates and proteins, but at neutral pH form stronger bonds with protein (McLeod, 1974). Reactions with protein have been widely investigated and found to be highly specific for different tannins as well as different proteins, which suggests that the differences in affinity are functionally significant (Asquith and Butler, 1986). CT-protein complexes are largely pH dependant and are stable and insoluble in the pH range 3.5-7.0, but are unstable and release protein at pH < 3.0 and at pH 8.0 (Jones and Mangan, 1977).

CT-protein bonds act to reduce protein degradation in the rumen, which is reflected in lower rumen fluid ammonia concentrations of sheep fed on CT-containing diets (Meissner *et al.*, 1993). Ruminants consuming CT-containing fresh forages have more non-ammonia N (NAN) leaving the rumen per unit of N eaten, compared to those consuming non CT-containing fresh forages. Barry and Manley (1984) established a significant linear relationship between dietary CT concentration and NAN per flow unit total N intake in sheep fed fresh *L.pedunculatus* and *L.corniculatus*. Barry and Manley (1984) showed that NAN flow out of the rumen per unit N eaten increased as ECT concentration of *lotus* species increased, and NAN flowing out of the rumen equalled N eaten at the value of about 40g ECT/kgDM.

Sheep fed *L.corniculatus* (22g ECT/kgDM) were found to have an increased abomasal amino acid (AA) (not including sulphur AA) flux due to CT (Waghorn *et al.*, 1987b). This study also showed that CT decreased AA degradation in the rumen, in favour of essential AA relative to non-essential AA, markedly increasing the essential AA flow to the abomasum (Waghorn *et al.*, 1987b). McNabb *et al.*, (1993) found CT in *L.pedunculatus* (ECT 55g/kg) reduced the proteolysis of forage sulphur AA in the rumen.

There is evidence to suggest that the action of CT in plants reduces post-ruminal N apparent digestibility (Barry and Manley, 1984; Waghorn *et al.*, 1987b; McNabb *et al.*, 1993; Waghorn *et al.*, 1994). However, low concentrations of CT in *L.corniculatus* greatly increased the quantitative absorption of AA(g/d), especially essential AA, from the small intestine (SI) (Waghorn *et al.*, 1987a) due to increasing abomasal flux. Wang (1995) found CT in *L.corniculatus* to reduce the true digestibility of methionine and cysteine and alter the site of digestion of <sup>35</sup>S-methionine and <sup>35</sup>S-cysteine in the SI. However, the flux of both AA's was increased in the mid and latter thirds of the SI which meant the action of CT increased the total amounts (g/g eaten) of plant methionine and

cysteine absorbed from the SI. CT also markedly increased utilisation of plasma cysteine for body synthetic reactions in sheep fed *L.corniculatus* (Wang, 1995). High concentrations of CT in *L.pedunculatus* did not increase the absorption of essential AA from the SI (Waghorn *et al.*, 1994).

#### **1.7.2.2. Carbohydrate Digestion**

CT-carbohydrate complexes are less stable than those of CT-protein. Usually only a small proportion of CT bind with carbohydrate (Terrill *et al.*, 1992a), and low concentrations of CT (up to 35gCT/kgDM) have been reported to have no effect on the digestion of both water soluble and structural carbohydrates in sheep (Ulyatt and Egan, 1979; Waghorn *et al.*, 1987a, b). Wang (1995) found 27gCT/kg DM in *L.corniculatus* slightly depressed apparent DM, OM and hemicellulose digestion, but had no effect on apparent cellulose digestion or rumen fermentation of carbohydrate to major volatile fatty acids. However a high concentration of CT (106gECT/kg DM) in *L.pedunculatus* caused a reduction in readily fermentable and structural carbohydrate and lignin digestibility in the rumen, but this was compensated for by increased digestibility in the intestine (Barry and Manley, 1984).

#### **1.7.3. Condensed Tannins and Animal Production**

All research on CT and animal production in NZ has involved sheep production. There has been limited work on CT in temperate pasture species on animal production outside NZ, because overseas research has concentrated on utilising tropical CT-containing shrubs and trees as supplementary feed for cattle, sheep and goats.

High concentrations of CT (50-100g/kg DM) have been shown to depress voluntary feed intake of ruminants (Reed *et al.*, 1982; Barry and Duncan, 1984; Chiquette *et al.*, 1988; Pritchard *et al.*, 1988). There is limited information on the effect of low levels of CT on the VFI of grazing ruminants, but research suggests a maximum dietary CT concentration of 40g/kgDM if VFI is to be maintained (Waghorn *et al.*, 1990). Dietary concentrations of CT as low as 1.7g/kgDM can affect protein solubility in the rumen, but concentrations up to 30g/kgDM seem optimal for sheep production (Waghorn *et al.*, 1990).

Polyethylene glycol (PEG) selectively binds to CT without affecting other aspects of the diet and can be used to assess the nutritional effects of CT. Sheep fed high concentrations of CT have exhibited reduced body and wool growth (Pritchard *et al.*,

1988, 1992) and Barry (1985) confirmed this was caused by CT, as oral administration of PEG increased body and wool growth of sheep grazing *L.pedunculatus* (76-90gECT/kgDM). However, Terrill *et al.*, (1992b) showed that sulla with 40-50gCT/kgDM increased body and wool growth of sheep during summer.

Wang (1995) found *L.corniculatus* (32-57gCT/kgCT) supported high levels of sheep productivity relative to lucerne (trace CT). The principal effect of CT in *L.corniculatus* on growing lambs was to increase wool growth without affecting VFI, thereby increasing the efficiency of wool production. Wang (1995) also found that for ewes rearing twin lambs, the action of CT in *L.corniculatus* increased milk yield and the secretion rates of protein and lactose without affecting VFI, thereby increasing the efficiency of milk production. CT-containing forages have been shown to boost mohair production and liveweight gain in goats (Hart and Sahlu, 1993).

Lower carcass fat concentrations have been reported in lambs grazing CT-containing forages *L.pedunculatus* (Purchas and Keogh, 1984) and sulla (Terrill *et al.*, 1992b). Wang (1995) reported increased carcass weight and dressing out percentage of lambs grazing lotus relative to lucerne, but no effect of CT on carcass fatness was evident.

Production levels of cattle grazing legumes such as white clover, RC, subterranean clover and lucerne can be seriously compromised by a widespread disorder known as bloat (Reid *et al.*, 1974). CT have been found to be an anti-bloat agent, due to the ability to reduce leaf protein solubility in the rumen (Jones *et al.*, 1973; Mangan *et al.*, 1976; Waghorn and Jones, 1989), which may also contribute to high ruminant animal productivity when grazing forages containing low levels of CT's. However, as the fractional outflow rate of solutes from the rumen is much faster for deer than for other farmed ruminants (see section 1.6.3.2.) it appears that deer do not suffer from bloat, even when grazing pure RC swards (Barry and Wilson, 1994).

Condensed tannins have also been shown to have effects on internal parasites of sheep (see section 1.9.2.).

#### **1.7.4. The Effect of Condensed Tannins in the Diets of Deer.**

There is little information in the literature concerning the effect of tannins (CT or HT) in the diets of deer on digestion or deer production. Goats have mixed feeding habits closer to that of deer than sheep so it could be expected that some deer species might respond to dietary CT in a similar way to goats, in comparison to sheep.



Goats (mixed feeders) have been found to have higher digestibilities than sheep (predominantly grazers) when fed high tannin diets (Wilson, 1977; Degen *et al.*, 1995). Goats appear to be more adapted to consuming CT than sheep by being able to detoxify tannins or their degraded products (Distel and Provenza, 1991), and by having tannase in the rumen (Begovic *et al.*, 1978).

It has been shown that the reduced cell wall (NDF) digestion due to tannins observed in sheep and *in-vitro* studies does not occur in mule deer (*Odocoileus hemionus*) or white-tailed deer, despite a reduction in apparent digestibility of protein and cell solubles at high tannin concentrations (Robbins *et al.*, 1987b; Robbins *et al.*, 1991). When mule deer and sheep are fed high tannin diets, the reduction in protein digestibility is greater for sheep than deer (Robbins *et al.*, 1991).

The classification of most deer as browsers or mixed adaptable feeders (Kay *et al.*, 1980) suggests extensive selection for adaptations dealing with tannins. Parotid salivary glands in ruminants, per unit body mass, are three times larger in browsers than in grazers (Kay *et al.*, 1980) and while large glands are induced by tannins in some species, all deer have large salivary glands. Combined with the effect of large salivary glands, mule deer have been found to secrete proline-rich, low molecular weight salivary proteins which neutralise tannins (Robbins *et al.*, 1987b; Austin *et al.*, 1989). These two factors alone will enable deer to utilise tannin-containing forages to a greater extent than other ruminant species, although the extent to which deer can withstand high dietary tannin concentrations would inevitably differ according to deer species. In a comparison of dietary preferences of intermediate feeders red and sambar deer, Semiadi *et al.*, (1995) found that sambar selected a total diet higher in CT than red deer and this difference in diet selection was thought to be linked with the greater CT-binding capacity of sambar deer saliva, relative to CT-binding capacity of red deer.

Hagerman and Robbins (1993) found that the tannin-binding salivary proteins of deer are not generalized scavengers of dietary tannins, but can be very specific for the types of tannins that are consumed in the preferred diet. Moose produce salivary proteins that bind only the linear CT common in their preferred foods such as willow, aspen or birch. Mule deer which have more generalised feeding habits (Kay *et al.*, 1980) produce salivary proteins that bind linear and branched-chain CT and gallotannins, but not ellagitannin (both HT).

Robbins *et al.*, (1987a) found in digestion trials with mule deer, white-tailed deer, moose, reindeer and elk that reductions in digestible protein were proportional to the

protein precipitating capacity of the dietary plant tannins fed. Tannins (majority HT) in dried flowers and forb, tree and shrub leaves markedly reduced protein availability, and a dramatic reduction in VFI was observed when deer were fed these high tannin diets, relative to the previously fed dried grass/pellet diets. Further research with mule deer using fresh forages containing mostly CT yielded similar effects on digestion (Hanley *et al.*, 1992), despite the hypothesis that hydrolysable and condensed tannins have different physiological roles when consumed (Zucker, 1983).

So although deer have adapted to more readily consume tannin containing diets, research suggests that deer are unable to prevent excessive protein binding by high concentrations of dietary tannins, which limits the extent to which high tannin diets can be consumed.

To date there has been little deer production and nutritional research reported with farmed deer fed temperate, CT-containing forages other than for birdsfoot trefoil (Adu, *et al.*, 1996; Min *et al.*, 1997) and the literature does not contain any information regarding the feeding of low tannin containing diets to deer other than that of Kusmartono (1996c). Kusmartono (1996c) found that small concentrations of CT (0.3-2.5g/kgDM) in perennial ryegrass and chicory reduced rumen protein degradation relative to PEG-drenched deer on both forages. However, further research is required to study the effect of different concentrations of CT on VFI, protein digestion and absorption, carbohydrate digestion, utilisation of absorbed nutrients, internal parasites and general farmed deer production. The minimum level of CT needed to increase production in deer and other farmed ruminants needs to be defined.

## 1.8. INTERNAL PARASITISM OF TEMPERATE DEER SPECIES

A list of helminth parasites identified from temperate species of deer worldwide is provided in Table 1.7. and includes helminths found in NZ deer.

### 1.8.1. Lungworm (*Dictyocaulus viviparus*)

*Dictyocaulus* sp. have a high prevalence in temperate deer worldwide, especially in red and wapiti deer in cooler, moist regions such as NZ. Under NZ farming conditions *Dictyocaulus* infection is potentially the most important parasitic disease of farmed deer, capable of causing high mortality rates in young animals if not controlled (Mason, 1979; Charleston, 1980; Gladden, 1981; Fletcher, 1982; Mason and Gladden, 1983; Mackintosh *et al.*, 1984; Orr, 1991). However, it appears that in some hotter areas of the world, such as Queensland, Australia, lungworm is less of a risk than gastrointestinal (GI) nematodes, especially to farmed deer (Winch, 1986).

There is some evidence for cross transmission of lungworm infection between cattle and deer (Presidente *et al.*, 1972; Corrigan 1985; Corrigan *et al.*, 1988), but they are currently not regarded as serious sources of infection for one another, as cattle and deer are each considered to have a host-adapted strain of *Dictyocaulus* (Watson and Charleston, 1985), although this has not been proven. In fact, there is some debate as to whether *Dictyocaulus* infecting cervids is different from that infecting cattle and hence should be taxonomically classified as *D.eckerti* (Jansen and Borgsteede, 1990). Schnieder *et al.*, (1996) found the lungworm of cattle and fallow deer to be genetically distinct, supporting the hypothesis that deer lungworm should be classified as a separate species. Using two molecular characterization methods, Epe *et al.*, (1996) found distinct differences between *D.viviparus* and *D.eckerti*, however, both species were found in fallow deer. No research of this type has been carried out on lungworm found in the various species of NZ deer, hence in NZ the lungworm of deer remains classified as *D. viviparus*.

#### 1.8.1.1. Epidemiology of Lungworm Infections

Figure 1.4. illustrates the life cycle of *D. viviparus*. The minimum prepatent period (time from ingestion of infective L<sub>3</sub> to maturation of adults to egg laying stage) in red deer is around 20 days (Corrigan *et al.*, 1980; Mason, 1985; Mason, 1994).

**Table 1.7. Helminth parasites identified from temperate deer species worldwide.**

PARASITE	COUNTRY & DEER SPECIES (reference)				
	NZ	EUROPE & UK	N. AMERICA	AUST- RALIA	S. AMERICA
<i>Spiculoptera</i> <i>asymmetrica</i>	S/SA/R/F/WT (19)	F (40)/ RO (110)/ R (70)	F (100, 88)/ RN (191)	F (264)/ R (111)	R (311)
<i>Spiculoptera</i> <i>spiculoptera</i>	S/W/R/WT (19)	RO (110)			R (311)
<i>Spiculoptera</i> <i>bohmi</i>	R (18)				
<i>Mazamastrongylus</i> spp			WT (191)		
<i>Skrjabinagia kolchida</i> ( <i>O. leptospicularis</i> )	R/W (19)	RO (110)/ R (70)	WT/RN (147)	F (264)	R (311)
<i>Skrjabinagia monodigitata</i>	R (17)				
<i>Skrjabinagia lyratiformis</i>	R (9)				
<i>Skrjabinagia odocoilei</i>			WT (268)		
<i>Skrjabinagia cervi</i>		R (63)			
<i>Apteragia quadrispiculata</i>	F/R (19)	F (40)/ RO (110)			
<i>Apteragia odocoilei</i>	WT (19)		WT/F (88)		
<i>Apteragia pursglovei</i>			F (88)/ WT (87)		
<i>Rinadia mathevossiani</i>	R (19)	RO (110)			
<i>Rinadia quadrifurcata</i>	R (17)				
<i>Marshallagia marshalli</i> ( <i>occidentalis</i> )		RN (61)	W (137)/ RN (191)		
<i>Ostertagia ostertagi</i> ( <i>O. lyrata</i> )	R (165)/W (19)	RO (110)/ RN (61)	WT (87)	F (264)/ WT (111)	
<i>Ostertagia circumcincta</i>	R (165)/W (19)	RO (110)			
<i>Ostertagia trifurcata</i>	WT (19)	RO (110)/ RN (61)			
<i>Ostertagia dikmansi</i> ( <i>mossi</i> )			WT (41)		
<i>Ostertagia gruehneri</i> ( <i>arctica</i> )		RN (61)	RN (147)		
<i>Ostertagia odocoilei</i>			WT (29)		
<i>Ostertagia rubricervi</i>	R (17)				
<i>Teladorsagia</i> spp		RO (110)/ RN (61)			
<i>Haemonchus contortus</i>	R (165)/WT (19)	RO (110)	WT (41)	R (382)/F (111)	
<i>Trichostrongylus axei</i>	R/F (216)	R (196)/ RO (110)		F (264)	
<i>Trichostrongylus dosteri</i>			WT (268)		
<i>Trichostrongylus askivali</i>			WT (88)		
<i>Cooperia curticei</i>	WT (19)	R/ F (213)			
<i>Cooperia oncophora</i>	R (216)	F (213)			
<i>Cooperia pectinata</i>	R (199)	RO (110)/ R (109)			
<i>Cooperia mcmasteri</i>	R (216)				
<i>Trichostrongylus capricola</i>		RO (110)			
<i>Trichostrongylus vitrinus</i>		RO (110)/ R/ F (213)			
<i>Trichostrongylus retortaeformis</i>		RO (110)			
<i>Trichostrongylus colubriformis</i>		R/ F (213)			
<i>Nematodirus</i> spp.	R (217)	RO (110)	WT (41)/F (88)		
<i>Nematodirella</i> spp.		/F/R/WT (213)			
<i>Nematodirella</i> spp.			W (137)		
<i>Bunostomum</i> spp.		RO (110)			
<i>Strongyloides</i> spp.		R/ F (213)	WT (125)		
<i>Capillaria</i> spp.	R/WT (19)	F (40)/ RO (110)	W (137)/ F/WT (88)		
<i>Oesophagostomum venulosum</i>	R/W/WT/F (19)		WT (41)	F (264)	
<i>Oesophagostomum radiatum</i>		R/ F (213)			
<i>Chabertia ovina</i>		RO (110)			
<i>Trichuris</i> spp. (=Buckleyuris)	F (218)/R (19)	RO (110)	W (137)/ WT (41)	F (264)	
<i>Dictyocaulus viviparus</i> ( <i>eckerti</i> )		R (79)/ F (40)/ RO (196)	W/R/WT/BT/ M (301, 137)	F (264)	
<i>Bicaulus sagittatus</i>		R (79)			
<i>Varestrongylus sagittatus</i>	R (216)				
<i>Moniezia</i> sp.	R (220)/F (218)				

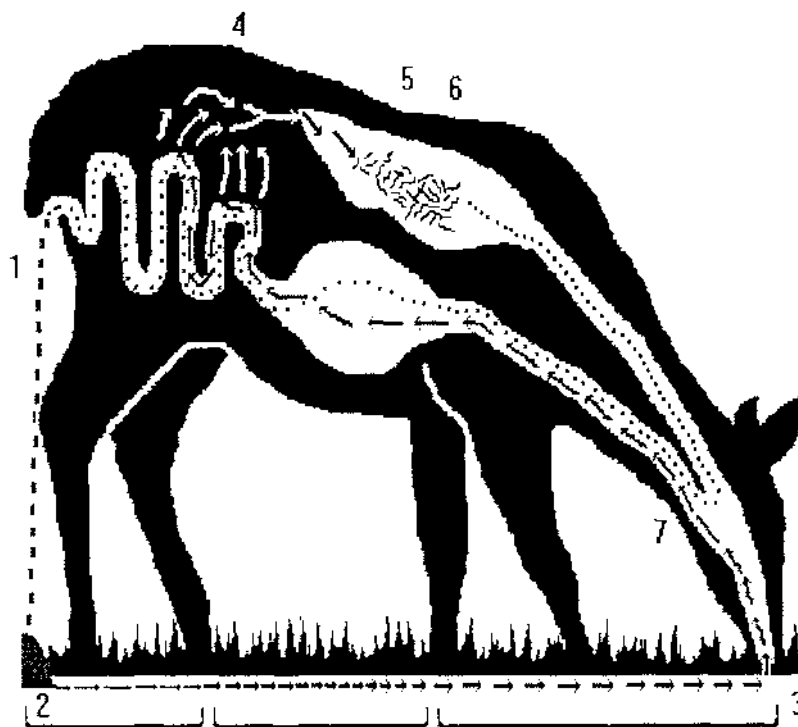
F=Fallow/WT=White-tailed/R=Red/W=Wapiti or Elk/M=Moose/BT=Black-tailed and Mule/RN=Reindeer/RO=Roe/SA=Sambar/S=Sika/RU=Rusa; () reference

There appears to be scant information on the duration of patency in deer, but continued shedding of larvae has been recorded for at least 10 months (Haigh and Hudson, 1993).

Under NZ pastoral conditions, small numbers of lungworm larvae are shed by deer calves 6-8 weeks old. Calves over two months old shed more larvae than their dams and thus become the major source of reinfection (Mason, 1985).

Attempts have been made to establish the relationship in farmed deer between faecal larval counts (FLC; Table 1.8.) via the Baermann technique and worm count in the lungs (Presidente, 1979; Mason and Gladden, 1983; Mackintosh *et al.*, 1984), with limited success. Audige (1995) reported that weaners can shed up to 1800 lungworm larvae per gram of faeces without showing clinical signs of disease. Further research into the relationship between FLC and worm burdens in NZ farmed deer is required.

Figure 1.4. Life cycle of *Dictyocaulus viviparus* in red deer



1. First stage larvae passed in faeces
2. Development from first to infective third stage on pasture (min 4 days)
3. Infective larvae consumed with herbage
4. Infective larvae penetrate intestinal mucosa and migrate via lymphatic and blood circulation to lungs (1-7 days)
5. Development to fourth stage and maturation to adulthood in lungs (min 12 days)
6. Adult worms inhabit bronchial tree and lay embryonated eggs
7. Eggs and hatched larvae are coughed up and swallowed

first stage larvae

second stage larvae

infective third stage larvae

### **1.8.1.2. Clinical Signs and Pathogenicity of Lungworm Infections**

Clinical signs of lungworm infection in deer are often non-specific, but include reduced food intake and weight gain (Corrigan *et al.*, 1982) and loss of condition, retarded growth and roughened coat (Mason, 1994). Severe debilitation and coughing are not common signs (Presidente, 1979), but in some cases a soft bronchial cough and/or raspy breathing can be heard after the animals have been physically exerted (Wilson, pers comm) which is unlike the paroxysmal coughing seen in bovine dictyocaulosis.

Auscultation of the lungs can reveal bronchovesicular breathing and adventitious sounds (Corrigan *et al.*, 1980). Severely affected animals can die suddenly (Charleston, 1980) due to asphyxiation caused by physical blockage of the air passages by worms which can pack the larynx, trachea and lower bronchial tree in a frothy exudate (Corrigan *et al.*, 1980). Damage to the lungs caused by *Dictyocaulus* increases the susceptibility to secondary bacterial infection which may result in pneumonia (van Reenen, 1982).

### **1.8.1.3. Diagnosis of Lungworm Infections**

The existence of patent *Dictyocaulus* infection can be diagnosed in the live animal by faecal examination using the Baermann technique, or modifications of it (Hendriksen, 1965), which give a measure of the number of lungworm larvae excreted in the faeces.

Necropsy of infected deer can show purulent bronchitis, eosinophilic infiltration of the lung tissue and patchy, red consolidation of parts of the cardiac and apical lobes (Mason, 1981). The disease process in red deer is essentially in and around the airways (Corrigan, 1985).

Red deer lung reacts relatively mildly to *Dictyocaulus* infection in comparison to bovine lung (Corrigan *et al.*, 1982). Corrigan *et al.*, (1982) found the reaction of alveolar tissue in red deer differed from that of bovine lung in that alveolar epithelialisation was limited and hyaline membrane formation and interstitial emphysema were not seen. Light to moderate infections (up to 20 worms) are reported to have little or no effect in deer (Haigh and Hudson, 1993).

## **1.8.2. Gastrointestinal Parasite Infections**

There is increasing concern about the negative effect of GI nematodes, especially abomasal nematodes, on farmed deer health and production (Connan, 1991; Waldrup and Mackintosh, 1992; Wagner and Mackintosh, 1993). However, little is known about the relationship between GI nematode burdens and deer production in NZ.

From Table 1.8. it can be seen that the majority of GI nematodes found in deer are trichostrongylids, present in the abomasum. Most of these are deer-specific Ostertagiinae or *Ostertagia*-type nematodes such as *Spiculopteragia sp.*, *Skrjabinagia sp.* etc. Necropsy of farmed deer occasionally reveals nematodes generally found in other farmed ruminants. When found, these are usually present in small numbers, suggesting that cross-transmission can occur to some extent. Experimental infection of weaner red deer with 30,000 *O.circumcincta*, *O.ostertagi* or *H.contortus* from sheep and cattle produced patent infections, but faecal egg counts remained low and the infections failed to produce significant differences in liveweight gain or feed intake relative to controls, and only the *H.contortus* infected animals exhibited a significant rise in plasma pepsinogen levels (Johnston *et al.*, 1984).

*Ostertagia*-type nematodes are the most prevalent GI species in NZ red deer (Wilson, 1981; Anderson 1985). Large burdens (up to 12,900 adult nematodes) of *T.axei* have also been recorded from adult deer (Anderson, 1985). Anderson (1985) reported that 97% of adult red deer surveyed were found to have abomasal worms, 27% had small intestinal worms and 46% had large intestinal worms. Of the abomasal nematodes recovered, *O. leptospicularis* and *Spiculopteragia sp.* were the most common. However, scant information is available in the literature concerning GI nematode burdens of NZ farmed deer under one year of age.

*Spiculopteragia asymmetrica* was found to have the widest host range and the highest prevalence of any parasite in Australian deer (Presidente, 1979; English, 1991). Dunn (1965) found *O.leptospicularis* to be the most prevalent parasite of roe deer in the UK. McDiarmid (1975) stated that *T.axei* was the most commonly found parasite in British red deer, but Connan (1996) found mixed *Ostertagia*-type infections to be dominant in red deer in Britain. *H.contortus* is thought to be the most pathogenic stomach worm of wild white-tailed deer in North America (Foreyt and Trainer, 1970; Eve and Kellogg, 1977). There has been no research to investigate the relative pathogenicity of different GI nematode species in deer.

### **1.8.2.1. Epidemiology of GI Nematode Infections**

Figure 1.5. illustrates the general life cycle of trichostrongylid GI nematode parasites in deer. The minimum prepatent period for GI nematodes in red deer has not been determined and is assumed to be similar to that of sheep (i.e. 18-26 days).

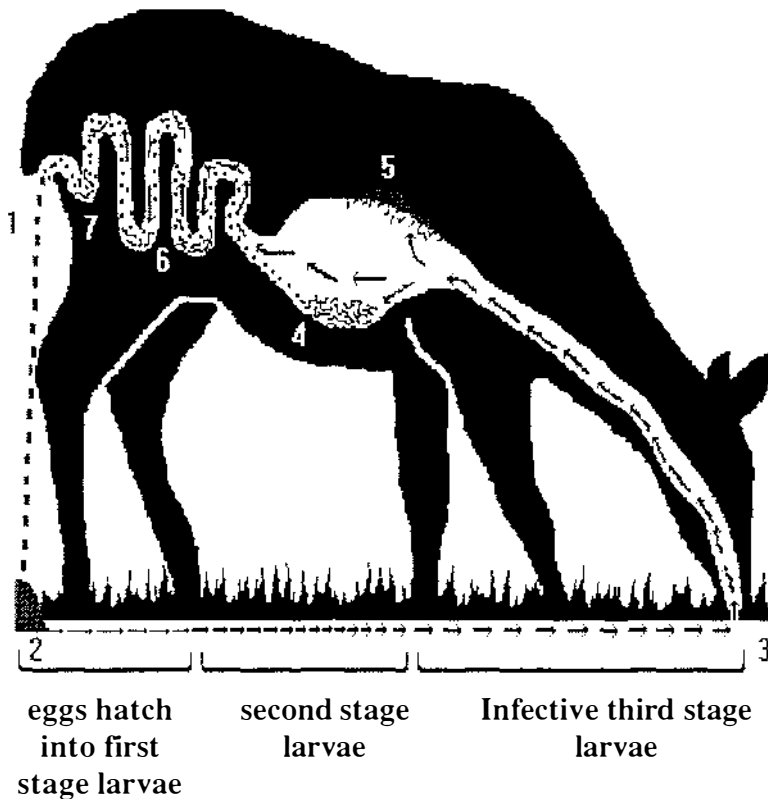
In the UK, the pattern of faecal egg counts (FEC) from hinds appeared to have a tri-modal distribution with peaks during spring and the periparturient period with a pronounced peak in late summer-autumn, with third stage larvae present on deer pastures throughout the year (Lancaster and Andrews, 1991; Rehbein *et al.*, 1995; Connan, 1996). There is currently no NZ data available on the annual pattern of faecal egg excretion of adult deer, or levels of infective larvae present on pasture.

Faecal egg counts in deer (Table 1.8.) are generally lower than are commonly found in sheep or cattle. Audige (1995) found the majority of FEC in weaner red deer to be below 200 eggs per gram faeces (epg), with a maximum of 650epg.

Attempts to correlate FEC with associated adult worm burdens have been complicated by low or zero FEC (even when up to 1000 adult worms were present; Wilson, 1981), low worm burdens associated with high FEC and very high variability of results (Schultz, 1993). Anderson and Wilson (1984) reported a linear relationship between total GI worm count and FEC, but of the deer that had worm burdens, less than half had detectable egg counts, and the majority of animals had either high or low FEC, with few in the mid-range. The interpretation of FEC as significant indicators of potential clinical disease in farmed deer has not been satisfactorily investigated, and egg counts should be interpreted with caution. English (1991) reported FEC of only 80-380epg in fallow deer that died from helminthosis in Australia.



Figure 1.5. Typical life cycle of trichostrongylid gastrointestinal parasites in red deer



1. Worm eggs passed in faeces
2. Eggs hatch in dung and develop from first to infective third stage (min 5 days\*)
3. Infective larvae consumed with herbage
4. Development to fourth stage and maturation to egg-laying adults in abomasum for *Ostertagia*-type and *T. axei* worms (18-26 days\*)
5. Some fourth stage *Ostertagia*-type larvae can become hypobiotic
- 6/7. Small and large intestinal larvae species mature to egg laying adults

\*There are no deer-specific data available so these time periods are from other ruminant animal species, based on *Ostertagia* spp.

### 1.8.2.2. Clinical Signs and Pathogenicity of GI Nematode Infections

Clinical signs of GI helminthiasis in deer include lowered growth or weight loss, rough hair or staring coat and loose faeces (Mylrea *et al.*, 1991) which can give a soiled tail and perineum. *Ostertagia*-type abomasal parasite infections have been associated with illthrift and/or death in red deer (Mason, 1977; Wagner and Mackintosh, 1993), farmed fallow deer (Presidente, 1979; English, 1991; Mylrea *et al.*, 1991) and associated with the so-called “fading elk (wapiti) syndrome” (Waldrup and Mackintosh, 1993).

Haemonchosis in deer has been associated with anaemia, lowered haemoglobin, packed cell volume and total serum protein (Foreyt and Trainer, 1970; Davidson *et al.*, 1980).

**Table 1.8. Percentage of weaner deer shedding faecal parasite eggs and lungworm larvae, and mean and range of geometric means (larvae/g) of positive faecal larval counts 1992-1994.**

(From Audige, 1995)

Year + month	No. weaners	Weaners with FEC>0 (%)			FLC>0 %	Geometric mean FLC>0		
		50	100-150	>=200		Mean	Min	Max
<b>February + March</b>								
1992†	129	19.4	13.2	9.3	65.9	14.5	0.3	162
1993‡	119	27.7	25.2	12.6	95.8	54.7	0.3	1807.5
1994‡	135	21.6	28.8	33.6	99.2	15.2	0.3	299.3
<b>June</b>								
1992	149	15.4	6.7	3.4	49	3.7	0.3	576.5
1993	139	20.1	15.8	2.2	48.9	3.6	0.3	173.3
<b>September</b>								
1992	150	18.7	6.7	1.3	70.7	4.3	0.3	108.5
1993	138	12.3	8	1.4	26.1	1.5	0.3	30.5
<b>November</b>								
1992	150	11.3	1.3	0	32.7	1.1	0.3	60.3

†Some farmers had drenched their calves before sampling ; ‡Calves were sampled before the commencement of anthelmintic treatment

FEC= faecal egg count (eggs per gram faeces); FLC= faecal larval count (larvae per gram faeces)

Detrimental effects of GI parasites of ruminants (not including deer) on feed intake, apparent digestibility and utilisation of energy and protein, skeletal mineral metabolism and tissue-protein metabolism which compromise animal growth and production have been extensively reviewed (Steel, 1974; Sykes, 1978; Holmes, 1985; Parkins and Holmes, 1989; Poppi *et al.*, 1990; Fox, 1993; Holmes, 1993; MacRae, 1993; Sykes, 1994; Coop and Holmes, 1996; Knox and Steel, 1996). However, the most significant effect of GI parasitism leading to reduced productivity of domestic ruminants is that of reduced N utilisation (Knox and Steel, 1996), and it is assumed that a similar situation exists for parasitised deer.

Lowered appetite alone has a pronounced effect on the N metabolism of infected animals. Increased N flow to the terminal ileum (Kimambo *et al.*, 1988; Poppi *et al.*, 1986) is caused by elevated endogenous protein loss from elevated gastroenteric plasma loss, increased turnover of intestinal epithelial cells and increased mucus production. This

incomplete reabsorption of proteins lost into the gut and increased protein turnover diverts amino acids away from productive processes. The overall effect on N metabolism is an increased urinary N excretion and a reduction in the efficiency of retention of apparently digested N and in total N-balance (Knox and Steel, 1996).

### **1.8.2.3. Diagnosis of GI Nematode Infections**

In the live animal, faecal egg counts (FEC) can be used to confirm patent GI nematode infections in deer but nematode species cannot be accurately determined from examining their eggs. Larval culture of eggs in faeces enables identification of larvae to generic level according to the criteria used for identifying larvae from other ruminants. However, it should be noted that the infective larvae of the individual species of deer-specific *Ostertagia*-type nematodes have not been described.

Elevated plasma pepsinogen levels have been associated with *H.contortus* (Johnston *et al.*, 1984) and mixed abomasal parasite infections in red deer (Wagner and Mackintosh, 1993) and elk (Mason, 1984).

At post-mortem, abomasal damage reported in deer includes varying degrees of hyperaemia, oedema, pitting and thickening of the abomasal mucosa or 'morocco leather appearance' (Presidente, 1979; Waldrup and Mackintosh, 1993); abomasal ulceration and fluid in the abdominal cavity (Prestwood and Kellogg, 1971); and abomasal haemorrhages (Conti and Howerth, 1987). Abomasal pH has been reported to be elevated in cases of helminthosis in deer (Connan, 1991; Wagner and Mackintosh, 1993; Waldrup and Mackintosh, 1993; Waldrup *et al.*, 1993).

Hypobiotic larvae of cervid *Ostertagia*-type nematodes have been found in the abomasal walls of red (Connan, 1991; 1997) and white-tailed deer (Baker and Anderson, 1975), as occurs in pre-type II ostertagiosis in cattle. Inhibition of larval development of *H.contortus* has also been seen in white-tailed deer (Foreyt and Trainer, 1970).

In sheep, small intestinal damage ranging from extensive villous atrophy, mucosal thickening and stunting of micro-villi in infections caused by *T.colubriformis*, to simple stunting of micro-villi caused by nematodiosis has been reported (Sykes, 1978). No descriptions of small intestinal damage have been reported for deer.

### **1.8.3. Susceptibility of Farmed Deer to Internal Parasitism**

The most susceptible deer are calves during their first autumn and early winter when both lungworm (Mason, 1994) and GI parasite larval availability is maximised on

pasture due to favourable environmental conditions. By July, weaners become more resistant to lungworm infection (Mason and Gladden, 1983) and this resistance persists in healthy, unstressed animals. By one year of age, young deer appear to have acquired some level of resistance to all internal parasites to which they have been exposed. Reduced intensity of infection with age is typical of *Dictyocaulus* infections in most cervids (Watson and Charleston, 1985). However, in a survey conducted by Schultz (1993) there was no age-related effect on abomasal parasite burdens in wild white-tailed deer. Schultz (1993) and Demarais *et al.*, (1983) found wild white-tailed bucks to have higher abomasal worm counts than does, but this sex difference has not been reported in any other deer species.

Young red, wapiti and fallow deer are all susceptible to lungworm infection, although there is some evidence to suggest that young fallow deer may be more resistant to infection than red or wapiti (Mackintosh, 1992). Both wapiti weaners and adults appear to be more susceptible to GI parasitism than red or fallow deer, and wapiti adults are thought to be more susceptible to lungworm infection than red and fallow deer adults (Mackintosh, 1992) although this is yet to be proven. "Fading elk syndrome" in Canadian wapiti (elk) and wapiti hybrids characterised by progressive weight loss, low serum albumin levels and, in some cases diarrhoea (Orr *et al.*, 1990) has in some situations been linked to *Ostertagia*-type parasitism (Waldrup and Mackintosh, 1993). Waldrup *et al.*, (1994a) found parasitism to have a greater effect on liveweight gains in wapiti hybrid weaner hinds than in red weaner hinds for three months post-weaning.

#### **1.8.4. Control of Internal Parasites in Farmed Deer**

Brunsdon (1980) stated that the aim of control is to ensure that parasite populations do not exceed levels compatible with economic production, as eradication of most helminth infections is not practical. This objective can be achieved by three interrelated approaches: grazing management, the use of anthelmintics and the utilization of natural or artificially-induced immunity (Brunsdon, 1980). Genetic selection for resistance may be an alternative approach to control (Watson, 1986). Effective, integrated control is dependent upon detailed understanding of the sequential inter-relationships between the various sources of pasture contamination, the availability of infective larvae and the build-up and decline of infections. A knowledge of the time course of events is also important (Brunsdon, 1980). Control measures should be designed to suit local environment and climatic conditions which largely govern the

overall patterns of larval availability, and cater for the type of animal production system, so will not necessarily be the same for every farm.

The higher the stocking rate the greater the risk of infection, although other factors such as altitude, climate and animal stress play a part in the epidemiology of parasite infections (Mason, 1985). Regular three-weekly faecal sampling and subsequent anthelmintic treatment (if required) combined with adequate animal nutrition and low animal stress should keep worm burdens adequately controlled using a minimum number of drenches (Wilson, 1984). Rotational grazing of deer on pasture and the use of safe pastures or crops (see section 1.8.4.2.) will help to ensure that exposure to infective larvae is minimised during an outbreak.

Live bovine lungworm vaccine has been evaluated for use in red deer in Scotland (Corrigall *et al.*, 1982; Corrigall *et al.*, 1986). However, results did not indicate any advantage to the vaccinated deer in weight gain or general health, and the use of this product has not become established.

#### **1.8.4.1. Anthelmintic Efficacy and Use**

There are a number of anthelmintics currently licensed for use in deer in NZ including benzimidazoles and the new generation milbemycin/ivermectin endectocides (McKellar and Benchaoui, 1996).

The literature reveals very few anthelmintic trials involving deer, relative to other farmed ruminants. Levamisole has been found to have only very limited activity against *Dictyocaulus* in red deer (Mason and Beatson, 1985) and black-tailed deer (Presidente *et al.*, 1973). Rhodes (1993) showed albendazole intra-ruminal sustained-release capsules lowered FEC compared with deer treated with oral albendazole, but did not prevent infection. Waldrup *et al.*, (1993, 1994b) also reported that albendazole-based sustained-release boluses did not prevent infection by lung and abomasal nematodes and found bolus-treated deer had significantly greater numbers of fourth stage *Ostertagia*-type larvae compared with deer treated with the liquid oral form of albendazole. Ivermectin has been reported to have a greater efficacy than benzimidazoles against lungworm in red deer (Mackintosh *et al.*, 1984; Mackintosh and Mason, 1985; Mackintosh *et al.*, 1990b) and in reducing FEC in fallow deer (Mylrea *et al.*, 1991).

Mackintosh and Mason (1985) reported 100% efficacy of injectable ivermectin (200µg/kg) against adult and immature lungworm. However, other research has shown doses of between 200µg/kg-500µg/kg of ivermectin to have poor efficacy against

predominantly immature lungworm (Mackintosh *et al.*, 1990b; Waldrup *et al.*, 1994a) and abomasal worms in deer (Connan, 1991; Mylrea *et al.*, 1991; Andrews *et al.*, 1993; Mackintosh *et al.*, 1993; Waldrup *et al.*, 1994a). Mackintosh *et al.*, (1990b) showed that topically applied ivermectin had a more persistent action than the oral formulation in young red deer, although the dose rate was more than twice that of the oral formulation. Ivermectin pour-on has been known to cause skin irritation and there are reports of both red and wapiti deer refusing to eat for a day or two after treatment (Haigh and Hudson 1993).

Mackintosh *et al.*, (1993) and Middleberg (1994) found that topical moxidectin (500µg/kg) is highly effective against adult and immature lungworm and abomasal parasites with no side-effects.

In order to control both lungworm and GI worms either in weaner deer on farms that have a history of parasite problems, or in situations where deer cannot be put onto safe pasture post-drenching, typically three-weekly drenching from weaning (March) until winter, and six-weekly drenching in spring has been advised when using benzimidazoles (Wilson, pers comm; Mason, 1979). This is assuming that the deer are becoming re-infected immediately after drenching, and that (lungworm) infections become patent in the minimum time of 20 days (Mason, 1994; Corrigan *et al.*, 1980). Extension of the three-week drenching interval is now possible given the use of the newer milbemycin/ivermectin anthelmintics which have persistent activity against lungworm (Bowie *et al.*, 1987; Mackintosh *et al.*, 1990a, 1990b, 1993; Mason *et al.*, 1990). However, different persistency claims are made for various products and different formulations of the same chemical, and this needs to be investigated in deer.

By drenching for lungworm infections, GI infections will also be controlled, as anthelmintics which control *Dictyocaulus* will remove GI nematodes as well.

Wilson (pers comm) has found that deer carrying burdens of lungworm sufficient to cause respiratory distress are best treated by giving one anthelmintic dose spread over three days (1/3 dose/day), because sudden death of a large number of adult *Dictyocaulus* worms in the lungs due to an anthelmintic can lead to blockages of bronchi and sudden death of the animal. Therapy which removes *Dictyocaulus* from the lungs will be effective and immediately restorative in red deer whereas in cattle severe lung lesions are likely to remain after worm removal (Corrigan, 1985).

Mason and Gladden (1983) conducted a survey of anthelmintic drenching practices on more than 100 NZ deer farms. Eighty-eight percent of farms had drenched

some or all of their stock in the past 12 months. The dose rates used differed markedly from the recommended dose rates, and there was a wide range in drenching frequency, which ranged from 0-17 times per year. The majority of farms drenched different classes of stock separately, with younger stock receiving the majority of drenches on an annual basis (see Table 1.9.). The majority of farms commenced treatment between February and April prior to the rut, with a smaller peak in June-July. Benzimidazoles were the most frequently used anthelmintics in 1983.

A 1992-94 survey of red deer farms (Audige, 1995) found weaners received their first drench anywhere from mid-January to the end of April, with the mean starting date being 9 March. A weaning weight increase of approximately 2.0-3.5 kilograms was estimated to be associated with each anthelmintic treatment administered before 1 April. The number of treatments ranged from 1-5 in autumn, 0-3 during winter and 0-2 in spring, or overall between 3-9 drenches in their first year. Drenching intervals ranged from 20-89 days, with some farms not drenching their weaners after mid-June; this was significantly associated with lower growth rates in winter and spring. The majority of farmers used avermectin/milbemycin-based anthelmintics, systematically or occasionally, while some used benzimidazole-based anthelmintics exclusively.

**Table 1.9. Mean number of annual anthelmintic treatments by farm and age/sex group**

(from Mason and Gladden, 1983)

	Sex/Age Group						
	Calves/Weaners		Hinds		Stags	Deer>1 year	
	R	F	R	F	R	R	F
<b>No Farms</b>	80	6	28	2	25	43	3
<b>Mean no. drenches/yr</b>	4.1	1.2	3.1	2.5	1.6	2.1	1.3

R=red deer, F=fallow deer

#### 1.8.4.2. Grazing Strategies

“Safe” pasture can be defined as pasture that has too few parasite larvae on it to be directly damaging to susceptible animals (Brunsdon, 1980). Pasture which is completely parasite free is almost impossible to achieve. Safe pasture can be achieved by

using pastures that have been cut for hay or silage, new pasture, fodder crops, by the use of mature cattle or sheep as ‘vacuum cleaners’ to clean up pastures following grazing by deer (Mason, 1985) or the use of older resistant deer to clean up after young deer (Brunsdon, 1980). It is common practice on NZ deer farms to use cattle for control of pasture growth and maintenance of pasture quality especially in late summer/autumn. The risk of cross-infection can be reduced by grazing adult cattle that should only carry very low numbers of parasites, or by drenching younger cattle before they are placed on deer pasture (Wilson and Collier, 1981).

For deer calves receiving their first drench at weaning when parasite burdens are already established (Audige, 1995), relatively “safe” pasture is that which has not yet been grazed by those calves in that year, which may only be a small proportion of the farm. However, if calves were to be regularly faecal sampled starting four weeks after birth and drenched when patent worm burdens are found then the issue of safe pasture would be avoided, and parasite infections can be more easily controlled for the rest of the year.

A farm survey by Wilson and Collier (1981) found the occurrence of lungworm in deer was less when rotational grazing methods were used, relative to set-stocked pastures. The benefits of rotational grazing are much greater again, if deer are put onto safe pasture after drenching.

## **1.9. THE USE OF FORAGE CROPS TO CONTROL INTERNAL PARASITES OF RUMINANTS**

The increasing incidence of drench resistance in farmed sheep, goats and cattle in NZ, combined with rising consumer concerns about chemical use on farms has encouraged research into alternative strategies to anthelmintic chemical control of internal parasites. Drench resistance has not yet been reported in deer, but the NZ deer industry promotes a clean, green, quality assured product CERVENA® which should therefore be produced using minimal chemical inputs.

Alternative management systems such as specialist forage crops may be the answer to decreasing anthelmintic use, and to reducing the incidence of drench resistance.

### **1.9.1. Plant Morphology and “Safe Crops”.**

Recent research has highlighted the potential use of different plant species grazed by ruminants to control GI parasitism.



Niezen *et al.*, (1993b) reported lower faecal egg counts from lambs grazing yorkshire fog or ryegrass than browntop or tall fescue despite similar grazing height in all species. In comparing forage species seeded with strongyle eggs Moss and Vlassoff (1993) observed fewer larvae on chicory and lucerne, compared with prairie grass or ryegrass. Scales *et al.*, (1995) also recovered fewer larvae from chicory than grass swards, suggesting that conditions in a chicory sward may be less suitable for larval development and/or migration. The authors suggested that the higher soluble carbohydrate and mineral content or low concentrations of condensed tannins in chicory may have better enabled the lambs to cope with parasitism.

The more erect, open, broadleaved growth habit of the herb chicory might not produce as suitable conditions for larval development in faeces at the base of the sward as occurs in a dense pasture sward and may lower the survival of infective larvae in water films on the leaves. The taller growth habit of forages like chicory or lucerne may also mean that animals may consume fewer larvae by not grazing as far down into the sward as they would on conventional grass-based pasture. Callinan and Westcott (1986) found that in ryegrass/clover pasture, 73% of infective stage larvae were recovered from within two centimetres of the soil-herbage interface and that larval migration was dependant on temperature and humidity. So distinct microclimates created within the canopy of plant species with differing morphology should be expected to affect larval migration, which in turn may affect animal uptake of infective larvae, and hence parasitism in grazing deer.

### **1.9.2. Condensed Tannins and Internal Parasites.**

It is generally accepted that animals on elevated planes of nutrition naturally express better resistance to infection and disease (Jagusch *et al.*, 1980; Watson, 1986). Parasitised animals exhibit reduce growth and production primarily due to the diversion of limited supplies of amino acids from productive processes into those essential for survival (see section 1.8.2.2.).

There is evidence to suggest that the effect of nutrition on parasitised animals varies with stage of infection. Dietary protein intake does not appear to influence initial parasite establishment in naive sheep, measured as worm burdens at slaughter, but the expression of disease has, in most cases, been found to be more severe in animals on lower levels of protein intake (Coop and Holmes, 1996). Protein intake has been shown to increase the resistance of sheep to haemonchosis (Wallace *et al.*, 1996), *O. columbianum* (Dobson and Bawden, 1974), and *T. colubriformis* (Kambara *et al.*, 1993;

van Houtert *et al.*, 1992) with the magnitude of effects even greater for ruminally protected protein. These effects of protein intake on resistance are influenced by the age of the animal. Limited information is available on the effects of protein supplementation on immune responses in parasitised ruminants (Coop and Holmes, 1996).

The infusion of protein directly into the abomasum of lambs infected with *T.colubriformis* resulted in a four-fold increase in N retention and improvements in the gross efficiency of metabolisable energy utilisation and reduced worm burdens in parasitised animals (Bown *et al.*, 1991a). Post-ruminal infusion of protein into lambs infected with *O.circumcincta* lowered faecal egg counts and mean worm burdens and accelerated the development of immunity to the parasite infection compared to lambs not supplemented with bypass protein (Coop *et al.*, 1995). This research clearly establishes that plane of nutrition and, in particular, duodenal protein supply can influence the ability of lambs to withstand the effects of parasitism and to mount an effective immunological response.

Low concentrations of condensed tannins (CT) have been shown to protect plant proteins against ruminal degradation (see section 1.7.3.) and to increase post-ruminal protein absorption in sheep (Waghorn *et al.*, 1987b). Therefore grazing animals on forages containing CT might reduce the effects of parasitism by increasing the amount of protein digested post-rationally. In addition, given the fact that CT bind specifically to proteins, it is conceivable that CT in the diet may have a direct effect on the parasites in the digestive tract.

Condensed tannin-containing forages, notably sulla and *Lotus* species have been shown to significantly boost the growth rate of parasitised lambs relative to non-CT containing forages, in the absence of anthelmintics (Waghorn and Niezen, 1994; Niezen *et al.*, 1994b, c, 1995; Robertson *et al.*, 1995). Parasitised lambs grazing CT-containing forages have exhibited significantly lower faecal egg counts (Niezen *et al.*, 1994b, 1994c, Waghorn and Niezen, 1994; Niezen *et al.*, 1995;) and abomasal and intestinal worm burdens than lambs grazing non-CT containing forages. The greatest responses in most trials was for animals grazing sulla (*Hedysarum coronarium*) and Robertson *et al.*, (1995) found lambs grazing sulla had 40% fewer worms at slaughter than lambs slaughtered pre-trial, which suggests that sulla can affect the persistence of established worm burdens. No effect on establishment of *O.circumcincta* and *T.colubriformis* in lambs fed either *Lotus corniculatus* or ryegrass was found by Neizen *et al.*, (1994, unpublished).

All research conducted to date investigating the effect of CT on internal parasites of ruminants has involved sheep only. The results indicate that although grazing forages containing CT will not prevent lambs from becoming parasitised, it can reduce the worm burden and negate many of the production losses due to parasitism and decrease the reliance on anthelmintic use for parasite control. However, the extent to which the results are due to the direct effects of CT alone, is not known. It is also to be established whether the effects seen in parasitised sheep will be similar with other ruminant species, such as deer.

Tannin containing legumes such as sulla, birdsfoot trefoil (*Lotus corniculatus*), lotus major (*Lotus pedunculatus*) and sainfoin (*Onobrychis viciifolia*) are all highly suitable for deer production given their high nutritive value, high summer-autumn DM production and drought tolerance, and the dietary preference deer have for legumes (see section 1.5.2.). They all have the potential to be used as specialist forage crops for venison production (see section 1.5.1.1.) with the added advantage of providing bypass protein for increased body protein deposition, and/or to overcome a protein deficiency caused by internal parasites. Incorporating these forages into a venison production system could potentially provide a means of producing venison under a system of reduced anthelmintic input.

## **1.10. CONCLUSION AND REQUIREMENTS FOR FURTHER RESEARCH**

**1.10.1.** The NZ deer industry is strongly export market-led and aims to produce quality-assured, natural, tender CERVENA® farm-raised venison from young deer all year round, with a premium paid for carcasses in the range 50-65kg. Most NZ deer farmers produce red deer for venison production for slaughter at an age of 15-24 months, through grazing convention PRG/WC pasture. However, it is preferable to produce carcass weights of 50-65kg during August-November, by slaughtering animals at and before one year of age. The NZ GIB marketing strategy, together with the on-farm quality assurance scheme presents NZ deer products as from a clean, green environment and focuses on natural production systems.

**1.10.2.** Temperate deer species exhibit strong seasonal patterns characterised by pronounced annual cycles of reproduction, VFI, growth and rumen metabolism. Because of the strong seasonality of VFI and LWG, optimum carcass weights for

efficient slaughter systems are not easily achievable in deer before one year of age. Young deer exhibit strong seasonality in N-deposition as body protein, which is maximised in spring and summer and minimised in winter.

**1.10.3.** The annual cycle of conventional PRG/WC pasture production and quality on NZ farms does not match that of annual deer feed requirements, which adds to the difficulty of achieving 50kg carcass weight by August-November by one year of age. Options for farmers include the use of specialist crops for deer that produce high DM yields of high nutritive value during the summer-autumn, or winter feed deficits, and/or the use of large terminal sires (wapiti or elk) to produce larger hybrid offspring with faster growth rates.

**1.10.4.** Combining the use of specialist forage crops during the summer, autumn and spring with 10cm PRG/WC pasture during winter, offers the maximum potential growth rates of young deer from birth to one year of age. Specialist crops evaluated for deer include chicory and red clover. However, other legume crops, especially those containing CT such as sulla, sainfoin and birdsfoot trefoil are yet to be evaluated with deer. Little research has been conducted into the use of winter forage crops.

**1.10.5.** Low concentrations of condensed tannins in forage plants have been shown to offer advantages to sheep production by protecting plant proteins against rumen degradation; increasing the flow of amino acids to the small intestine; and increasing the absorption of amino acids from the small intestine. However, research is required to study the effect of different concentrations of CT in temperate CT-containing forages on digestion and utilisation of absorbed nutrients, and general production of farmed deer in NZ. The minimum level of CT needed to increase ruminant animal production and the optimum level of CT for deer production needs to be defined, given that deer are more adapted to consuming tannin-containing diets than sheep.

**1.10.6.** Young deer in their first autumn and early winter are highly susceptible to internal parasites. Lungworm is considered to pose the greatest risk, followed by GI nematodes, especially the deer-specific abomasal *Ostertagia*-type nematodes. Control of these parasites in young deer is by systematic anthelmintic treatment. Little is

known about the epidemiology and pathogenicity of lungworm infections in young deer, and even less is known about GI nematode infections of deer. Scant data are available on the effect of various numbers of lungworm or GI nematodes on pathophysiology in deer. There are few published reports of experimental nematode infections of deer and the majority of artificial infections of deer with GI nematodes have not involved deer-specific parasites. The most significant effect of GI parasitism leading to reduced productivity of farmed ruminants is that of altered N-utilisation, leading to reduced protein deposition. However, research is required into the effect of internal parasites of young deer on production, and specifically on digestion, absorption, utilisation and deposition of protein. The dose rate of infective-stage lungworm and GI nematodes required to produce sub-clinical infection in weaner deer needs to be defined.

**1.10.7.** Forages containing CT have been shown to significantly increase the performance of parasitised lambs. It is thought that the increased by-pass protein supply caused by the action of CT in these forages helps counteract the protein deficiency caused by nematode infections, however more research is required to confirm this. It is also thought that CT may have a direct effect on inhibiting internal parasites *in-situ*, and research is needed to investigate this possibility. There has been no research on the effect of CT on the internal parasites of deer, or on lungworm infections of any farmed ruminant species. Given the emphasis the NZ deer industry places on a clean, green, natural product, research into use of CT-containing forages as alternative methods of parasite control to anthelmintics should be initiated.

## CHAPTER 2.

# Growth and carcass production of young farmed deer grazing sulla (*Hedysarum coronarium*), chicory (*Cichorium intybus*) or perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture

SIMONE ●. HOSKIN<sup>1</sup>, T.N. BARRY<sup>1</sup>, P.R. WILSON<sup>2</sup>, W.A.G. CHARLESTON<sup>2</sup> & P.D. KEMP<sup>3</sup>

<sup>1</sup>Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand

<sup>2</sup>Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand

<sup>3</sup>Institute of Natural Resources, Massey University, Palmerston North, New Zealand

### 2.1. ABSTRACT

A grazing experiment was conducted to compare the liveweight gain (LWG), voluntary feed intake (VFI) and carcass production of 33 pure red and hybrid (0.75 red:0.25 elk) deer calves grazing sulla (*Hedysarum coronarium*, cv Necton), with those grazing chicory (*Cichorium intybus*, cv Grasslands Puna) and perennial ryegrass (*Lolium perenne*, cv Nui)/white clover (*Trifolium repens* cv Huia) pasture from weaning in March to slaughter in December and to determine the proportion producing carcasses in the range 50-65kg by one year of age. Deer rotationally grazed all three forages at a constant daily dry matter allowance during autumn and spring, but chicory was not grazed during winter. Measurements were made of pre- and post-grazing herbage mass, botanical composition of feed on offer, chemical composition and *in-vitro* organic matter digestibility of feed on offer and diet selected, VFI, LWG, carcass weight (CW), carcass dressing out percentage (DR%), carcass fat depth (GR), weight and date of velvet antler harvested.

Organic matter digestibility (OMD) of diet selected was similar for all forages during autumn, but OMD of chicory (88.4%) was greater than both sulla (78.3%) and pasture (83.8%) during spring ( $P<0.05$ ). Sulla diet selected contained 5.1% condensed tannin (CT) in autumn and 8.4% CT in spring ( $P<0.05$ ), while pasture and chicory contained 0.14-0.26% CT. VFI of deer grazing sulla was greater than for deer grazing chicory in autumn (2027 versus 1014gOM/d;  $P=0.07$ ), but not spring (2029 versus 2251gOM/d). In autumn, deer gained 293g/d on sulla, 218g/d on pasture ( $P<0.01$ ) and 183g/d on chicory ( $P<0.001$ ). In winter, deer gained 150g/d on sulla and 133g/d on pasture. Final liveweight of deer grazing sulla was 106kg, which was significantly higher than 97kg for deer on pasture or 95kg for deer on chicory ( $P<0.01$ ). Deer grazing sulla had greater carcass weights (59.9kg) than deer grazing pasture (52.3kg) or chicory (52.1kg) ( $P<0.01$  hinds;  $P<0.05$  stags). The proportion of deer reaching 50-65kg carcass weight by one year of age was 100% for sulla and 89% for pasture and chicory. Carcass DR% was greater for deer grazing sulla (56.4%) than pasture (54.3%;  $P<0.05$ ).

Hybrid stags had significantly greater carcass weights than red stags (64.1kg versus 56.3kg;  $P<0.01$ ), and hybrid hinds (52.8kg;  $P<0.001$ ). Velvet antler production did not differ between forages, but red stags produced velvet 33 days earlier than hybrid stags ( $P<0.05$ ). It was concluded that the increased growth and carcass weight of young deer grazing sulla was caused by a higher feeding value of sulla, with a component of this being increased utilisation of digested nutrients.

## 2.2. INTRODUCTION

One of the New Zealand (NZ) deer industry aims is to produce quality-assured, tender, farm-raised venison (CERVENA®) from young deer all year round. A premium is paid for carcasses in the range 50-65kg. Most NZ farmers achieve optimum carcass weights for venison production at an age of 15-24 months (Drew, 1985; Barry and Wilson, 1994), through grazing perennial ryegrass/white clover (PRG/WC) pastures. However, it is more efficient to produce carcass weights of 50-65kg before 12 months of age, during August-November (spring), which attract a seasonal premium related to market demand.

Red deer exhibit seasonal patterns of voluntary feed intake (VFI) and liveweight gain (LWG) (Kay, 1979; Suttie *et al.*, 1987), making achievement of optimum carcass weights before 12 months of age difficult (Audige, 1995). Most PRG/WC pastures are unable to produce the quality and quantity of feed required for high deer growth rates during summer and autumn, the period of inherent maximum potential VFI and LWG in deer.

Special purpose forages such as red clover (*Trifolium pratense*) (Niezen *et al.*, 1993a; Semiadi *et al.*, 1993) and chicory (*Cichorium intybus*) (Kusmartono *et al.*, 1996a), which produce high dry matter (DM) yields of high nutritive value particularly during summer and autumn enable 100% of red deer stags to reach 92kg liveweight (50kg carcass weight) by one year of age, compared with 73% (range 25-90%) of those grazing PRG/WC pasture of 10cm surface height.

Sulla (*Hedysarum coronarium*) is an erect, tap-rooted, biennial forage legume adapted to semi-arid areas, and is the main forage legume in Italy and Sicily, used for hay, silage and greenfeed (Watson, 1982). Sulla was introduced into NZ for soil conservation purposes but was subsequently recognised as a palatable, high quality herbage capable of producing high lamb growth rates (Terrill *et al.*, 1992b). Sulla contains condensed tannins (30-70g/kgDM) and significantly increases the growth rate of parasitised lambs compared with grazing non-tannin containing forages (Niezen *et al.*, 1994b, 1995). Sulla is of interest for deer production because of its tall growth habit, high summer growth rates, drought tolerance, high annual DM production (Rys *et al.*, 1988; Krishna *et al.*, 1990) and ready acceptance by grazing deer (Hunt and Hay, 1990). Unlike other forage legumes, sulla exhibits some winter growth (Krishna *et al.*, 1990), but current cultivars exhibit poor persistence under grazing.

The objectives of this study were to compare the growth, VFI and carcass weight of red and hybrid deer (0.75 red: 0.25 elk) calves grazing sulla, with those grazing chicory and perennial ryegrass/white clover pasture from weaning to slaughter at one year of age when internal parasites were controlled by regular anthelmintic treatment. This study was conducted at the same time and on the same experimental area as Chapter 3., hence the same chicory and pasture were used in both investigations.

## **2.3. MATERIALS AND METHODS**

### **2.3.1. Trial Design**

A rotational grazing experiment was conducted with 33 weaner deer (3-4 months old) grazed on either PRG/WC pasture, chicory, or sulla (each forage n=11). Deer grazing each forage were balanced for genotype (pure red v. 0.75 red:0.25elk hybrid), sex and liveweight. Measurements included LWG, VFI, velvet production and carcass characteristics at slaughter. The trial was carried out at the Massey University Deer Research Unit (DRU),



Palmerston North from 17 March to 28 November 1994, and was divided into autumn (71 days), winter (116 days) and spring (68 days) periods.

### 2.3.2. Forages

The forages grazed were: established perennial ryegrass (cv Nui)/ white clover (cv Huia) pasture (Plate 2.1.); chicory (cv Grasslands Puna), sown December 1992 (Plate 2.2.); and sulla (cv Necton) sown November 1993 (Plate 2.3.). Potassic super-phosphate (9%P:10%S:7%K) was applied onto all forages in late April 1994 at 250kg/ha, corresponding to 22.5kgP/ha. Nitrogen fertiliser (Urea; 46%N) was applied to chicory (76kgN/ha), pasture (56kgN/ha) and sulla (53kgN/ha) in late March, and to pasture (60kgN/ha) and chicory (76kgN/ha) in early August. In autumn, chicory paddocks were mechanically topped following initial grazing to remove reproductive stem material to maintain the vegetative state. In winter 1994, the chicory and one sulla paddock were sprayed with herbicide (Galant; Dow-Elanco, NZ Ltd; 3L/ha) to control grasses. In spring, pasture paddocks becoming long and rank were removed from the rotation.

### 2.3.3. Animals

Seven pure red hinds, eight pure red stags, 10 0.75 red: 0.25 elk hybrid hinds and eight 0.75 red:0.25 elk hybrid stags were used. Mean initial liveweight ( $\pm$  SD) was 51.0 ( $\pm$  6.19)kg. On 28 February 1994, the calves were weaned, weighed, sexed, vaccinated against clostridial infections (Clostridial 5 in 1; "Ultravac" CSL Ltd, NZ) and yersiniosis (Yersiniavax; AgVax Developments Ltd, NZ) by subcutaneous injection into the anterior of the neck, and treated orally with ivermectin ("Ivomec" 0.4%w/v at 200 $\mu$ g/kg liveweight; Merck, Sharp and Dohme, NZ). Booster vaccinations were given 30 days later. On 15 March, the calves were ear tagged and reweighed, and were randomly assigned to the three forages on 17 March, with the three groups balanced for liveweight, sex and genotype. On 3 October, all deer were given 12g cupric oxide/animal orally (Copper Needles, Bayer NZ Ltd) and a 3ml injection of vitamin B12 (Prolaject 1mg vitB12/ml; Bomac Laboratories Ltd, NZ) subcutaneously in the anterior of the neck. All deer were treated three-weekly with ivermectin as above until six weeks before slaughter and were weighed at three-weekly intervals and immediately prior to slaughter.

Velvet antler was removed when 20cm in length by the method described by Semiadi *et al.*, (1993), and weight and date of removal recorded.

#### **2.3.4. Grazing Management**

Deer were rotationally grazed throughout the trial, with allowances (excluding dead matter) set at 5kgDM/hd/day from 17 March – 1 September (autumn and winter), 6kgDM/hd/day from 1 September – 19 September (late winter), and 7kgDM/hd/day from 19 September to slaughter on 28 November (spring). Rotation length was 4-7 weeks, with grazing periods of 4-7 days for chicory and 5-10 days for pasture and sulla.

In autumn and spring, the deer grazed either chicory (1.20ha), pasture (1.20ha) or sulla (1.25ha autumn; 0.84ha spring). In winter, the chicory and pasture groups were combined on pasture (3.66ha) because chicory is dormant during winter. Deer were only able to graze sulla for 26 days during winter despite adequate available forage, because of wet soil conditions causing pugging damage to the crop. They grazed pasture (0.90ha) during the remaining winter.

In spring, the chicory-grazed group returned to chicory and the sulla-grazed group to sulla, while the pasture group returned to paddocks they had grazed in autumn.

#### **2.3.5. Forage Sampling and Measurements**

Pre- and post-grazing herbage mass was measured by taking cuts to soil level from six quadrats (0.1m<sup>2</sup>) from each paddock for DM determination (100°C, 18hrs); enabling calculation of grazing days (Semiadi *et al.*, 1993) according to the allowance set. Samples of feed on offer were taken from each paddock at the commencement of grazing, mixed and divided into two 200g parts and stored at -20°C. Samples for botanical composition were dissected into grasses, clover (red and white together), dead matter and weed (PRG/WC pasture), and for sulla and chicory, stem and leaf (separately), clover, dead matter and weed. Each component was separately oven-dried (100°C, 18hrs) and weighed. During autumn and spring, hand-plucked samples estimating deer diet selected were taken daily (Kusmartono *et al.*, 1996a), pooled per paddock and stored at -20°C for chemical analysis.

#### **2.3.6. Voluntary Feed Intake**

In late autumn and spring, intra-ruminal, slow-release chromium capsules (CRDC, Cr<sub>2</sub>O<sub>3</sub> matrix, Nufarm, NZ) were administered to all deer to estimate faecal organic matter output (Parker *et al.*, 1989). Rectal faecal samples were collected at two-day intervals from days 8-22 post CRDC insertion. Samples were oven dried (100°C, 36hrs; minimum 2g dry weight/animal), pooled per animal, and ground for chromium analysis.

### 2.3.7. Slaughter

Twenty-six deer were slaughtered at the Feilding Deer Slaughter Premises (Venison Packers NZ Ltd) on 28 November 1994, with seven red hinds remaining at Massey University Deer Research Unit as breeding replacements. Final liveweight was measured before transport. Hot carcass weight, carcass grade and carcass GR, an indirect measure of subcutaneous fat depth measured as soft tissue depth over the 12th rib, 16cm from the dorsal midline, were recorded for each animal.

### 2.3.8. Laboratory Analysis

Samples of feed on offer and diet selected were freeze dried and ground to pass a 1mm sieve (Wiley Mill, USA). Organic matter (OM) content was determined by ashing overnight at 555°C. *In vitro* organic matter digestibility (OMD) was determined by incubation with fungal cellulase and hemicellulase enzymes (Roughan and Holland, 1977). Total nitrogen (N) was determined by the Kjeldahl method (Kjeltec Auto 1030 Analyser, Tectator, Sweden). Extractable and bound condensed tannins were determined by the modified butanol-HCl procedure of Terrill *et al.*, (1992a), and extractable condensed tannins were also determined by the vanillin-HCl procedure of Broadhurst and Jones (1978). Extractable CT values (%OM; Butanol-HCl procedure) were deducted from *in vitro* OMD determinations because extractable CT would be solubilised in the initial *in vitro* steps, but is known to be indigestible *in vivo* (Terrill *et al.*, 1994). Faecal chromium concentration was determined by atomic absorption spectrometry, as described by Costigan and Ellis (1987).

### 2.3.9. Calculations and Data Analysis

Faecal output (FO) was calculated according to Equation 2.1.

$$\text{Equation 2.1.} \quad \text{FO (gOM/day)} = \frac{\text{Cr release rate (RR)(mg/day)}}{\text{Faecal Cr concentration (mg/gOM)}}$$

Voluntary feed intake (VFI) was calculated using Equation 2.2., using organic matter digestibility (OMD) from estimated diet selected (hand-plucked samples), as described by Kusmartono *et al.*, (1996a).

**Equation 2.2.**

$$\text{VFI (gOM/day)} = \text{FO (gOM/day)} / \text{1 - OMD}$$

Liveweight gain and VFI were compared within each season (autumn, winter and spring) using Generalised Linear Models (GLM; SAS 6.11, 1996; SAS Institute Inc. USA), with forage type, animal genotype, sex and any interaction as factors. Other factors analysed were final liveweight, carcass weight, velvet antler weight and removal date. Carcass weight was used as a covariate for carcass GR measurements. There were no interactions involving forage, sex and genotype for liveweight, liveweight gain or for any of the carcass measurements; hence only main effects are presented.

## **2.4. RESULTS**

### **2.4.1. Herbage Mass and Botanical Composition**

Pre- and post-grazing herbage masses are presented in Table 2.1.. The pre- and post-grazing herbage mass of sulla was higher than for chicory and pasture, particularly during spring when sulla became reproductive and produced thick stems up to 1m tall. However, the average daily DM allowance per animal was constant for all forages. The pre- and post-grazing herbage mass of chicory was slightly lower than that of pasture in autumn and spring.

Botanical composition of swards is presented in Table 2.2.. The sulla sward increased in purity during the trial, while the ratio of sulla leaf:stem decreased from 8.2:1 in autumn to 1.1:1 in spring, with the sulla sward reaching 10-15% flowering by late spring. The white plus red clover components of the sulla sward decreased from autumn to spring, while the dead matter content decreased from autumn to winter and spring.

The chicory sward decreased in purity from autumn to spring. This was accompanied by an increase in the white and red clover content of chicory. The ratio of chicory leaf:stem increased from 1.9:1 in autumn to 20:1 in spring. The dead matter content of chicory decreased from autumn to spring, whilst the maximum weed content of both sulla and chicory was c.10%.

Perennial ryegrass constituted 60% of the pasture sward in autumn, increasing to 80% in winter and spring. The white clover decreased from autumn to winter and spring, whilst dead matter content remained relatively constant.

**Table 2.1. Seasonal pre- and post-grazing herbage mass (kgDM/ha  $\pm$ S.E.) of perennial ryegrass/white clover pasture, chicory or sulla grazed deer.**

Season	PASTURE			CHICORY			SULLA		
	n	Pre-grazing	Post-grazing	n	Pre-grazing	Post-grazing	n	Pre-grazing	Post-grazing
<b>Autumn</b>	6	2960	1852	8	2852	1777	8	4008	2626
S.E.		352.9	128.4		160.1	79.4		258.7	183.0
<b>Winter</b>	28	2377	1413	-	-	-	4	5225	3588
S.E.		80.8	40.3		-	-		1486.4	663.4
<b>Spring</b>	6	3130	2185	11	2819	1801	3	9658	4664
S.E.		332.6	413.0		95.4	62.8		1235.9	1311.7



**Plate 2.1. Perennial ryegrass/white clover pasture grazed in early spring.**



Plate 2.2. Chicory grazed in early spring.



Plate 2.3. Sulla grazed in early spring.

**Table 2.2. Seasonal botanical composition (% DM  $\pm$  S.E.) of perennial ryegrass/white clover pasture, chicory or sulla on offer.**

	<b>PASTURE</b>	<b>CHICORY</b>	<b>SULLA</b>
<b>Season/Species</b>		<b>Autumn</b>	
No. samples	6	8	8
Ryegrass	58.9 $\pm$ 3.53	-	-
Sulla/Chicory leaf	-	46.0 $\pm$ 3.51	50.1 $\pm$ 3.71
Sulla/Chicory stem	-	24.5 $\pm$ 4.55	6.1 $\pm$ 1.97
Clover*	27.6 $\pm$ 2.04	4.1 $\pm$ 1.21	12.8 $\pm$ 2.57
Weed	3.5 $\pm$ 1.49	5.9 $\pm$ 1.57	9.0 $\pm$ 2.25
Dead matter	10.0 $\pm$ 4.94	19.5 $\pm$ 2.79	22.0 $\pm$ 3.70
		<b>Winter</b>	
No. samples	28	-	4
Ryegrass	78.1 $\pm$ 1.50	-	-
Sulla/Chicory leaf	-	-	57.8 $\pm$ 6.17
Sulla/Chicory stem	-	-	19.8 $\pm$ 7.03
Clover*	7.2 $\pm$ 0.98	-	6.8 $\pm$ 1.82
Weed	1.1 $\pm$ 0.34	-	9.9 $\pm$ 2.68
Dead matter	13.6 $\pm$ 1.26	-	5.7 $\pm$ 1.85
		<b>Spring</b>	
No. samples	6	11	3
Ryegrass	81.8 $\pm$ 1.92	-	-
Sulla/Chicory leaf	-	53.2 $\pm$ 3.10	41.6 $\pm$ 4.28
Sulla/Chicory stem	-	2.7 $\pm$ 1.06	38.6 $\pm$ 4.67
Clover*	6.6 $\pm$ 0.27	24.1 $\pm$ 2.24	3.2 $\pm$ 1.37
Weed	2.4 $\pm$ 0.71	9.4 $\pm$ 2.41	10.0 $\pm$ 1.59
Dead matter	9.2 $\pm$ 1.44	10.6 $\pm$ 2.74	6.6 $\pm$ 0.59

Clover for pasture = white clover; clover for chicory/sulla = white and red clover

#### 2.4.2. Nutritive Value of Forages

For all forages, diet selected was generally higher in total N, organic matter (OM) and organic matter digestibility (OMD) than the feed on offer (Table 2.3). Sulla diet selected in autumn was higher in OM content than chicory, but pasture had a higher OM content of diet selected in autumn than both sulla and chicory ( $P<0.01$ ). There was a trend for OMD of diet selected in autumn for chicory to be greater than both sulla and pasture ( $P=0.11$ ), but there was no difference in OMD of diet selected in autumn between sulla and pasture. Sulla diet selected in autumn was significantly higher in extractable-CT (Butanol-HCl,  $P<0.05$ ; Vanillin-HCl,  $P<0.01$ ), protein and fibre-bound CT ( $P<0.01$ ) and total CT ( $P<0.05$ ) than both chicory and pasture, which contained only trace levels of CT (Table 2.4.). There were no significant differences in total N, OMD or OM content of feed on offer of pasture and sulla grazed during winter.

The OM content of diet selected in spring did not differ significantly between sulla and pasture, both of which had a higher OM content than chicory ( $P<0.01$ ). The OMD of diet selected in spring for chicory was greater than sulla and pasture ( $P<0.05$ ), but there was no difference in OMD of spring diet selected between sulla and pasture ( $P=0.14$ ). Sulla diet selected in spring was significantly higher in extractable-CT (Butanol-HCl,  $P<0.001$ ; Vanillin-HCl,  $P<0.01$ ), protein and fibre-bound CT ( $P<0.05$ ) and total CT ( $P<0.01$ ) than chicory and pasture (Table 2.4.). The total CT content of sulla increased from 5.1% in autumn to 8.4% in spring, associated with a rise in extractable-CT. Protein-bound and fibre-bound CT concentrations of sulla exhibited a decrease from autumn to spring.



**Table 2.3. Chemical composition (mean  $\pm$  S.E.) of forage on offer and diet selected by deer grazing either perennial ryegrass/white clover pasture, chicory or sulla.**

Season	PASTURE		CHICORY		SULLA	
	On Offer	Selected	On Offer	Selected	On Offer	Selected
<b>Total Nitrogen (% DM)</b>						
Autumn	3.02 $\pm$ 0.27	4.16 $\pm$ 0.17	3.01 $\pm$ 0.11	3.79 $\pm$ 0.11	3.29 $\pm$ 0.61	4.00 $\pm$ 0.19
Winter	3.69 $\pm$ 0.11	-	-	-	3.91 $\pm$ 0.27	-
Spring	2.35 $\pm$ 0.25	3.08 $\pm$ 0.11	2.91 $\pm$ 0.12	3.36 $\pm$ 0.23	2.17 $\pm$ 0.10	3.27 $\pm$ 0.22
<b>Organic Matter (% DM)</b>						
Autumn	89.0 $\pm$ 0.67	90.1 $\pm$ 0.55	83.1 $\pm$ 0.55	82.1 $\pm$ 0.55	85.7 $\pm$ 0.60	87.4 $\pm$ 0.55
Winter	86.5 $\pm$ 0.79	-	-	-	87.0 $\pm$ 1.29	-
Spring	87.6 $\pm$ 1.12	91.6 $\pm$ 0.38	86.3 $\pm$ 0.85	87.0 $\pm$ 0.38	89.3 $\pm$ 1.13	91.0 $\pm$ 0.38
<b>Organic Matter Digestibility (%OM)</b>						
Autumn	67.7 $\pm$ 4.03	82.7 $\pm$ 0.54	83.1 $\pm$ 0.55	85.2 $\pm$ 1.05	72.0 $\pm$ 2.80	81.9 $\pm$ 0.98
Winter	79.0 $\pm$ 0.95	-	-	-	77.0 $\pm$ 2.38	-
Spring	78.4 $\pm$ 1.06	83.8 $\pm$ 2.34	86.3 $\pm$ 0.85	88.4 $\pm$ 0.03	73.9 $\pm$ 1.30	76.7 $\pm$ 0.20

Chicory was dormant during winter  
n=5, 12, 4 for pasture on offer during autumn, winter and spring respectively; n=7, 8 for chicory on offer in autumn and spring; n=5, 3, 4 for sulla on offer during autumn, winter and spring respectively; n=4, 3 for diet selected in autumn and spring respectively for all forages.

**Table 2.4. Condensed tannin concentration (%DM  $\pm$  range) of diet selected by deer grazing either perennial ryegrass/white clover pasture, chicory or sulla.**

	Season	PASTURE	CHICORY	SULLA
<b>Extractable</b>	Autumn†	0.89 $\pm$ 0.015	0.78 $\pm$ 0.070	4.35 $\pm$ 0.680
	Spring†	0.64 $\pm$ 0.025	0.49 $\pm$ 0.105	7.41 $\pm$ 0.670
	Autumn‡	0.06 $\pm$ 0.000	0.05 $\pm$ 0.005	3.51 $\pm$ 0.101
	Spring‡	0.06 $\pm$ 0.010	0.07 $\pm$ 0.015	7.34 $\pm$ 0.535
<b>Protein-bound ‡</b>	Autumn	0.17 $\pm$ 0.030	0.14 $\pm$ 0.015	1.35 $\pm$ 0.110
	Spring	0.05 $\pm$ 0.010	0.07 $\pm$ 0.025	0.91 $\pm$ 0.180
<b>Fibre-bound ‡</b>	Autumn	0.04 $\pm$ 0.005	0.07 $\pm$ 0.005	0.24 $\pm$ 0.025
	Spring	0.02 $\pm$ 0.000	0.04 $\pm$ 0.005	0.18 $\pm$ 0.050
<b>Total ‡</b>	Autumn	0.26 $\pm$ 0.020	0.26 $\pm$ 0.015	5.10 $\pm$ 1.145
	Spring	0.14 $\pm$ 0.005	0.17 $\pm$ 0.025	8.42 $\pm$ 0.770

n=2 for all forages in both seasons

†=Vanillin-HCl

‡=Butanol-HCl

### 2.4.3. Voluntary Feed Intake

During autumn, VFI (Table 2.5.) of deer grazing sulla was greater than for deer grazing chicory ( $P=0.07$ ), with the pasture group being intermediate and not significantly different from either sulla or chicory. In spring, there were no significant differences in VFI between the forage groups. There was no effect of genotype on VFI in either season. VFI of stags was greater than hinds, but this effect only reached significance during spring ( $P=0.07$ ).

### 2.4.4. Liveweight and Liveweight Gain

Liveweight gain (LWG; Table 2.5.) of stags was higher than that of hinds during winter and spring ( $P<0.001$ ), and the growth of hybrid deer was greater than pure red deer during winter ( $P<0.05$ ) and spring ( $P<0.001$ ). During autumn, LWG of deer grazing sulla was significantly higher than that of deer grazing chicory ( $P<0.001$ ) or pasture ( $P<0.01$ ).

Table 2.5. Voluntary feed intake (gOM/d), liveweight (kg) and liveweight gain (g/d) of deer grazed on perennial ryegrass/white clover pasture, chicory or sulla.

Forage...	PASTURE		CHICORY		SULLA		S.E. D.F.= 27
	Stags	Hinds	Stags	Hinds	Stags	Hinds	
Number of deer	5	6	5	6	6	5	
<b>Voluntary feed intake</b>							
Autumn	1900	1494	1029	1011	2224	1831	282.4
Spring	2408	1868	2365	2173	2556	1763	225.1
<b>Mean liveweight</b>							
Initial (15/3/94)	50.9	50.8	54.8	48.3	51.7	50.1	1.08
End autumn (25/5/94)	66.5	62.1	64.3	61.8	69.2	66.8	1.33
End winter (19/9/94)	87.1	73.5	84.5	71.6	94.3	80.6	1.83
End spring (27/11/94)	106.2	87.5	105.8	85.8	116.3	95.1	2.48
<b>Liveweight gain</b>							
Autumn (71 days)	224	214	154	203	315	268	11.4
Winter (116 days)	172	95	166	66	196	106	9.5
Spring (68 days)	289	211	344	236	333	220	13.6

During winter, LWG of both sulla and pasture groups was significantly higher ( $P<0.01$ ) than the chicory group. In spring, LWG of deer grazing all three forages was similar. Despite the higher LWG gain of the deer grazing sulla during autumn, there was no significant difference in liveweight (Table 2.5.) at the end of autumn, between the forage groups. However, at the end of winter and spring the sulla group had a higher liveweight than both the pasture and chicory groups ( $P<0.01$ ), with the latter two groups being similar. Stags were heavier than hinds at the end of both winter and spring ( $P<0.001$ ) and hybrid deer were heavier than pure red deer at the end of spring ( $P<0.05$ ).

#### **2.4.5. Carcass Production**

Data in Table 2.6. show that 100% of deer grazing sulla reached the target of 92kg liveweight (50kg carcass weight) by one year of age, whereas 88% and 89% of deer grazing chicory and pasture respectively, met this target. Deer grazing sulla had greater carcass weights than deer grazing chicory or pasture ( $P<0.01$  hinds;  $P<0.05$  stags). Regardless of forage grazed, hybrid stags had significantly higher carcass weights than hybrid hinds ( $P<0.001$ ), and carcass weights of hybrid stags were higher than for pure red stags (64.1 versus 56.3kg;  $P<0.01$ ). After being adjusted to equal carcass weight, there were no differences in carcass subcutaneous fat depth (GR) due to forage, sex or genotype. Carcass dressing out percentage (DR) was greater for stags grazing sulla, than those grazing both chicory ( $P<0.05$ ) and pasture ( $P<0.01$ ), with hybrid stags on all forages having a higher DR than pure red stags ( $P<0.05$ ). Carcass DR of hinds was greater on sulla than on pasture ( $P<0.05$ ), but there was no difference between GR of hinds that grazed chicory and any other forage ( $P=0.20$ ).

#### **2.4.6. Velvet Antler Production**

Velvet antler production shown in Table 2.7. did not differ significantly ( $P>0.10$ ) between forages. Date of first cut was earlier for red stags than hybrid stags (33 days;  $P<0.05$ ) and was negatively correlated with liveweight at the end of winter, with each 10kg increase in liveweight advancing date of velvetting by an average of  $6.8 \pm 8.4$  days (SE). The difference between red and hybrid stags was even greater when date of first cut was corrected to equal liveweight at the end of winter (49 days;  $P<0.01$ ). There was a tendency for stags on sulla to be velvetted earlier than stags on chicory and pasture, but this effect disappeared when the data was corrected for equal liveweight.

Table 2.6. Carcass production of red and hybrid stags and hybrid hinds grazed on perennial ryegrass/white clover pasture, chicory or sulla.

Sex...	STAGS (red + hybrid)			S.E.	HINDS (hybrid)			S.E.
	Pasture	Chicory	Sulla		Pasture	Chicory	Sulla	
Forage...				D.F. =12				D.F.=5
No. deer	5	5	6		4	3	3	
No. reaching target CW	5	4	6		2	3	3	
Carcass weight (kg)	57.9	57.1	65.6	2.29	50.0	50.1	58.2	1.45
Dressing percent (%)*	54.2	54.9	56.4	0.40	55.1	56.0	57.0	0.39
GR tissue depth (mm)#	5.9	6.5	9.7	1.72	4.0	4.3	8.7	1.42

\*dressing percentage calculated using final liveweight off pasture prior to being trucked

#adjusted to equal carcass weight

**Table 2.7. Velvet antler production from red and hybrid yearling stags grazing either perennial ryegrass/white clover pasture, chicory or sulla.**

Forage...	PASTURE		CHICORY		SULLA		S.E.#
	R	H	R	H	R	H	
Total number of stags	3	2	2	3	3	3	
Stags velveted	3	1	2	3	3	3	
Weight first cut (g)	153	301	166	148	125	186	15.8
<b>Mean date (first cut)</b>							
Uncorrected	14 Oct	24 Nov	27 Oct	14 Nov	25 Sept	7 Nov	13.8
Corrected*	7 Oct	12 Dec	7 Oct	10 Nov	2 Oct	25 Nov	10.0

\* Corrected by covariate to equal liveweight at end of winter; #D.F.=9

## 2.5. DISCUSSION

This is the first report of production from farmed deer grazing sulla. The most significant result from this experiment was the greater autumn LWG and carcass weight achieved from weaner deer grazing sulla, compared with deer grazing pasture or chicory. In addition, 100% of red and hybrid stags and hybrid hinds grazing sulla reached the target of at least 92kg carcass weight (50kg carcass weight or greater) by one year of age, compared with 89% of deer grazing chicory and pasture. The increased carcass production on sulla was mostly due to higher LWG during autumn and early winter. The advantage of sulla in increasing deer LWG during late autumn and winter is in part agronomic, in that it has capacity for cool season growth, relative to other special-purpose forages for deer production such as chicory and red clover which are dormant during winter.

Increases in animal productivity or feeding value of a forage can result from increased VFI, higher digestibility or improved utilisation of digested nutrients (Ulyatt, 1973). Terrill *et al.*, (1992b) reported greater VFI in lambs grazing sulla than perennial ryegrass/white clover pasture and there was some indication of this during autumn in the present study (Table 2.5.), but this did not reach statistical significance due to the high variability found. Feed intakes of deer grazing pasture and sulla in spring were similar, and despite the high pre- and post-grazing herbage mass/ha of sulla caused by its large, fibrous, reproductive stem fraction, forage allowances were maintained at a constant level. It seems that OMD was not

a factor in the superior feeding value of sulla in this study, as apparent digestibility of organic matter (OMD) of both pasture and sulla were similar in autumn, with digestibility of sulla slightly lower than pasture in spring.

The high feeding value for deer on sulla compared with pasture could be partially due to improved utilisation of digested nutrients. Condensed tannins in forage legumes (*Lotus* sp.) fed to sheep have been shown to reduce degradation of protein in the rumen, to increase amino acid absorption from the small intestine (Barry *et al.*, 1986; Waghorn *et al.*, 1987b) and to increase utilisation of plasma cysteine for body synthetic reactions (McNabb *et al.*, 1993; Wang *et al.*, 1994). Hart and Sahlu (1993) reported greater LWG and mohair production, and reduced rumen ammonia concentration from young goats grazing sainfoin (moderate CT content) compared with lucerne (containing traces of CT). Stienezen *et al.*, (1996) found CT in sulla reduced apparent N-digestibility, rumen ammonia and plasma urea concentration when fed to sheep and it is proposed that similar effects could occur in deer. Wang *et al.*, (1996a,b) found the action of CT improved the efficiency of wool and milk production in sheep grazing *Lotus corniculatus* (35 gCT/kgDM) without affecting VFI. The CT in sulla may similarly have improved the efficiency of the growth process in young deer.

It is not known whether VFI of deer grazing sulla in spring was affected by the high total CT content (8.4%). Total CT concentrations of 5-10% in *Lotus pedunculatus* and mulga (*Acacia aneura*) have been shown to reduce VFI in sheep (Barry and Duncan 1984; Waghorn *et al.*, 1990; Pritchard *et al.*, 1992). However, the relationship between CT concentration and VFI of deer has not been established. Any depression of VFI by high dietary CT concentrations in deer may not be as marked as in sheep and this needs to be determined in future studies. Deer species such as mule deer and moose have adapted to consuming tannin-containing diets by the production of CT-binding salivary proteins (Austin *et al.*, 1989; Hagerman and Robbins, 1993). These CT-binding proteins are present in red deer saliva (Semiadi *et al.*, 1995), at lower concentrations than mule deer, but the nutritional significance of this for red deer is unknown.

Velvet antler production did not differ between forages in this experiment, although only a small number of stags were used. The effect of genotype on velvet harvest date in this experiment was similar to that reported by Kusmartono *et al.*, (1996a), except that in this experiment the magnitude of the effect was greater.

In contrast to the results of Kusmartono *et al.*, (1996a), which found deer grazing chicory had superior carcass production, greater autumn and spring LWG and VFI and higher total velvet weight than deer grazing pasture, there were no differences in production

between deer grazing those forages in the present experiment. The chicory used in this experiment differed from that used by Kusmartono *et al.*, (1996a) in several respects; the most important being the lower levels of post-grazing herbage mass (1789kgDM/ha versus 2386kgDM/ha), lower autumn forage allowance/animal (5kgDM/deer/day versus 7kgDM/deer/day) and the chicory stand used in this experiment was older (3 years versus 1 year) and therefore contributed a lower percentage of sward DM. These factors probably combined to lower VFI for deer grazing chicory compared with pasture in autumn in the present experiment, whereas the reverse occurred in the study of Kusmartono *et al.*, (1996a). The low LWG of the chicory group compared with the pasture group during winter cannot be explained.

Deer grazing chicory exhibited lower LWG than deer grazing sulla during autumn and winter, but not spring. Possible factors contributing to this include the low ratio of leaf:stem in autumn and high ratio of leaf:stem in spring for chicory compared with sulla, the significantly higher OMD of diet selected for chicory compared with sulla in spring, and the low herbage mass/ha which probably restricted VFI of the deer grazing chicory during autumn.

The effects of sex and genotype on LWG, VFI and carcass production seen in this experiment are comparable to other reports (Semiadi *et al.*, 1993; Kusmartono *et al.*, 1996a), although there were no significant interactions between forage and sex or forage and genotype in this experiment.

Given the high carcass weights and DR% of deer grazing sulla, there is a place for it to be incorporated into commercial deer farms in NZ, especially in areas that experience dry summer conditions. However, sulla is slow to establish and only persists under grazing for one-two years. More research is required to investigate management strategies to increase persistency before this new forage crop can be successfully used for deer production.

To maximise deer production from chicory it is essential to graze to a greater post-grazing herbage mass/ha than used in the present study. A post-grazing herbage mass of >2100kgDM/ha as used by Kusmartono *et al.*, (1996a) is recommended. It is also important reproductive stem development is controlled prior to autumn grazing by weaner deer and chicory sward purity is maintained.

In conclusion, carcass weights of 50-65kg can be achieved by 12 months of age by grazing young deer on sulla. The highest carcass weights were from hybrid stags grazed on sulla, as the genetic potential for growth in hybrid deer is best expressed with superior nutrition (Kusmartono *et al.*, 1996a). Research is required to investigate the effects of CT in



sulla on VFI, digestibility and efficiency of utilisation of absorbed nutrients for growth and carcass production.

## CHAPTER 3.

# Effects of reducing anthelmintic input upon growth and faecal egg and larval counts in young farmed deer grazing chicory (*Cichorium intybus*) and perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture.

SIMONE O. HOSKIN<sup>1</sup>, T.N. BARRY<sup>1</sup>, P.R. WILSON<sup>2</sup>, W.A.G. CHARLESTON<sup>2</sup>, J. HODGSON<sup>3</sup>

<sup>1</sup> Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand

<sup>2</sup> Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand

<sup>3</sup> Institute of Natural Resources, Massey University, Palmerston North, New Zealand

### 3.1. ABSTRACT

A rotational grazing experiment using weaner deer was conducted at Palmerston North, New Zealand, during the autumn, winter and spring of 1994 to compare the voluntary feed intake (VFI), liveweight gain (LWG), carcass and velvet production of deer grazing chicory with those grazing perennial ryegrass/white clover pasture. Deer were either treated with anthelmintic three-weekly (T) or anthelmintic was withheld until trigger-treatment (TT) criteria were attained. Pure red and 0.75 red:0.25 elk hybrid stags and hinds were given forage allowances of 5kgDM/deer/day in autumn and early-mid winter, 6kgDM/deer/day in late winter and 7kgDM/deer/day in spring. Deer grazed chicory or pasture in autumn and spring, with all deer combined on pasture during winter when chicory was dormant.

Perennial ryegrass comprised 60-80% of pasture on offer, whilst chicory comprised 56-70% of forage on offer. Organic matter digestibility of diet selected was greater for

chicory than pasture in both autumn and spring.

Withholding anthelmintic treatment significantly reduced VFI and LWG of deer grazing pasture in autumn, contributing to lower carcass weights of pasture TT deer compared with pasture T deer, but had no effect upon the productivity of deer grazing chicory. Clinical signs of lungworm were evident in pasture TT deer during autumn and winter, and in chicory TT deer grazing pasture during winter. Faecal egg counts (FEC) were significantly greater for pasture TT deer during autumn and early winter than all other groups. Faecal lungworm larval counts (FLC) were significantly greater for chicory TT deer following transfer to pasture, than for all other groups in early winter, although both FEC and FLC were low. Faecal larval counts were poorly related to clinical signs of lungworm infection during autumn, but were a better guide in winter. Plasma pepsinogen concentrations appeared unrelated to gastrointestinal parasite infection. Trigger-treated deer grazing pasture required five anthelmintic treatments during autumn and winter. The chicory TT group required no anthelmintic treatment when grazing chicory, but required two treatments after transfer from chicory to pasture during winter. There was no effect of anthelmintic regime or forage on velvet antler production, VFI and LWG in spring. Hybrid deer had greater carcass weights than red deer when grazing chicory, but similar carcass weights when grazing pasture.

Further research is needed to investigate the epidemiology and pathogenicity of internal parasite infections and the influence of plant morphology and chemical composition on parasitism in deer grazing different forage species.

### **3.2. INTRODUCTION**

One of the New Zealand (NZ) deer industry aims is to produce quality-assured, natural, tender, farm-raised venison (CERVENA®) from young deer all year round. A premium is paid for carcasses in the range 50-65kg. Most NZ farmers produce red deer for slaughter at an age of 15-24 months (Drew, 1985; Barry and Wilson, 1994), through grazing perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) (PRG/WC) pastures, with the regular use of anthelmintics to control internal parasites. However, it is more efficient to produce carcass weights of 50-65kg before 12 months of age, during August-November (spring), which attract a seasonal premium. It is also desirable to minimise anthelmintic usage, to lower cost and to reduce the risk of development of anthelmintic resistance and carcass chemical residues.

Red deer exhibit seasonal patterns of voluntary feed intake (VFI) and liveweight gain

(LWG) (Kay, 1979; Suttie *et al.*, 1987), making achievement of optimum carcass weights before 12 months of age difficult (Audige, 1995). Most PRG/WC pastures are unable to produce the quality and quantity of feed required for high deer growth rates during the summer-autumn period of inherent maximum potential VFI and LWG in deer.

Young deer in their first autumn and early winter are highly susceptible to internal parasites (Audige *et al.*, 1998). Lungworm (*Dictyocaulus viviparus*) is considered to pose the greatest risk (Wilson, 1984), followed by gastrointestinal nematodes, especially deer-specific abomasal *Ostertagia*-type (*Spiculopteragia sp.*, *Skrjabinagia sp.* etc) nematodes (Connan, 1991; Waldrup and Mackintosh, 1992). Audige *et al.*, (1998) observed that frequency of anthelmintic treatment and the interval between treatments varied markedly between NZ red-deer farms. A significant liveweight increase was associated with each autumn anthelmintic treatment for calves grazing conventional ryegrass-based pasture rates (Audige *et al.*, 1998). Deer calves not treated after mid-June exhibited lower winter and spring growth rates (Audige *et al.*, 1998).

Special purpose forages such as red clover (*Trifolium pratense*; Niezen *et al.*, 1993a; Semiadi *et al.*, 1993) and chicory (*Cichorium intybus*; Kusmartono *et al.*, 1996a), which produce high dry matter (DM) yields of high nutritive value particularly during summer and autumn, enable 100% of red deer stags to reach 92kg liveweight (50kg carcass weight) by one year of age, compared with 73% (range 25-90%) of those grazing PRG/WC pasture of 10cm surface height. Three-weekly anthelmintic treatment was administered during autumn and winter to prevent a confounding effect of parasitism in those trials.

Moss and Vlassoff (1993) and Scales *et al.*, (1995) observed fewer parasite larvae on chicory compared with several different grass-based swards, suggesting that conditions in a chicory sward caused by the different plant morphology may be less suitable for larval development and migration. Scales *et al.*, (1995) suggested that the higher soluble carbohydrate and mineral content and presence of condensed tannins (CT) in chicory may have better enabled lambs grazing chicory to cope with parasitism, compared with lambs grazing different grass-based swards. There are no reports on the effect of chicory on parasitism in deer.

The objective of this study was to determine the growth, VFI, carcass weight and faecal excretion of gastrointestinal nematode eggs and lungworm larvae in young red and hybrid (0.75 red:0.25 elk) deer calves grazing chicory or PRG/WC pasture from weaning to slaughter at one year of age, with and without regular anthelmintic treatment to control internal parasites. This study was conducted at the same time and on the same experimental

area as that described in Chapter 2.; hence the same chicory and pasture grazed by treated (but not trigger-treated) deer were used in the two investigations.

### **3.3. MATERIALS AND METHODS**

#### **3.3.1. Experimental Design**

A rotational grazing experiment was conducted with 44 weaner deer (three-four months old) grazed on either PRG/WC pasture (n=22) or chicory (n=22). Deer grazing each forage were balanced for genotype (pure red deer *versus* 0.75 red:0.25 elk hybrid), liveweight and sex. Half the deer were regularly treated at three-weekly intervals with an oral anthelmintic to control internal nematode parasites. Half remained untreated until pre-defined trigger levels of faecal gastrointestinal parasite eggs or lungworm larvae were reached or deer exhibited clinical signs of parasitism. Treated (T) and trigger-treated (TT) deer were grazed as separate groups and each was rotated around different halves of the eight paddocks of each forage used.

Factors investigated included LWG, VFI and velvet antler production of the deer, faecal gastrointestinal nematode egg (FEC) and lungworm larval counts (FLC), serum pepsinogen levels and carcass characteristics at slaughter. The experiment was carried out at the Massey University Deer Research Unit (DRU), Palmerston North, for 255 days from 15 March 1994 to 27 November 1994, and was divided into autumn (71 days), winter (116 days) and spring (68 days) periods.

#### **3.3.2. Forages**

The forages grazed were established perennial ryegrass (cv Nui)/ white clover (cv Huia) pasture (Plate 2.1.) and chicory (cv Grasslands Puna) (Plate 2.2.), sown December 1992. Both forages were grazed by hinds and calves and yearling deer in the six months prior to the start of the trial. Potassic superphosphate (9%P:10%S:7%K) was applied to both forages in late April 1994 at 250kg/ha, corresponding to 22.5kgP/ha. Nitrogen fertilizer (Urea; 46%N) was applied to both forages in late March (chicory 76kgN/ha; pasture 56kgN/ha) and early August (chicory 76kgN/ha; pasture 60kgN/ha). In autumn, chicory paddocks were mechanically topped following initial grazing to remove reproductive stem material to maintain the vegetative state. In winter 1994 the chicory was sprayed with herbicide (Galant; Dow-Elanco, NZ Ltd; 3L/ha) to control grasses. In spring, pasture paddocks becoming long and rank were removed from the rotation.

### 3.3.3. Animals

Nine pure red hinds, 10 pure red stags, 13 0.75 red:0.25 elk hybrid hinds and 12 0.75 red:0.25 elk hybrid stags were used. Mean initial liveweight ( $\pm$  SD) was 50.6 ( $\pm$  6.23)kg. On 28 February 1994, the fawns were weaned, weighed, sexed, vaccinated against clostridial infections (Clostridial 5 in 1; “Ultravac” CSL Ltd, NZ) and yersiniosis (Yersiniavax; AgVax, AgResearch, NZ) by subcutaneous injection into the anterior of the neck, and treated orally with ivermectin (“Ivomec” oral, 0.4%w/v at 200 $\mu$ g/kg liveweight; Merck, Sharp and Dohme, NZ). Booster vaccinations were given 30 days later. On 15 March the weaners were ear tagged and reweighed. On 17 March they were randomly assigned to the four treatment groups based on liveweight, and balanced as far as possible for sex and genotype. On 3 October all deer were given 12g cupric oxide/animal orally (Copper Needles, Bayer NZ Ltd) and a 3ml injection of vitamin B12 (Prolaject 1 mg vitB12/ml; Bomac Laboratories Ltd, NZ) subcutaneously in the anterior of the neck.

Deer were weighed and a rectal faecal sample was taken for FEC and FLC at three-week intervals. Plain blood samples (10ml) for serum pepsinogen analysis were taken six-weekly by jugular venipuncture using vacutainers (Hemogard, Becton-Dickinson, New Jersey, USA). Samples stood at room temperature for two hours before being centrifuged (20min; Minifuge-T, Heraeus, Separationstechnik, Gispshuhlenweg, Germany). Sera were stored at -20°C. Spiker velvet antler was removed when 20cm in length by the method described by Semiadi *et al.*, (1993), and weight and date of removal recorded.

Treated animals (T) were administered oral anthelmintic three-weekly, until six weeks before slaughter. All deer in a trigger-treated (TT) group were given anthelmintic (“Ivomec” oral, 0.4%w/v at 200 $\mu$ g/kg liveweight; Merck, Sharp and Dohme, NZ) when one or more of the following conditions were met:

- a) One or more animals in the group exhibited clinical signs of parasitism.
- b) The group mean average for lungworm larvae exceeded 150 larvae/g faeces, with 100% prevalence.
- c) An individual animal in the group exceeded 500 lungworm larvae/g faeces.
- d) Eggs of strongyle nematodes exceeded a group mean average of 1000/g faeces.

Anthelmintic usage was recorded.

### 3.3.4. Grazing Management

Deer were rotationally grazed throughout the trial, with allowances (excluding dead matter) for all animals being set at 5 kgDM/hd/day from March 17 until September 1

(autumn and winter), 6 kgDM/hd/day from September 1 to September 19 (late winter), and 7 kgDM/hd/day from September 19 to slaughter on November 28 (spring). Rotation length was 4-7 weeks, with grazing periods of 4-7 days for chicory and 5-10 days for pasture. Each paddock was bisected with an electric fence (5-strand, Gallagher Electronics, Hamilton, NZ), and the T and TT groups were randomly allocated to one half each (Plate 3.1.). Treatment groups rotated to return to the same side of each paddock, so that no cross-grazing occurred.

In autumn and spring the deer grazed either chicory (2.39ha), or pasture (2.46ha). In winter the chicory and pasture groups were combined on pasture (3.66ha) due to chicory becoming dormant, with the two anthelmintic treatment groups continuing to graze separately.



**Plate 3.1. Method of paddock bisection using a five-strand electric fence. This shows winter grazing with TT groups on the left and T groups on the right.**

### **3.3.5. Forage Sampling and Measurements**

Pre- and post-grazing herbage mass was measured by taking cuts to soil level from six quadrats (0.1m<sup>2</sup>) per each half paddock for DM determination enabling calculation of grazing days (Semiadi *et al.*, 1993) according to the allowance set. Samples of herbage on offer were taken from each whole paddock at the commencement of grazing, mixed and divided into two 200g parts and stored at -20°C. Samples for botanical composition were dissected into grasses, clover (red plus white), dead matter and weed (for PRG/WC pasture); and chicory stem and leaf separately, clover, dead matter and weed (for chicory). Each component was separately oven-dried at 100°C for 18hrs, and weighed. During autumn and spring, hand plucked samples estimating deer diet selected were taken daily (Kusmartono *et al.*, 1996a), pooled per paddock and stored at -20°C for chemical analysis.

### **3.3.6. Voluntary Feed Intake**

In autumn and in spring intra-ruminal, slow release chromium capsules (CRDC, Cr<sub>2</sub>O<sub>3</sub> matrix, Nufarm, NZ) were administered to all deer to estimate faecal organic matter output (Parker *et al.*, 1989). Rectal faecal samples were collected at 2-day intervals during days 8-22 post CRDC insertion. Samples were oven dried at 100°C for 36hrs (minimum 2g dry weight/animal), pooled per animal, and ground for chromium analysis.

### **3.3.7. Slaughter Procedure**

Thirty five deer were slaughtered at the Fielding Deer Slaughter Premises (Venison Packers NZ Ltd) on 28 November 1994, with 9 pure red hinds remaining at Massey University Deer Research Unit as breeding replacements. Final liveweight was measured before transport. Hot carcass weight, carcass grade and carcass GR, an indirect measure of subcutaneous fat depth measured as soft tissue depth over the 12th rib, 16cm from the dorsal midline, was recorded for each animal.

### **3.3.8. Laboratory Analysis**

Samples of diet selected were freeze dried and then ground to pass a 1mm sieve (Wiley Mill, USA). Organic matter (OM) content was determined by ashing overnight at 555°C. *In vitro* organic matter digestibility (OMD) was determined by incubation with fungal cellulase and hemicellulase enzymes (Roughan and Holland, 1977) and total nitrogen (N) was determined by the Kjeldahl method (Kjeltec Auto 1030 Analyzer, Tectator, Sweden). Total condensed tannins were determined by the modified butanol/ HCl procedure



of Terrill *et al.*, (1992a). Faecal chromium concentration was determined by atomic absorption spectrometry, as described by Costigan and Ellis (1987). Serum pepsinogen levels were assayed according to the method described by Pomroy and Charleston (1989).

Faecal samples for FEC were refrigerated within 2hrs of sampling (4°C) and FEC were determined within 7 days of sampling using a modified McMaster technique (Stafford *et al.*, 1994), where a count of one egg was equivalent to 50 eggs/g faeces. Faecal lungworm larval counts were determined using a modified Baermann technique (Hendriksen, 1965). Within 2hrs of sampling, 4g of fresh faeces were suspended in muslin cloth in a 40ml vial of tepid water to allow larvae to migrate to the bottom of the vial, to be counted the following day.

### **3.3.9. Calculations and Data Analysis**

Voluntary feed intake was calculated using organic matter digestibility (OMD) from estimated diet selected (hand-plucked samples) and faecal output as described in Chapter 2. (section 2.3.8.), using Equations 2.1. and 2.2. Carcass dressing-out percentage (DR) was calculated using final liveweight when the animals were transported to slaughter, prior to overnight fasting preceding slaughter.

Live weight gain, faecal egg and larval counts, serum pepsinogen concentration and VFI were compared within each season (autumn, winter and spring) using Generalised Linear Models (GLM; SAS 6.11; 1996, SAS Institute Inc. USA), with forage type, anthelmintic treatment, animal genotype, sex and interactions as factors. Liveweight gain at three-weekly intervals throughout the experiment was also analyzed using repeat measures analysis. Other factors analyzed at the end of the experiment were final liveweight, carcass weight and velvet antler weight and harvest date. Initial liveweight was used as a covariate in the analyses for live weight and carcass weight, and carcass weight was used as a covariate for carcass GR.

## **3.4. RESULTS**

### **3.4.1. Herbage Mass and Botanical Composition**

Table 3.1. shows similar pre- and post-grazing herbage mass of both chicory and pasture swards in autumn. However, in spring the pre- and post grazing herbage mass of chicory was lower than for pasture. There was no difference ( $P=0.10$ ) in pre- and post grazing herbage mass between areas grazed by T and TT deer for both forages.

Detailed seasonal botanical composition of chicory and pasture has been presented in Chapter 2. (Table 2.2.), and only a brief summary is given here. Perennial ryegrass constituted  $59 \pm 3.5(\text{SE})\%$  of the pasture sward in autumn, and  $82 \pm 1.9\%$  in spring. The white clover component of pasture ranged from  $28 \pm 2.0\%$  in autumn to  $7 \pm 0.3\%$  in winter and spring, whilst dead matter comprised 10-14%. Chicory content of feed on offer ranged from  $71 \pm 4.0\%$  in autumn to  $56 \pm 2.1\%$  in spring. The ratio of chicory leaf:stem was 1.9:1 in autumn and 20:1 in spring. The dead matter content of chicory was  $20 \pm 2.8\%$  in autumn and  $11 \pm 2.7\%$  in spring. The weed component of chicory increased from  $6 \pm 1.6\%$  in autumn to  $9 \pm 2.4\%$  in spring, while the weed component of pasture was consistently lower than for chicory and was similar in autumn and spring ( $3 \pm 1.1\%$ ).

**Table 3.1. Seasonal pre- and post-grazing herbage mass (kgDM/ha  $\pm$ S.E.) of perennial ryegrass/white clover pasture or chicory.**

Season	PASTURE			CHICORY			
	Treatment	n	Pre-grazing	Post-grazing	n	Pre-grazing	Post-grazing
<b>Autumn</b>							
	Treated	6	2960 $\pm$ 352.9	1852 $\pm$ 128.4	8	2852 $\pm$ 160.1	1777 $\pm$ 79.4
	Trigger-treated	6	2879 $\pm$ 236.1	1909 $\pm$ 169.2	8	2813 $\pm$ 189.3	1799 $\pm$ 114.7
<b>Winter</b>							
	Treated	14	2377 $\pm$ 80.8	1413 $\pm$ 40.3	-	-	-
	Trigger-treated	14	2310 $\pm$ 110.5	1377 $\pm$ 50.1	-	-	-
<b>Spring</b>							
	Treated	6	3130 $\pm$ 332.6	2185 $\pm$ 413.0	6	2819 $\pm$ 95.4	1801 $\pm$ 62.8
	Trigger-treated	6	3219 $\pm$ 401.9	2105 $\pm$ 447	6	3011 $\pm$ 126.4	1771 $\pm$ 144.8

**Table 3.2. Seasonal chemical composition (%DM  $\pm$  S.E.) of diet selected by deer grazing either perennial ryegrass/white clover pasture or chicory.**

	Season	PASTURE	CHICORY
Total N	Autumn	4.16 $\pm$ 0.17	3.79 $\pm$ 0.11
	Spring	3.08 $\pm$ 0.11	3.36 $\pm$ 0.23
OM	Autumn	90.1 $\pm$ 0.55	82.1 $\pm$ 0.55
	Spring	91.6 $\pm$ 0.38	87.0 $\pm$ 0.38
OMD (% OM)	Autumn	82.7 $\pm$ 0.54	85.2 $\pm$ 1.05
	Spring	83.8 $\pm$ 2.34	88.4 $\pm$ 0.03
Total CT	Autumn	0.26 $\pm$ 0.020	0.26 $\pm$ 0.015
	Spring	0.14 $\pm$ 0.005	0.17 $\pm$ 0.025

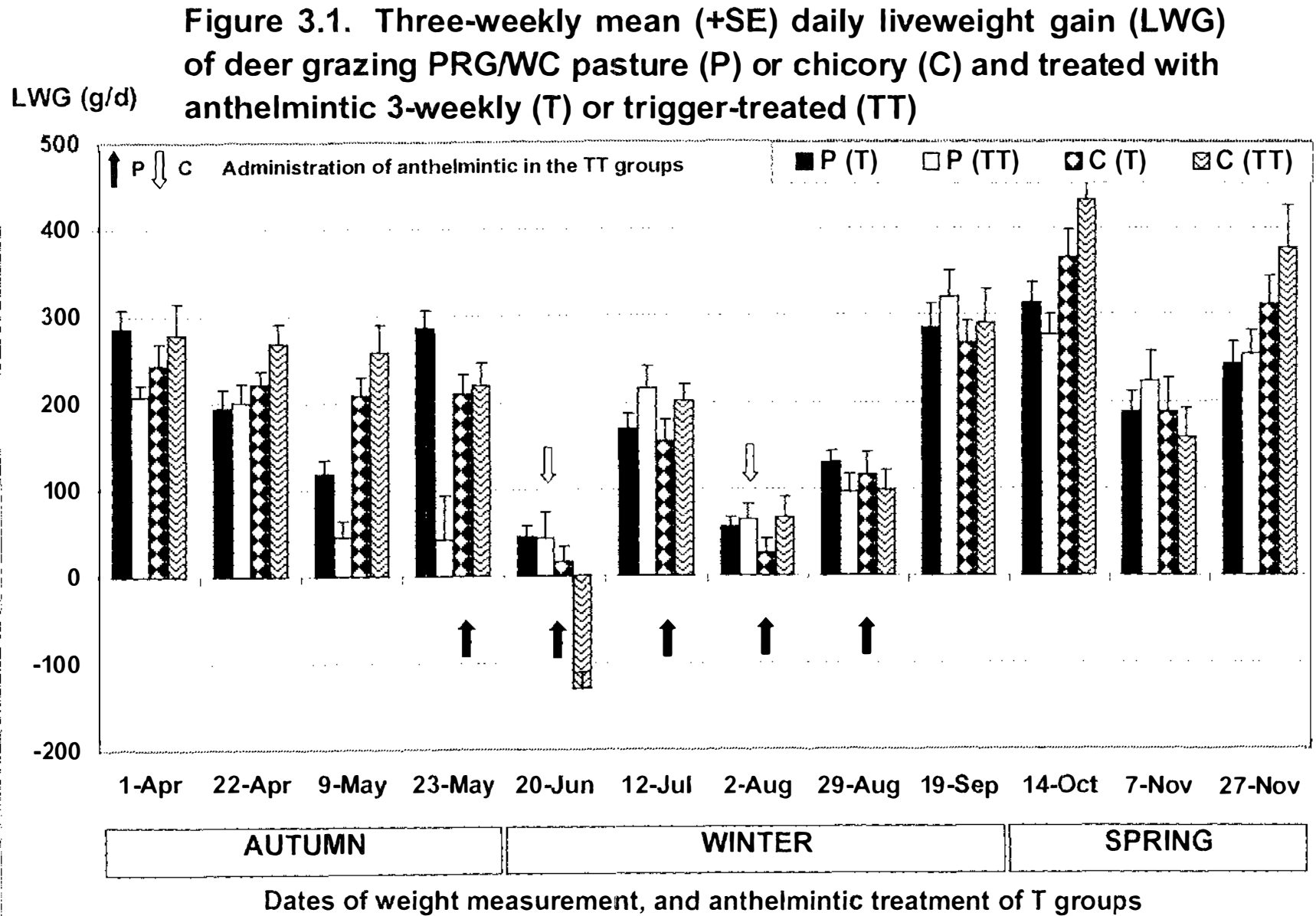
n=4 for each forage in each season

### 3.4.2. Nutritive Value and Chemical Composition of the Diet Selected

Seasonal chemical composition of diet selected is presented in Table 3.2. Chicory diet selected had a significantly lower OM content than pasture in autumn ( $P<0.01$ ) and spring ( $P<0.01$ ). Organic matter digestibility of diet selected for chicory was higher than pasture in both autumn ( $P=0.08$ ) and spring ( $P<0.05$ ). There was no difference in total nitrogen (N) content between chicory and pasture diet selected in autumn or spring ( $P=0.10$ ). Low concentrations of condensed tannins (CT) were measured in both pasture and chicory selected. The total CT content of both forages were very similar in both seasons, with total CT concentration being higher in autumn than in spring.

### 3.4.3. Liveweight Gain and Anthelmintic Treatment

Figure 3.1. shows the times of trigger treatment in the TT groups and LWG at three-weekly intervals throughout the trial. Treated deer on both forages received anthelmintic on 11 occasions, at 3 week intervals. Repeat measures analysis showed a significant effect of time on LWG ( $P<0.01$ ) and a significant forage\*anthelmintic treatment\*time interaction ( $P<0.01$ ). The latter can be explained by LWG decreasing in the TT pasture group during



late autumn and in the chicory TT group grazing PRG/WC pasture during early winter. Trigger-treated deer grazing pasture were first treated with anthelmintic on May 17 (late autumn) because of weight loss of some animals, bouts of coughing and laboured breathing and reduced VFI. Treatment was repeated on June 20 (early winter) after further reduced weight gain and coughing. Following this, it was decided to treat the pasture-fed TT group at four further three-weekly intervals, as per the pasture T group during the remaining winter period (Fig. 3.1.). The TT chicory group received two anthelmintic treatments, both during winter when they were grazing PRG/WC pasture, when clinical signs were observed as in the pasture TT group.

Table 3.3. shows the average autumn LWG and final autumn liveweight of the TT pasture group was lower than that of the other three groups ( $P < 0.01$ ). Over the entire winter period the average LWG of both pasture groups was significantly higher than both chicory groups ( $P < 0.01$ ), with no effect of anthelmintic regime. The winter final liveweight of the pasture TT group remained significantly lower than the pasture T group ( $P < 0.01$ ). During spring, the mean LWG of deer grazing chicory was greater than for deer grazing pasture ( $P < 0.05$ ), with no effect of anthelmintic regime. The final spring liveweight of the pasture TT group was significantly lower than the pasture T group ( $P < 0.05$ ) only, with no significant differences between the two chicory groups. There was no effect of genotype on winter LWG and final liveweight at the end of winter. However, during autumn hybrid deer gained  $200 \pm 9.1(\text{SE})\text{g/d}$  compared with red deer that gained  $170 \pm 9.9\text{g/d}$  ( $P < 0.05$ ) and during spring hybrid deer gained  $296 \pm 10.1\text{g/d}$ , compared with red deer that gained  $237 \pm 11.6\text{g/d}$  ( $P < 0.001$ ). A significant forage\*genotype interaction for spring LWG ( $P < 0.05$ ) was found where hybrid deer on chicory gained  $331 \pm 13.0\text{g/d}$ , red deer on chicory gained  $238 \pm 14.5\text{g/d}$ , hybrid deer on pasture gained  $260 \pm 13.0\text{g/d}$  and red deer on pasture gained  $233 \pm 15.2\text{g/d}$ . Hybrid deer reached  $96 \pm 1.2\text{kg}$  liveweight at the end of spring, compared with  $91 \pm 1.4\text{kg}$  for red deer ( $P < 0.05$ ). Stags gained weight faster than hinds during autumn ( $188 \pm 9.5$  v  $181 \pm 9.5\text{g/d}$ ;  $P < 0.05$ ), winter ( $159 \pm 5.7$  v  $81 \pm 5.9\text{g/d}$ ) and spring ( $323 \pm 10.8$  v  $209 \pm 10.9\text{g/d}$ ;  $P < 0.001$ ). Final liveweight was greater for stags than hinds ( $P < 0.001$ ) at the end of winter ( $81 \pm 0.9$  v  $73 \pm 0.9\text{kg}$ ) and spring ( $102 \pm 1.3$  v  $86 \pm 1.3\text{kg}$ ), but not autumn ( $61 \pm 0.8$  v  $62 \pm 0.8\text{kg}$ ). There were no interactions involving sex, genotype, forage or anthelmintic treatment for final liveweight for any season, or for LWG during autumn and winter.

**Table 3.3. Seasonal voluntary feed intake (gOM/d), liveweight (kg) and liveweight gain (g/d) of deer grazed on perennial ryegrass/white clover pasture or chicory and treated with anthelmintic 3-weekly or trigger-treated.**

Forage...	PASTURE		CHICORY		S.E.
	Treated	Trigger-treated	Treated	Trigger-treated	
Number of deer	11	11	11	11	
<b>Voluntary feed intake</b>					
Autumn	1920	835♦	1015	1150♦	127.3
Spring	1539	1739	1765	1631	55.2
<b>Mean liveweight</b>					
Initial (15/3/94)	50.4	49.8	51.1	49.2	0.95
End autumn (25/5/94)	63.8	57.2	62.0	64.0	1.20
End winter (19/9/94)	80.0	74.5	77.0	76.7	1.94
End spring (27/11/94)	96.4	90.0	94.7	94.5	1.42
<b>Liveweight gain</b>					
Autumn (71 days)	217	125	184	212	8.7
Winter (116 days)	133	138	115	95	7.4
Spring (68 days)	249	238	288	291	12.4

D.F. = 40. ♦ VFI was measured prior to anthelmintic treatment being given to these groups.

#### 3.4.4. Voluntary Feed Intake

Voluntary feed intake (Table 3.3.) of deer grazing chicory was similar for both anthelmintic treatments during autumn and spring. The VFI of the pasture TT group was lower than the pasture T group in autumn ( $P<0.01$ ), but not spring. During autumn, the VFI of both chicory TT ( $P<0.05$ ) and treated ( $P<0.01$ ) groups was lower than the pasture T group, but in spring VFI of all groups was similar. There was a trend towards higher VFI of hybrid deer than red deer in autumn ( $1443 \pm 150.7$  v  $997 \pm 167.8$ g/d;  $P=0.06$ ) and spring ( $1748 \pm 56.6$  v  $1588 \pm 66.0$ g/d;  $P=0.07$ ). Stags exhibited significantly higher VFI than hinds in spring ( $1884 \pm 62.0$  v  $1453 \pm 59.9$ g/d;  $P<0.001$ ), but not autumn ( $1366 \pm 158.5$  v  $1073 \pm 158.6$ g/d;  $P=0.20$ ). There were no interactions involving sex, genotype, forage or anthelmintic treatment for VFI during autumn or spring.

#### 3.4.5. Carcass Production

Carcass data is presented in Table 3.4. For deer grazing chicory, 88% of both T and TT deer reached the target of 92kg liveweight (50kg carcass weight) by one year of age, whilst for pasture, 56% of TT and 78% of T deer reached this target. An initial analysis of carcass weight (CW) data revealed no treatment effects, despite treatment effects on final liveweight. The statistical analysis was repeated with initial liveweight as a covariate, when a significant forage\*anthelmintic interaction was found ( $P=0.011$ ), with CW of the pasture TT group being significantly lower than the pasture T group ( $P<0.01$ ), but there was no difference between chicory groups. Carcass weight was significantly greater for hybrid stags than pure red stags ( $59.5 \pm 1.89$  v  $52.2 \pm 2.05$ kg;  $P<0.01$ ), and was greater for hybrid stags than hybrid hinds ( $48.5 \pm 1.30$ kg;  $P<0.001$ ). A significant forage\*genotype interaction was found for CW ( $P<0.05$ ), with hybrid deer on chicory having a greater carcass weight than red deer ( $54.5$  v  $47.9 \pm 1.58$ kg;  $P<0.01$ ), whilst CW of hybrid and red deer grazing pasture was similar ( $51.1$  v  $51.9 \pm 1.70$ kg). There were no significant differences in carcass subcutaneous fat depth (GR; adjusted to equal CW) due to forage grazed or anthelmintic treatment. The dressing out percentage (DR) of the pasture TT group was significantly lower than that of the other three groups ( $P<0.05$ ). There was no effect of sex or genotype on GR or carcass DR.

**Table 3.4. Carcass measurements of stags and hinds, treated (T) or trigger-treated (TT) with anthelmintic and grazed on perennial ryegrass/white clover pasture or chicory.**

Sex...	STAGS				S.E.	HINDS				S.E.		
	PASTURE		CHICORY			D.F.	PASTURE		CHICORY		D.F.	
	<u>T</u>	<u>TT</u>	<u>T</u>	<u>TT</u>			<u>T</u>	<u>TT</u>	<u>T</u>			<u>TT</u>
Anthelmintic...				=20					=12			
No. deer	5	6	5	6		4	3	3	3			
No. reaching target CW	5	4	4	6		2	1	3	1			
Carcass weight (kg)	57.9	51.3	57.1	57.0	1.52	50.0	46.5	50.1	47.3	0.90		
Dressing percent (%)*	54.2	52.9	54.9	55.3	0.49	55.1	54.0	56.0	54.1	0.32		
GR tissue depth (mm)#	5.4	6.2	6.0	5.9	0.33	3.4	5.9	3.7	5.9	0.50		

\*dressing percentage calculated using final liveweight off pasture prior to being trucked

#adjusted to equal carcass weight



### 3.4.6. Velvet Antler Production

There were no significant differences ( $P=0.10$ ) in velvet antler weight produced between forage and anthelmintic treatments. 100% of chicory T stags, 67% of chicory TT stags, 80% of pasture T stags and 67% of pasture TT stags produced velvet antler prior to slaughter.

### 3.4.7. Serum Pepsinogen

The serum pepsinogen concentration (Table 3.5.) of both T and TT pasture groups was higher than the chicory T group in mid autumn ( $P=0.07$ ), but there was no difference in mean pepsinogen concentration between groups during early and mid winter. However, in late winter the serum pepsinogen concentration of the chicory TT group was higher than all other groups ( $P=0.07$ ).

**Table 3.5. Serum pepsinogen concentration (mU tyrosine/l  $\pm$ SE) from deer grazing either perennial ryegrass/white clover pasture or chicory and treated with anthelmintic 3-weekly (T) or trigger-treated (TT).**

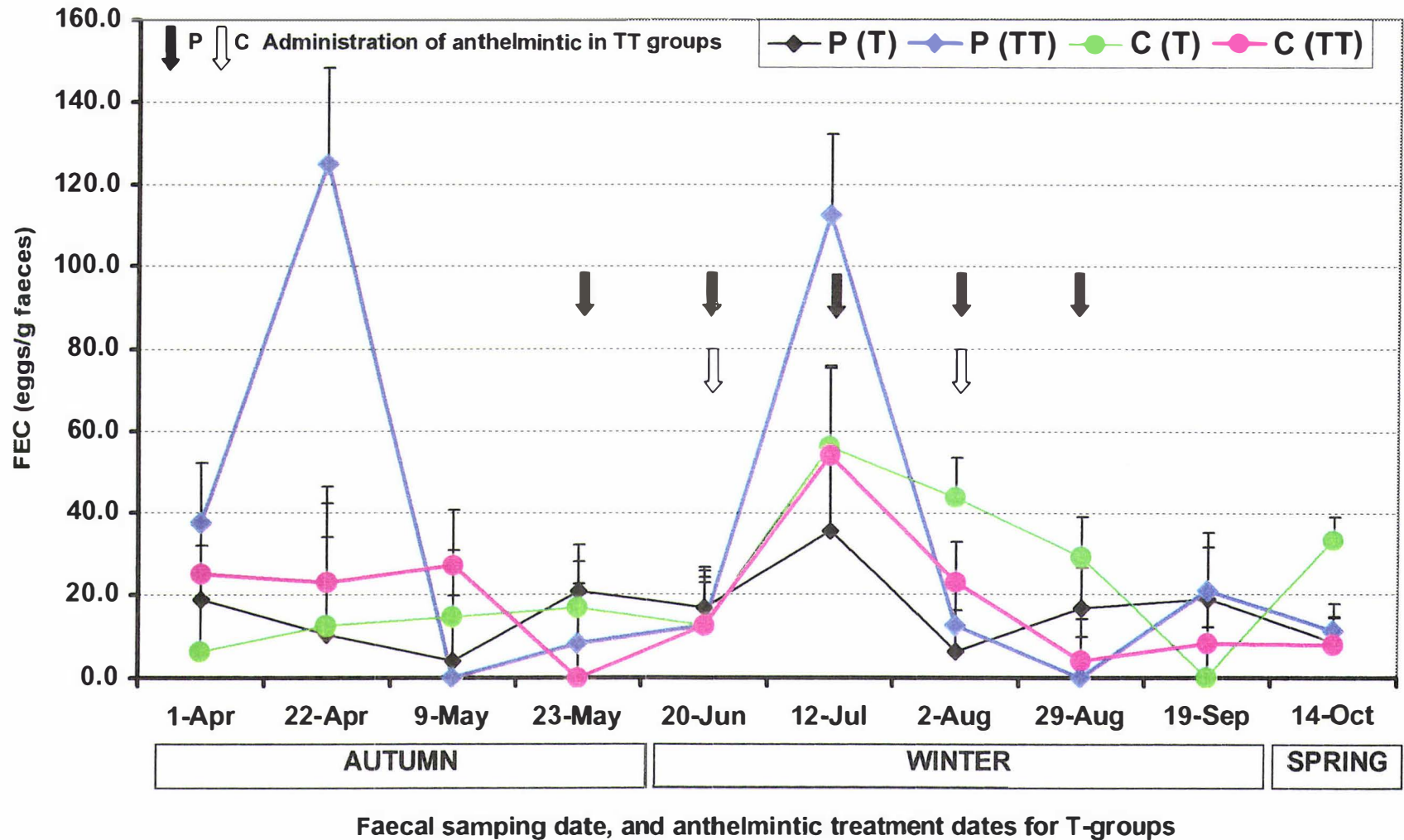
Forage...	PASTURE		CHICORY	
	T	TT	T	TT
<b>Anthelmintic...</b>				
<b>Sample date...</b>				
<b>Autumn</b>				
26 April	301 $\pm$ 28.6	274 $\pm$ 28.8	194 $\pm$ 28.6	246 $\pm$ 28.6
<b>Winter</b>				
30 May	302 $\pm$ 30.0	269 $\pm$ 30.3	257 $\pm$ 30.0	263 $\pm$ 30.0
12 July	321 $\pm$ 31.9	283 $\pm$ 32.2	308 $\pm$ 31.9	355 $\pm$ 31.9
29 August	335 $\pm$ 31.9	346 $\pm$ 32.2	330 $\pm$ 31.9	439 $\pm$ 31.9

D.F. = 40

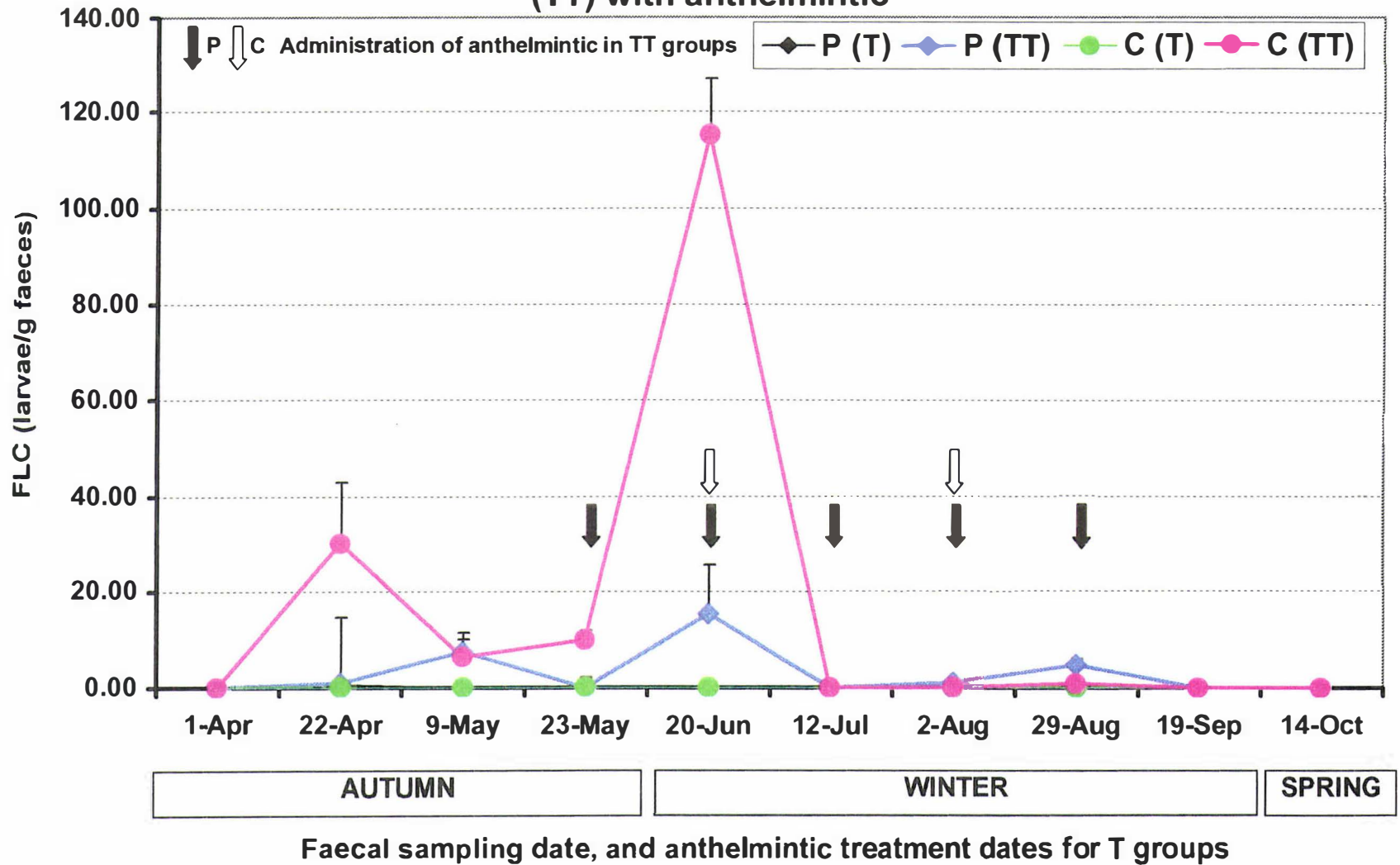
#### **3.4.8. Faecal Egg and Larval Counts**

Regular anthelmintic administration to T deer grazing both forages maintained FEC at low levels and maintained FLC at levels close to zero. Faecal egg counts (Fig. 3.2.) from the pasture TT group were significantly higher than all other groups in early autumn ( $P<0.01$ ) and higher than the pasture T group ( $P<0.01$ ) and both chicory groups ( $P=0.06$ ) in early winter. FEC did not differ between chicory groups at any time during the experiment. Faecal lungworm larval counts (Fig. 3.3.) of the chicory TT group were significantly higher than all other groups during early winter ( $P<0.001$ ), with no differences found in FLC between pasture groups.

**Figure 3.2. Mean (+SE) faecal egg counts of deer grazing PRG/WC pasture (P) or chicory (C) and treated 3-weekly (T) or trigger-drenched (TT) with anthelmintic**



**Figure 3.3. Mean (+SE) faecal larval counts of deer grazing PRG/WC pasture (P) or chicory (C) and treated 3-weekly (T) or trigger-treated (TT) with anthelmintic**



### 3.5. DISCUSSION

This experiment has shown that venison production from young deer by one year of age could be achieved with reduced anthelmintic input when deer are grazed on chicory in autumn, but not when they are grazed on PRG/WC pasture. This is the first such observation for deer and suggests that the use of different herbage species may play an important role in parasite control on deer farms. Further evaluation is now needed on both research and commercial deer farms.

Withholding anthelmintic treatment resulted in clinical parasitism in deer grazing pasture during autumn, associated with reductions in LWG, VFI, carcass weight and a 22% reduction in the number of deer reaching 92kg liveweight (50kg CW) by one year of age, despite trigger treatment. There was no effect of withholding anthelmintic treatment on autumn LWG, VFI or carcass weight of deer grazing chicory, but clinical parasitism became apparent once these deer were transferred to pasture for the winter period. This may have been due to the parasite larval challenge on pasture, but may also have been a result of failure to develop resistance in autumn because of low exposure to parasite larvae on chicory. These results are similar to those of Scales *et al.*, (1995) who found that lambs grazing chicory in autumn were unaffected by gastrointestinal nematodes, whilst parasitised lambs grazing grass pastures exhibited lower carcass weights than anthelmintic-treated lambs. Chicory therefore may offer the potential for reduced anthelmintic use in autumn, whilst simultaneously increasing LWG in autumn and spring when fed under higher herbage allowance (7kgDM/deer/day) and higher post-grazing herbage mass (>2100kgDM/ha; Kusmartono *et al.*, 1996a) than used here. The similarity in LWG, VFI and velvet production of deer on both anthelmintic treatment regimes in spring indicates that deer were unaffected by internal parasites by this time, probably due to the development of host resistance or the effect that treatment of all groups during late winter had on parasite life-cycles.

No studies have been performed to investigate the relationship between potentially diagnostic parameters such as FEC and FLC or serum pepsinogen concentration and nematode burdens or the likelihood of clinical or sub-clinical effects. Therefore the trigger treatment criteria chosen were based on first principles clinical judgement. During autumn in the pasture TT group, a combination of weight loss, reduced VFI and clinical signs of coughing occurred in the absence of significant FEC and FLC. The decision to treat was followed by a rapid reduction of coughing and return to expected LWG. That FEC and FLC were low at that time could indicate that the majority of nematodes were immature, yet were

of sufficient numbers to cause clinical signs, or that the fecundity of adult nematodes was low. This could only be determined post-mortem, and it is clear that future experiments of this type will need to include slaughter of sub-groups at defined time intervals or when clinical symptoms are observed. Hypobiotic larvae of deer *Ostertagia*-type nematodes have been found in the abomasal walls of red (Connan, 1991; 1997) and white-tailed deer (Baker and Anderson, 1975) similar to type-II ostertagiasis in cattle and it is possible that hypobiotic larvae could have caused or contributed to the clinical signs seen in this trial.

Faecal egg counts and FLC appeared not to be of diagnostic or prognostic value during autumn, when larval challenge may be high and host resistance low. Counts appeared to be more useful during winter, particularly for FLC in the chicory TT group. However, some animals on both forages exhibited severe clinical signs of lungworm, despite FLC's of less than 10 larvae/g faeces. The extent to which gastrointestinal nematode burdens were contributing to the clinical signs observed is not known.

Attempts to correlate FEC with associated adult nematode burdens in deer have been complicated by low or zero FEC in infected animals (Wilson, 1981), low infection levels associated with high FEC and high variability of results (Anderson, 1985; Schultz *et al*, 1993). Similar problems exist for FLC. Serum pepsinogen measurement has been used to attempt to confirm *H. contortus* (Johnston, 1984) and mixed abomasal nematode infections in red deer (Wagner and Mackintosh, 1993) and elk (Mason, 1984). In this study serum pepsinogen concentrations were inconclusive as a diagnostic aid. More research is needed to evaluate the use of both individual deer and herd mean serum pepsinogen levels as indicators of parasitism (Audige *et al*, 1998).

There was some evidence that gastrointestinal nematodes contributed to the reduced production of pasture TT deer in autumn and early winter (Figure 2). However, clinical signs, especially coughing in the absence of diarrhoea, suggests that lungworm infection, not gastrointestinal nematode infection, was the major factor in the reduced LWG of the chicory TT group in early winter after they were transferred to pasture (Figure 3). Whilst anthelmintic treatment was completely effective at eliminating lungworm larvae from faeces, it did not completely eliminate the presence of gastrointestinal nematode eggs in faeces.

Possible plant characteristics by which deer grazing chicory had reduced parasite infection compared with deer grazing pasture include differing plant morphology and sward structure and/or effects of plant chemical composition. Chicory has a broad-leaved, taller, and more open growth habit than PRG/WC pasture and this may affect the micro-climate within the sward and hence larval development, migration and survival (Knapp, 1964). This

may in turn influence larval intake by grazing animals. Moss and Vlassoff (1993) seeded different herbage species with strongylate eggs and recovered fewer nematode larvae from chicory than grass swards. Niezen (1996) recovered fewer nematode larvae from chicory than PRG/WC pasture swards infected with sheep faeces. A higher ratio of *Trichostrongylus:Ostertagia* sp. larvae was found on chicory than other herbage species (Moss and Vlassoff, 1993; Niezen, 1996). Deer-specific *Ostertagia*-type nematodes are the most prevalent gastrointestinal species in NZ red deer (Wilson, 1981; Anderson, 1985) associated with illthrift and/or death (Mason, 1977; Connan, 1991; Wagner and Mackintosh, 1993). An advantage of chicory for deer production with reduced anthelmintic input may therefore be a less suitable micro-climate, particularly for development and survival of *Ostertagia*-type deer nematodes.

Chicory contains trace levels of CT and other phenolic compounds including sesquiterpene lactones, coumarins and caffeic acid derivatives (Rees and Harborne, 1985). These are part of the chicory plants' defensive chemistry and deter feeding by insects; they could potentially affect other organisms including perhaps gastrointestinal nematode and lungworm larvae. Cultivars of chicory selected for high and low concentrations of sesquiterpene lactones will be available for research in 1999 (W Green, personal communication). Larval migration and survival should be studied on these cultivars. Forages containing moderate to high levels of CT have been shown to increase the production of parasitised lambs compared with non CT-containing forages (Niezen *et al.*, 1993c; 1995), but it is unlikely that the trace amounts of CT in chicory observed in this study had any effect, because there was no difference in CT content between chicory and pasture.

Research has indicated that young hybrid deer are more susceptible to parasitism than pure red deer (Mackintosh, 1992; Waldrup *et al.*, 1994), but the absence of any significant genotype\*anthelmintic treatment interactions suggests that in this study hybrid deer with 0.25% elk genes were susceptible to internal parasites to the same extent as pure red deer. The significant genotype\*forage interaction found for carcass weight is similar to that reported by Kusmartono *et al.*, (1996a), confirming the advantage for venison production of grazing weaner hybrid deer on chicory in autumn and spring to allow best expression of the elk genes for superior growth rate. Other sex and genotype differences on deer production shown in this study are similar to those reported by Kusmartono *et al.*, (1996a) and in Chapter 2.

This study has shown the potential for reduced anthelmintic input without a reduction in growth and carcass weight with weaner deer grazing chicory during autumn. Withholding

anthelmintic treatment from weaner deer grazing PRG/WC pasture during autumn appears to increase the risk of sub-clinical and clinical parasitism. Further studies on the epidemiology and pathogenicity of mixed and single species gastrointestinal and lungworm infections in young deer grazing different forage species are required. Further research is also required into methods of diagnosis of internal parasite infections in deer, including FEC, FLC, serum biochemistry and haematology in relation to number of nematodes present and the pathogenicity of such infections. Such studies are essential if deer farmers are to minimise anthelmintic useage, in order to safely monitor the effectiveness of management and the risk of parasitism.



## CHAPTER 4.

# Development of a model for study of lungworm (*Dictyocaulus* sp.) and gastrointestinal nematode infection in red deer (*Cervus elaphus*).

SIMONE O. HOSKIN<sup>1</sup>, P.R. WILSON<sup>2</sup>, W.A.G. CHARLESTON<sup>2</sup> & T.N. BARRY<sup>1</sup>.

<sup>1</sup>Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand

<sup>2</sup>Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand,

### 4.1. ABSTRACT

This study describes the development of a model of sub-clinical parasitism in weaner red deer, which can be used for a multiplicity of purposes, involving concurrent trickle infections of lungworm (*Dictyocaulus viviparus*) and mixed GI nematodes of deer origin. Parasite-free deer calves (n=20) were artificially reared indoors from four days of age and at 3-4 months were individually housed and dosed 3x/week at four levels of infection with infective larvae of lungworm and GI nematodes cultured from deer faeces. A further five calves were naturally reared on pasture with their dams, treated with anthelmintic, brought indoors and allocated to one of the four levels of infection, in order to evaluate suitable rearing systems of young deer for use in parasitological experiments. Liveweight, voluntary feed intake, faecal egg and larval counts were measured weekly and serum biochemistry and haematology was examined weekly. Nematodes were allowed to mature for 3-4 weeks following the end of trickle infection, then deer were euthanased and lung and GI nematodes recovered.

The sub-clinical infections resulted in reduced liveweight, VFI and serum albumin concentration, elevated serum pepsinogen, gastrin and globulin concentrations and elevated peripheral eosinophil counts and slight haemoconcentration, despite low

nematode counts. The reduction in liveweight was thought to be largely a result of the reduction in VFI. Animal growth, GI nematode burdens and FLC were related to infection rate. Relationships between lungworm burdens, FEC, serum biochemistry and haematology were less consistently related to infection rate. Naturally reared deer had similar liveweight, VFI and nematode counts to artificially-reared deer, but significant differences in serum biochemistry were found between rearing methods. This suggested that young naturally-reared deer could be used for parasitological studies involving nutrition, but are not suitable for use in immunological studies. A suitable level of infection to produce concurrent sub-clinical lungworm and GI nematode parasitism in weaner deer is defined, and the relevance of data collected in this study to diagnosis of parasitism in young deer is discussed.

## 4.2. INTRODUCTION

Farmed red deer are particularly susceptible to internal parasites in their first autumn/ early winter (Mason, 1985). Lungworm (*Dictyo-caulus viviparus*) is considered to be the parasite of greatest risk to farmed deer (Charleston, 1980; Wilson and Collier, 1981), although there is concern about the effect of gastro-intestinal (GI) parasites, especially deer-specific abomasal *Ostertagia*-type nematodes (Mason, 1977; Mason, 1984; Connan, 1991; Waldrup and Mackintosh, 1992,1993; Wagner and Mackintosh, 1993; Connan, 1996; Connan, 1997; Wilson *et al.*, 1997; Audige *et al.*, 1998).

Little is known about the epidemiology and pathogenicity of lungworm infections, and even less is known about GI nematode infections of young deer. Experimental infections of deer with lungworm have been restricted to evaluation of the lungworm vaccine "Dictol®" (Corrigall *et al.*, 1980, 1982, 1986), and comparisons of infection with lungworm larvae of bovine and cervine origin (Presidente *et al.*, 1972; Corrigall, 1985; Corrigall *et al.*, 1988;). Experimental infections of deer with abomasal nematodes have involved nematode species that are not deer specific, cultured from other ruminant species (Foreyt and Trainer, 1970; McGhee, 1981; Johnston 1984; English, 1991), with the exception of Davidson *et al.*, (1980) who used infective larvae derived from deer.

Current methods used for the control of internal parasites in deer are chemical (Mackintosh and Mason, 1985; Mason and Beatson, 1985; Bowie *et al.*, 1987; Andrews *et al.*, 1993; Mackintosh *et al.*, 1993), combined with grazing management (Wilson, 1984). However, there is increasing interest in non-chemical parasite control methods for grazing ruminants including specialty forage crops containing condensed tannins

(Robertson *et al.*, 1995; Niezen *et al.*, 1996).

To investigate alternative methods of control or treatment of deer nematodes, a deer-specific parasitological model is desirable. Such a model would involve individually housed, parasite-free deer that are infected with a known number of infective-stage larvae of deer-origin to produce a predictable parasite burden. However, there are no reports of such a model in the literature.

The objectives of this study were firstly to develop a parasitological model for weaner red deer that identified suitable infection rates of deer-origin lungworm and GI nematode larvae given simultaneously to produce sub-clinical infections, and secondly to define a suitable rearing system of young deer for use in parasite control investigation. The application of this model in determining the effect of feeding forages containing different levels of condensed tannins on establishment of internal parasites in weaner deer is presented in Chapter 6.

### **4.3. MATERIALS AND METHODS**

#### **4.3.1. Experimental Design**

The experimental design is summarised in Table 4.1. Twenty artificially-reared, individually-penned, weaned red deer calves (4-4.5 months of age) were allocated to receive four treatment groups, three of which received different dose rates of infective ( $L_3$ ) deer lungworm (*Dictyocaulus* sp.) and gastrointestinal nematode larvae. In addition, five calves reared naturally on pasture were allocated to the medium rate infection group, to compare the effect of rearing method.

Measurements included liveweight, voluntary feed intake (VFI), haematology and serum biochemistry, faecal gastrointestinal nematode egg and lungworm larval counts and numbers of gastrointestinal (GI) and lung parasites at slaughter. The trial was carried out at Massey University from 1 April to 10 August 1995 (Table 4.2.), and was divided into an adjustment phase (29 days), a pre-parasite infection period (nine days), a trickle parasite infection period (64 days), and a post-infection period prior to slaughter (26-29 days). The post-infection period allowed maturation of nematodes, to aid recovery and identification. Also a series of nutritional measurements were taken, presented in Chapter 5.

**Table 4.1. Experimental design.**

Treatment	No. Deer		Dose rate of infective larvae*	
	Stags	Hinds	<i>Dictyocaulus</i> sp.	GI
C (AR)	2	3	0	0
LT (AR)	2	3	100	500
MT (AR) <sup>ψ</sup>	3	2	200	1000
MT (NR)	3	2	200	1000
HT (AR)	3	2	400	2000

\*Given 3x per week. AR=artificially-reared; NR=naturally reared.

C = control; LT = low dose treatment; MT = medium dose treatment; HT = high dose treatment.

<sup>ψ</sup> This group was used both in the investigation of rate of infection (as MT) and the comparison of rearing methods (as AR).

**Table 4.2. Sequence of experimental events.**

Week*	Dates	Events
<b>1995</b>		
-2	1-18/4	Deer allowed to adjust to individual cages and lucerne chaff diet
-1	19-29/4	VFI + liveweight measured for covariate
0	30/4-8/5	Deer weighed, allocated to treatments 30/4/95. VFI + liveweight measured, blood sampled for covariate, faeces sampled
1-9	9/5-12/7	Trickle infection 3x/week, deer weighed + VFI measured weekly, blood + faeces sampled weekly
10-13	13/7-6/8	Nematodes allowed to mature, weekly measurements last taken 26/7
14	7-10/8	Deer euthanased

\*Reference to week is consistent with Figures 1-17.

#### **4.3.2. Animals and Housing**

The artificially-reared deer (AR) were kept indoors and fed ewe milk replacer (Anlamb, Anchor Milk Products, NZ) from four days of age, to ensure they were parasite-free (see Plate 4.1.). The five naturally-reared calves (NR) on pasture, were treated with oral ivermectin at 400µg/kg liveweight (“Ivomec” 0.4%w/v, Merck, Sharp and Dohme Ltd, NZ), along with their dams on three occasions, at 10-14 day intervals from 1-28 February. They were weaned, brought indoors and treated again with oral ivermectin on

12 and 26 March before being joined with the artificially-reared calves and accustomed to handling for one week. All deer were weaned onto chaffed lucerne (*Medicago sativa*) hay and deer pellets (Harvey Farms, NZ) on 28 February 1995 when 2.5-3.5 months of age. At weaning, all calves were vaccinated against clostridial infections (Clostridial 5 in 1; "Ultravac" CSL Ltd, NZ) and yersiniosis ("Yersiniavax"; AgVax, AgResearch, NZ) by injection into the anterior of the neck, with booster vaccinations given 30 days later.

Prior to trial commencement, the deer were housed in pens indoors on sawdust in groups of 4-10. Faecal gastrointestinal nematode egg counts and lungworm larval counts taken at weaning, and on 12 and 25 March 1995 were found to be zero, confirming animals were parasite-free.

Twelve deer were individually housed in specially designed deer metabolism crates (Milne *et al.*, 1978) and 13 in modified sheep crates, under constant lighting conditions of 14h light and 10h dark.

The artificially-reared animals were randomly assigned to the four treatment groups (Table 4.1.), on 30 April 1995, based on liveweight and sex. During the experimental period all deer were fed chaffed lucerne hay *ad-libitum* at 0900hrs, and had free access to water and a multi-mineral salt block. The deer were removed from their cages once per week for weighing and blood sampling.

One NR deer was euthanased in week five of larval dosing due to depression, low VFI and rapid weight loss. Post-mortem examination indicated parapox virus infection. One week prior to slaughter, another NR deer exhibited clinical signs and post-mortem examination at slaughter confirmed parapox virus.

#### **4.3.3. Infective Larvae**

Larvae were cultured from faeces collected from nine infected, commercially-farmed donor red deer stag calves. Donors were weaned on 1 March 1995 and transported to the Animal Health Services Centre Jennersmead Research Farm, Bunnythorpe and grazed together on pasture. These animals were ranked and paired according to initial parasite egg and larval excretion rate. Starting with the pair with highest egg and larval counts, dexamethasone trimethylacetate was given 3x per week (0.05mg/kg liveweight, "Opticortenol", Ciba-Geigy NZ Ltd). Faeces were collected using sheep collection bags and harnesses, five days per week, with bags changed every 24hrs at 0800hrs for 4-6 weeks or until animals showed moderate clinical signs of parasitism, such as weight loss. They were then treated with oral ivermectin at 200µg/kg liveweight

("Ivomec" 0.4%w/v, Merck, Sharp and Dohme Ltd, NZ) and the next pair given dexamethasone as above. Mean ( $\pm$ SE) FEC and FLC pre-dexamethasone treatment were FEC=206 ( $\pm$ 59.2)epg, FLC=157 ( $\pm$ 63.4)lpg and post-dexamethasone treatment were FEC=406 ( $\pm$ 125.1)epg, FLC=3311 ( $\pm$ 1286.2)lpg.

Each day, fresh faeces were first placed in a Baermann apparatus (Thienpont *et al.*, 1979) and left over night to allow lungworm larvae ( $L_1$ ) to migrate out of the faeces and be tapped-off in 1-200ml water 12-18hrs later. This was then sieved (250  $\mu$ m) to remove any debris and added to 200ml distilled water in a measuring cylinder and allowed to settle for 1-2hrs. This was then siphoned down to 100ml, added to 100ml distilled water in a measuring cylinder and again allowed to settle. The process was repeated until larvae were concentrated in 1-200ml distilled water with no debris, and then placed in a 500ml conical flask and gently aerated using an air pump (Elite-801, Aquarium Products, Rydalmere, NSW, Australia) away from direct sunlight at room temperature for eight days or until >95% of larvae had reached the infective ( $L_3$ ) stage.

Larvae were then counted by taking 10 0.1 ml aliquots and counting the larvae in a McMaster slide under a binocular microscope after addition of 1-2ml Lugol's solution (Weigart's variation; Pritchard and Kruse, 1982). The apparent viability of larvae was also assessed by counting the percentage of motile larvae or larvae in a curled-up position, in a drop of water placed on a slide and examined under a binocular microscope. Larval cultures of <95% viability were discarded. Larvae were then stored in 250ml lots in 600ml culture bottles placed horizontally at 10°C for up to three weeks before being administered to the experimental animals.

Faeces remaining after lungworm larvae had been extracted were mixed with medium grain vermiculite until the mixture reached a moist crumbly consistency. The mixture was then covered with a plate of glass and incubated at 23°C for 10 days to allow GI nematode eggs to hatch and develop to the infective stage ( $L_3$ ), with the mixture being thoroughly stirred every day. After incubation, the mixture was left overnight in a Baermann apparatus as above. Two-hundred ml of water, containing larvae, was then tapped-off, and larvae cleaned and reduced to 100-200ml of distilled water as described for lungworm ( $L_3$ ) larvae above. These larvae were then counted and assessed for viability and genus before storage (10°C). GI nematode larval cultures containing >50% abomasal nematode larvae were pooled prior to the first week of larval dosing and all other cultures discarded. All deer received larvae from this pooled culture throughout the trial because subsequent cultures contained high numbers of large-intestinal nematode

larvae and were therefore unsuitable. GI larvae given in the final week of dosing had been stored for up to 12 weeks, but apparent viability remained >95%.

Doses of infective larvae were made up of *Dictyocaulus* and GI nematode larvae combined in 5ml distilled water to within 7% of the required dose rate (Table 4.1.). Control deer received a placebo dose of distilled water only. The infection rates were given irrespective of calf bodyweights. Deer were infected for nine weeks by trickle infection on Monday, Wednesday and Friday of each week. The GI larvae comprised 44% *Ostertagia*-type (*Spiculoptera* sp, *Skrjabinagia* sp, etc), 33% *Trichostrongylus*, 5% *Cooperia* and 18% *Oesophagostomum*; (average of 10 counts of 100 larvae).

#### **4.3.4. Voluntary Feed Intake**

Voluntary feed intake (kgDM/day) of chaffed lucerne hay (DM%  $\pm$  SD = 83.8  $\pm$  1.08%) was recorded for each animal daily and calculated weekly. Individual samples of feed on offer and refusal were pooled and mixed, and triplicate 200g subsamples of each were taken for DM determination (100°C, 18hrs) twice per week. Individual VFI recorded during the adjustment period, once VFI had stabilised, was used as a covariate in all subsequent analyses of VFI data to correct for any inherent differences in size and appetite between deer.

Samples of feed offered (200g) were taken daily and stored at -20°C. These were later mixed to form weekly samples and duplicate 200g subsamples taken for freeze-drying and chemical analysis.

#### **4.3.5. Blood Sampling, Serum Biochemistry and Haematology**

Blood samples were taken weekly from 4 May-26 July via jugular venipuncture using one 10ml plain and one 5ml EDTA vacutainer (Hemogard, Becton-Dickinson, New Jersey, USA). After centrifugation, 4ml of serum was frozen (-20°C) for pepsinogen and gastrin assay, and 0.5ml used fresh for determination of serum total protein, albumin and globulin concentrations using an automatic analyser (Cobas Mira, F. Hoffman-La Roche & Co Ltd, Diagnostica, Basle, Switzerland). Serum pepsinogen levels were assayed according to the method described by Pomroy and Charleston (1989) and serum gastrin was determined by radio-immunoassay (Simpson *et al.*, 1993) based on the method of Hansky and Cain (1969).

Haematology was carried out on the fresh EDTA blood samples within three hours of sampling. White cell counts (WBC), red cell counts (RBC), haemoglobin

concentration (HB) and packed cell volume (PCV) were determined using an automatic analyser (Cobas Minos-Vet, Roche, Montpellier, France). Blood smears were stained for differential leucocyte counts.

Fibrinogen concentration was determined from fresh EDTA blood samples from the control and high dose treatments only, after centrifugation of microhaematocrit tubes, and using a refractometer (Reichert, Cambridge Instruments Inc. Buffalo, USA).

#### **4.3.6. Euthanasia and Organ Processing**

Six deer, balanced for treatment groups, were euthanased daily from 7-10 August by intravenous injection of sodium pentobarbitone. Immediately following euthanasia, the digestive tract was ligated at the omasal/abomasal junction, pyloric/duodenal junction, ileo-caecal junction and distal rectum. The digestive tract from the omasal/abomasal junction to distal rectum was then sectioned into the separate organs and the mesentery removed. The intact respiratory tract, heart and pericardial sac were removed from the thoracic cavity for perfusion of the lungs to recover lungworm, within 1 hr of slaughter.

The contents of each abomasum was removed and the total weight recorded. A 100g sub-sample of digesta was taken for nutritional analyses (Chapter 5.) and the proportion (%) of the total contents remaining for counting of nematodes was calculated and recorded. Each abomasum and remaining contents were then frozen (-20°C) for later worm counts. A 100g sample of digesta from the terminal ileum of each small intestine was removed for nutritional analyses, prior to storage (-20°C) for later worm counts. The lower part of the large intestine from the point at which faecal pellets form was discarded, and the remainder stored at -20°C.

#### **4.3.7. Parasitology**

##### **4.3.7.1. Faeces**

Faecal samples (10g) were collected weekly from trays beneath individual deer cages. Samples for faecal egg counts (FEC) were refrigerated (4°C) and counts were determined within three days using a modified McMaster technique (Stafford *et al.*, 1994), where a count of one egg was equivalent to 50 eggs/g of faeces. Faecal lungworm larval counts (FLC) were determined using a modified Baermann technique (Hendriksen, 1965), in which within 2hrs of sampling, 4g of fresh faeces was suspended in muslin cloth in a 40ml vial of tepid water to allow larvae to migrate to the bottom of the vial, to be counted the following day.



#### **4.3.7.2. Lungs**

Within 1hr of slaughter the lungs were perfused using a modification of the method described by Inderbitzen (1976). Water (30L) was flushed through a tube inserted through an incision into the right ventricle of the heart and fixed in the pulmonary artery before the point of bifurcation by ligation, exiting the trachea into a collection bucket. The free end of the tube was attached to a mains water tap fitted with a pressure gauge to provide a constant pressure of 2.11kg/cm<sup>2</sup>. Octanol (2ml; 1-Octanol MW 130.23g/mol, Merck, Schuchardt, Germany) was added to the lung flushings to remove excess foam prior to sieving (53µm). The nematodes were stored in 200ml water to which 20ml of 40% formalin (stabilised formaldehyde 400g/L, methanol 98.5g/L; Mallinckrodt Veterinary, Upper Hutt, NZ) was added to preserve the sample for later counting. Following perfusion, the lung was cut into 2cm<sup>3</sup> pieces and incubated in saline at 37°C for 3hrs to obtain any remaining worms. The saline was sieved (53µm) and the sievings added to the preserved fluid from the lung perfusion for worm counting. Preserved samples were later washed with tap water through a 53µm sieve to remove formalin and adult and immature lungworm were counted on a petri dish under a dissecting microscope (10x magnification) and differentiated by sex.

#### **4.3.7.3. Abomasum**

After thawing at room temperature overnight, the abomasal contents were washed into a bucket. Abomasal mucosa was scraped under running water to recover any adherent material. Fluid and contents were diluted to 2 litres with tap water and a 10% aliquot was taken. Of this, 1% was sieved through a 53µm sieve for counting of all stages of nematodes including larvae, with the remaining 9% passed through a 250µm sieve for counting of adult nematodes. The number of nematodes counted from abomasal contents was adjusted according to the proportion of total abomasal contents that was used for worm counts (see section 4.3.6.). The washed abomasum was then digested for 2hrs at 37°C in 600ml tap water, 10g pepsin (1200E/g; Riedel-de-Haen, Hannover, Germany) and 10ml hydrochloric acid (37%; AnaLar, BDH Laboratory Supplies, Poole, UK). Following digestion the abomasum was washed under warm running water and then discarded. The washings were then diluted to 2 litres and a 10% aliquot was taken and sieved (53µm) prior to counting of all stages of nematodes.

#### 4.3.7.4. Small and Large Intestines

The small intestines were opened along their entire length and washed under running water. The contents and washings were then processed as for the abomasa. The intestines were then subjected to pepsin-HCl digestion as previously described. The large intestines were opened and the entire contents washed out through a 710 $\mu$ m sieve; worms were counted without magnification against a black background and identified to species level using a dissecting microscope.

Female nematodes from the abomasa and small intestines were identified to genus level, whereas males were identified to species level where possible after clearing in polyvinyl lactophenol (Pritchard and Kruse, 1982).

#### 4.3.8. Dietary Chemical Analyses

Samples of feed on offer were analysed for organic matter, *in-vitro* organic matter digestibility and total nitrogen (N) as described in Chapter 5. *In-vitro* organic matter digestibility and total nitrogen only are presented in this chapter.

#### 4.3.9. Statistical Analyses

All data from the naturally-reared deer euthanased early due to parapox virus were omitted from statistical analysis. Data from the second naturally-reared deer found to have a less severe parapox infection when euthanased at the intended time was included in final statistical analysis as data values obtained from this animal were not outlying. Analysis of variance by repeated measures was used to examine the effects of time, time x level of infection, time x rearing method and sex on VFI, liveweight, FEC, FLC, serum biochemistry and haematology (SAS 6.11; 1996, SAS Institute Inc. USA). One-way analysis of variance was used to assess the effect of level of infection, rearing method and sex on worm counts. There were no significant effects of sex or significant interactions involving sex on all parameters measured, so sex was subsequently removed from the model. Differences between means were considered to be statistically significant if  $P < 0.05$ , but differences between means of  $P = 0.05-0.10$  are mentioned in the text. Initial liveweight, VFI and blood data prior to infection were used as covariates for subsequent analyses of liveweight, VFI and blood data respectively. Faecal lungworm larval counts were log transformed to normalise the data prior to analysis of variance. Linear regression was used to examine the relationships between liveweight gain and VFI, group mean and individual numbers of GI nematodes and serum pepsinogen concentration,

individual GI nematode and lungworm counts and both peripheral eosinophil counts and serum gastrin concentration (Microsoft Excel; 1996, Microsoft Corporation, USA).

## **4.4. RESULTS**

Summary and statistical data are presented in the figures, tables and text. Means, minima and maxima of raw data FEC, FLC, blood data and nematode counts are presented in Appendices 4.1. and 4.2.

### **4.4.1. Diet**

The chaffed lucerne hay fed contained  $3.2 \pm 0.05\%$  ( $\pm$ SE) of the dry matter as nitrogen and had an organic matter digestibility of  $61.7 \pm 0.44\%$  (%OM). A more detailed description of the chemical composition of the hay fed is presented in Chapter 5. (Table 5.2.).

### **4.4.2. Investigation of Infection Rate**

#### **4.4.2.1. Voluntary feed intake and liveweight**

Voluntary feed intake and liveweights are summarised in Figures 4.1. and 4.2., respectively. There was a decline in VFI for all infected groups from weeks 3-6 and for the C group from weeks 4-6. VFI increased for all groups from week 6 to week 11, with a drop in VFI across all groups during week 9. Mean VFI of the HT group was lower than the C group during weeks 6-7 ( $P < 0.05$ ), 8 ( $P = 0.07$ ), 10 ( $P < 0.05$ ) and 11 ( $P < 0.01$ ). Mean VFI of the LT group was lower than the C group during weeks 8 ( $P = 0.07$ ), 10 and 11 ( $P < 0.05$ ). The pattern of liveweight change showed a significant time\*infection rate interaction ( $P < 0.01$ ). Thus liveweight for C animals steadily increased with time and liveweight of infected animals was lower than for control animals, with the difference increasing with time. Mean liveweight of the HT group was lower than for the control group during weeks 7-8 ( $P = 0.08$ ), 9-10 ( $P < 0.05$ ) and 11 ( $P = 0.06$ ), with low and medium groups being intermediate.

Figure 4.1. Mean (+SE) voluntary feed intake

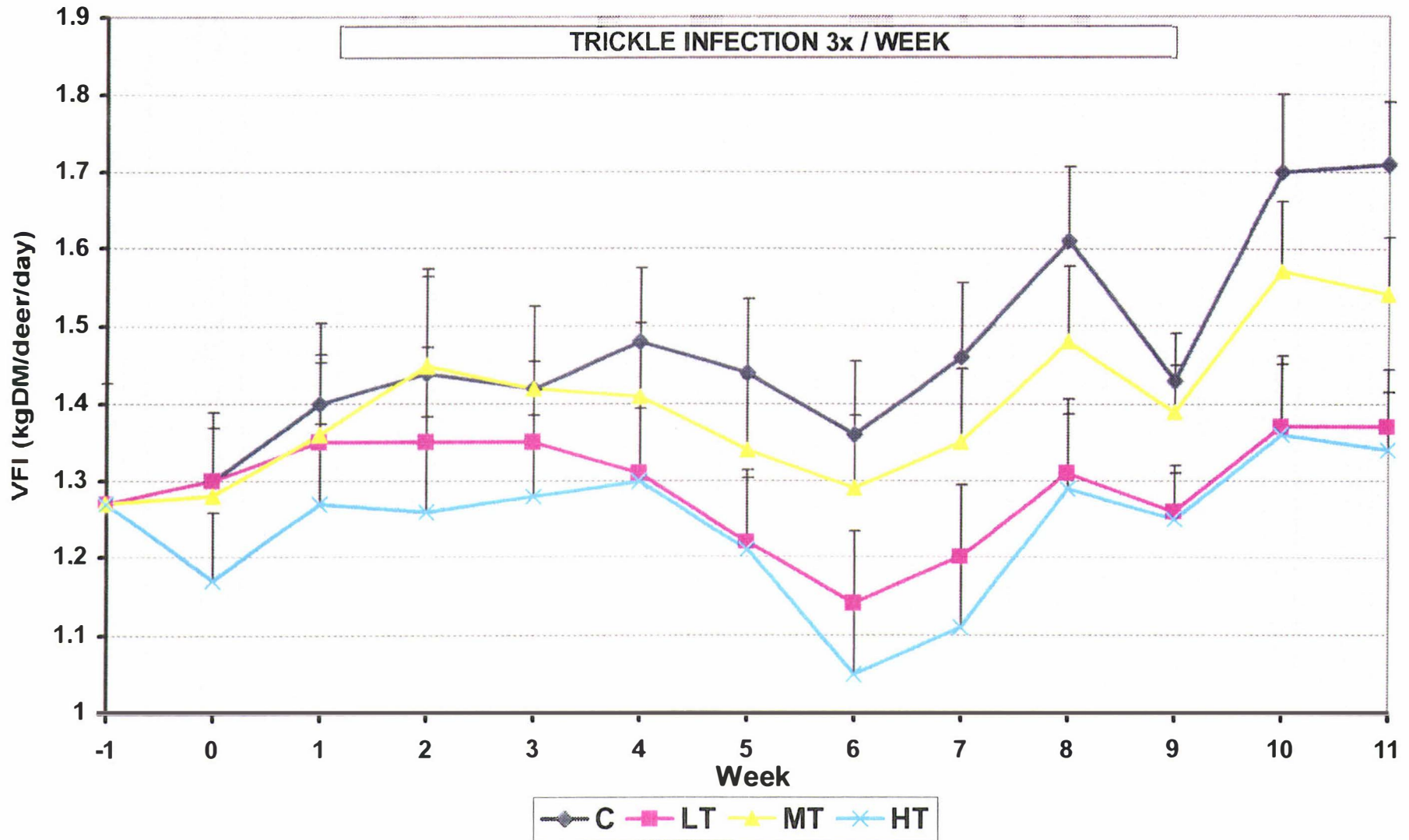
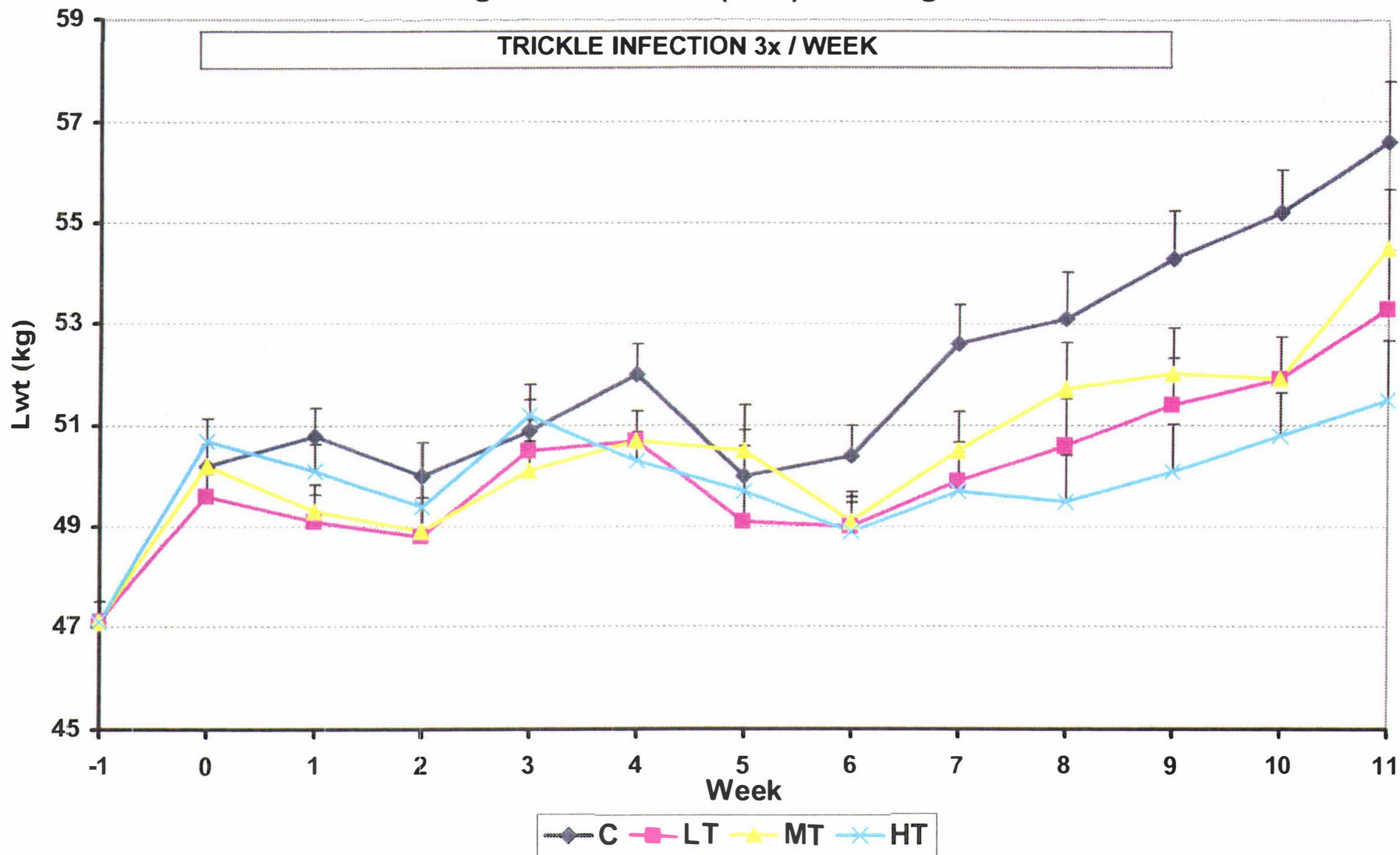


Figure 4.2. Mean (+SE) liveweight

TRICKLE INFECTION 3x / WEEK

110



#### 4.4.2.2. Faecal egg and larval counts

Faecal egg and lungworm larval count data are presented in Figures 4.3. and 4.4., respectively. All FEC and FLC for the C group was zero. Lungworm and GI nematodes in infected deer became patent by week 4. Faecal egg counts for all groups peaked between weeks 5-6, with a smaller secondary peak during weeks 9-10. There were no significant differences in FEC between treatment groups. Mean FLC for all groups peaked between weeks 6-8, with a significant time\*infection rate interaction ( $P<0.001$ ), the HT group peaking later than the other infected groups. Mean FLC of the HT group was higher than the LT group during weeks 4 and 7 ( $P<0.05$ ). All groups showed large individual animal variation in both FEC and FLC. At the final sampling, mean FEC and FLC were low and in many animals no eggs and/or larvae were detected (see Appendix 4.1.).



Plate 4.1. Artificial rearing of deer calves using ewe milk replacer.

Figure 4.3. Mean (+SE) faecal egg counts

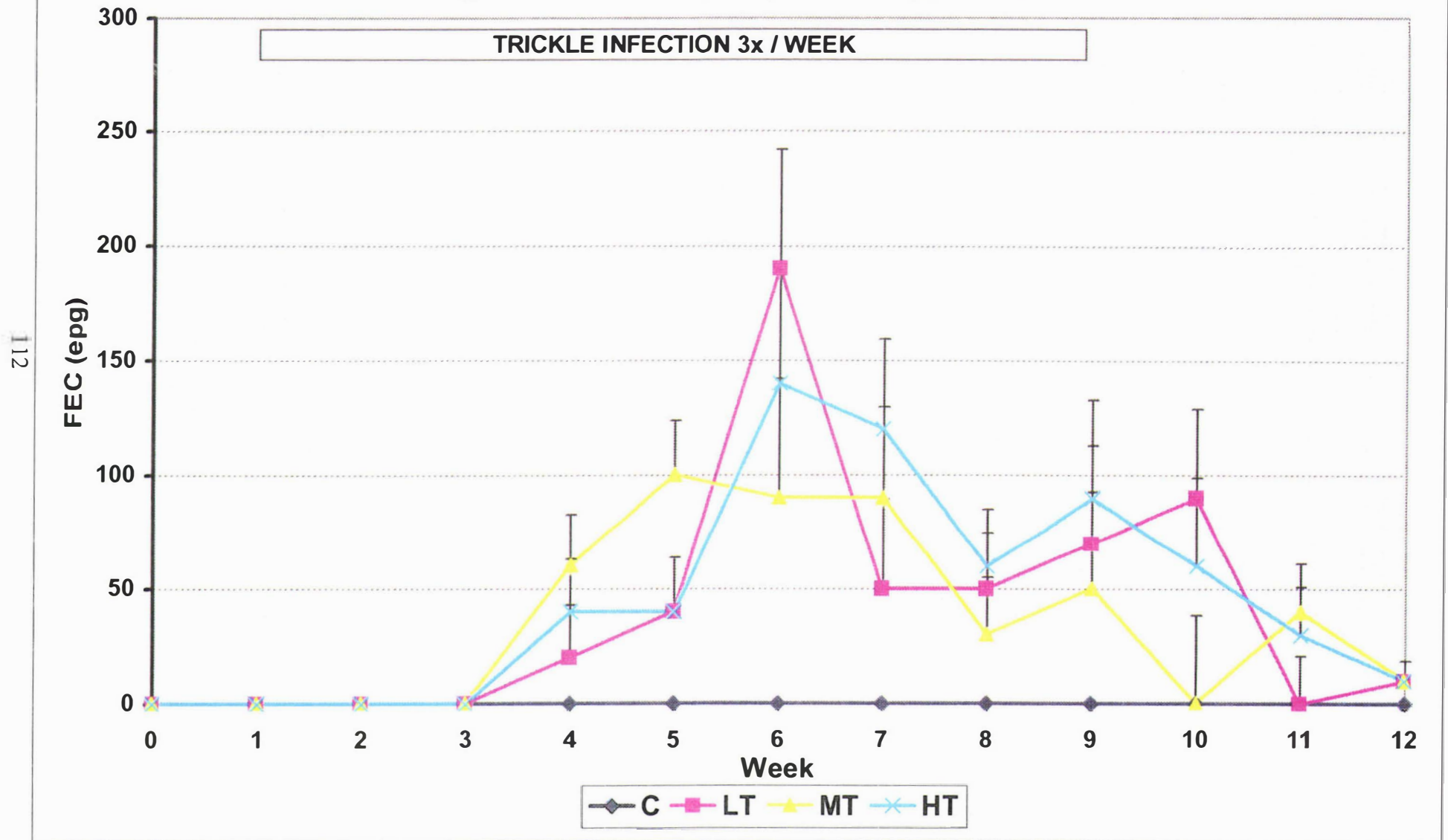
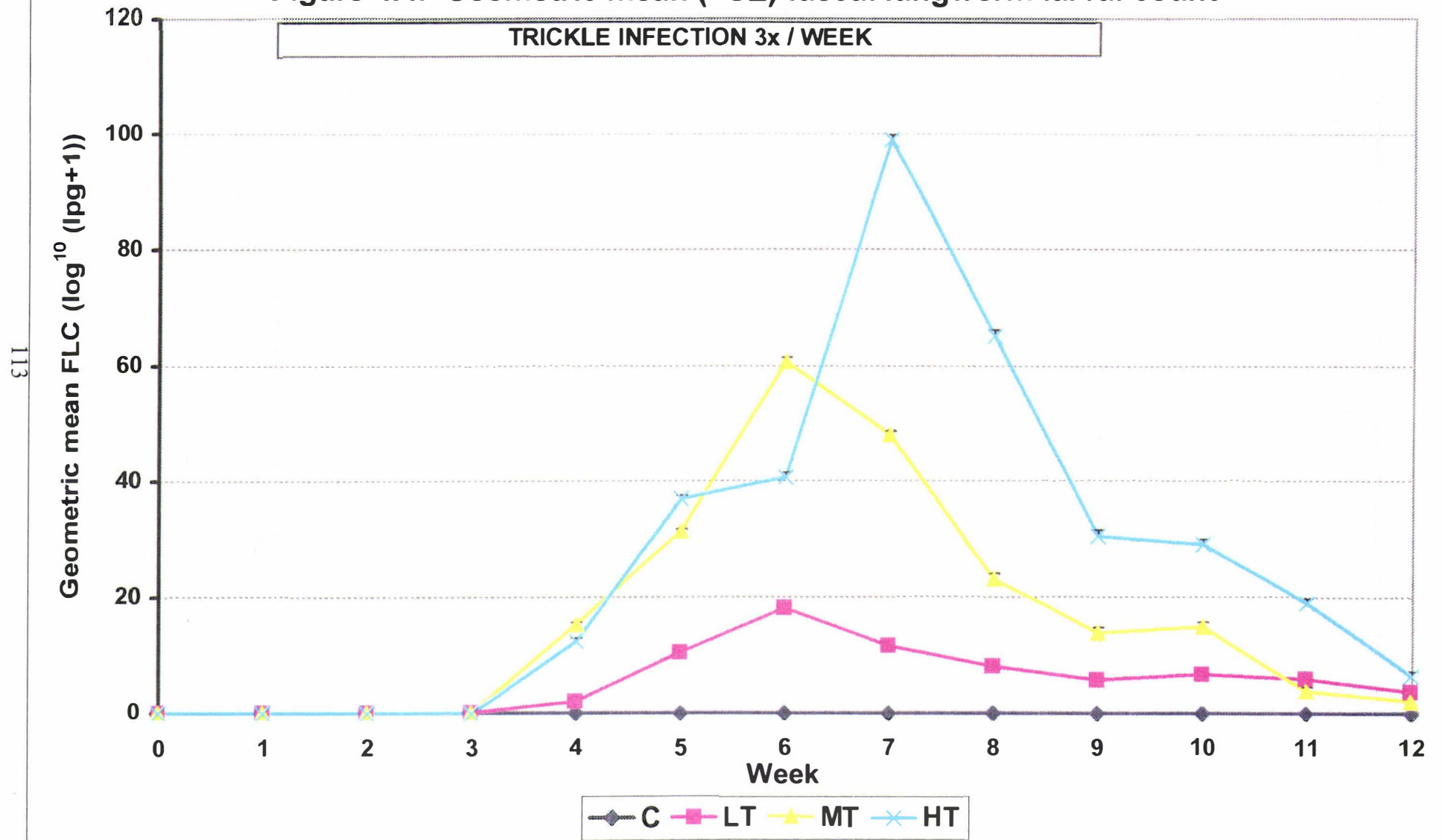


Figure 4.4. Geometric mean (+SE) faecal lungworm larval count





#### 4.4.2.3. Serum biochemistry

Figure 4.5. shows the serum total protein (STP), serum albumin (SA) and serum globulin (SG) concentrations. Mean STP of the HT group was greater than the C group in week 6 only ( $P=0.05$ ). In weeks 6 and 9 the STP of the HT group was higher than the LT group ( $P<0.05$ ). A significant time\*infection rate interaction was found for SA ( $P<0.05$ ), which is due to the difference between HT and C groups increasing with time. Mean SA of the HT group was significantly lower than the C group during weeks 4 ( $P<0.05$ ), 6 ( $P<0.01$ ), 7 ( $P=0.06$ ) and 9-10 ( $P<0.01$ ), respectively. Mean SA of the HT group was significantly lower than the LT group during weeks 4 ( $P<0.05$ ), 6 ( $P<0.01$ ) and 10 ( $P<0.05$ ). A significant time\*infection rate interaction was also found for SG ( $P<0.001$ ) with differences in SG between the HT and C groups also increasing with time. Mean SG of the HT group was higher than in the C group during weeks 6-8 ( $P<0.05$ ) and 10 ( $P=0.09$ ).

Mean ( $\pm$  range) serum fibrinogen concentrations are presented in Appendix 4.1. Values for the HT group tended to be higher than for the C group during weeks 9 and 10 ( $P=0.09$ ).

Figure 4.6. shows the serum pepsinogen concentrations (SPC). Mean SPC of the HT group was greater than that of the C group during weeks 4 ( $P<0.01$ ), 5 ( $P<0.05$ ), 6 ( $P<0.01$ ), 8 ( $P<0.05$ ), 9 ( $P=0.08$ ) and 11 ( $P<0.01$ ), respectively. Mean SPC of the HT group was higher than both LT and MT during weeks 4-6, 8 and 11 ( $P<0.05$ ). Mean SPC of the control group remained below 220 mU Tyrosine/L throughout the experiment.

Figure 4.7. shows the serum gastrin concentration (SGC). Mean SGC was higher for the HT group than the C group during week 6 ( $P=0.05$ ), 7 ( $P=0.09$ ), 8 ( $P=0.06$ ) and 9 ( $P=0.07$ ). Mean SGC of the MT group was higher than the C group during weeks 5-6 ( $P<0.05$ ), with a trend towards higher SGC for the MT group compared with the C group during weeks 7 ( $P=0.09$ ), 8 ( $P=0.06$ ) and 9 ( $P=0.07$ ).

Figure 4.5. Mean (+SE) serum total protein, albumin and globulin concentration

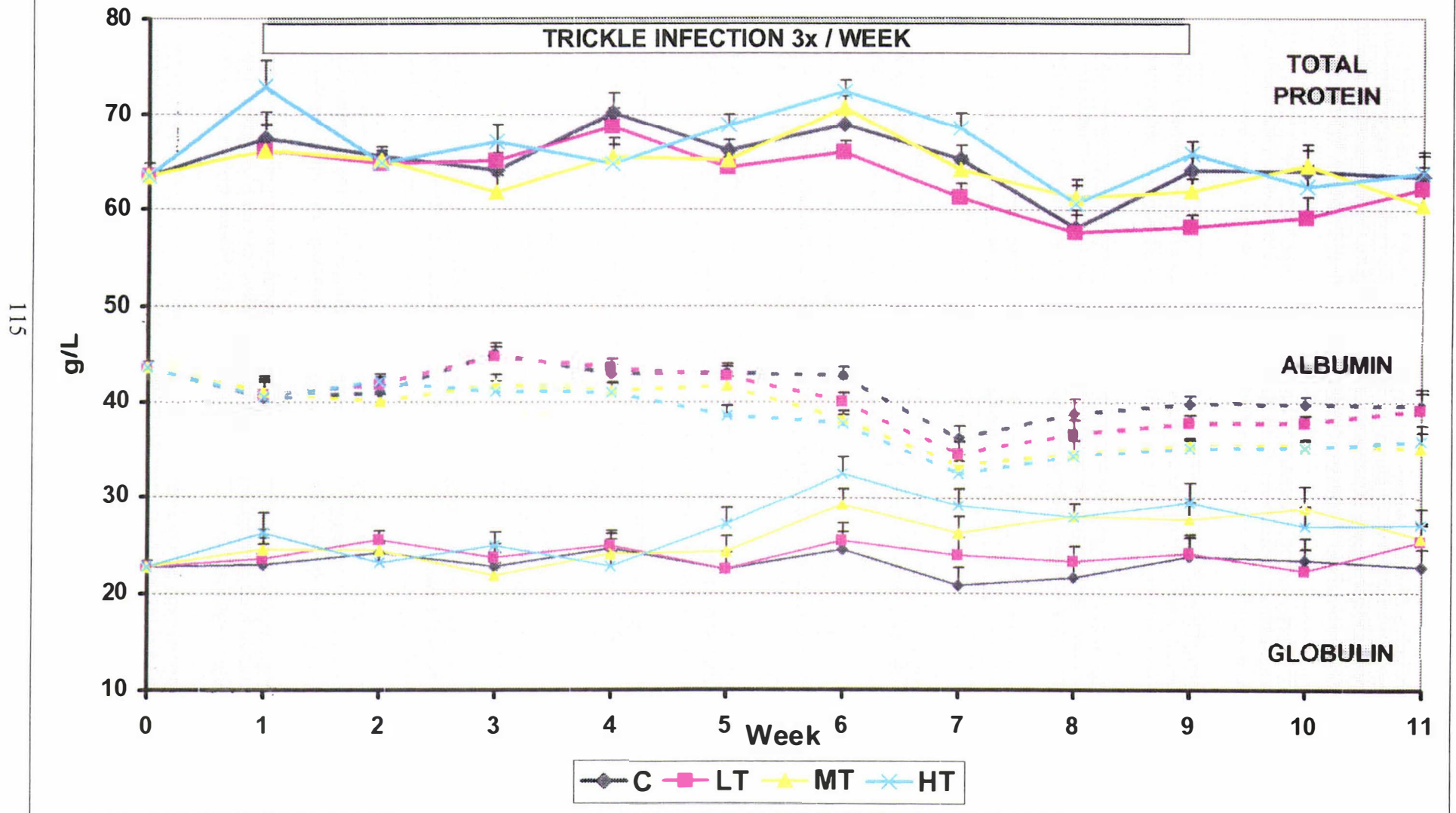


Figure 4.6. Mean (+SE) serum pepsinogen concentration

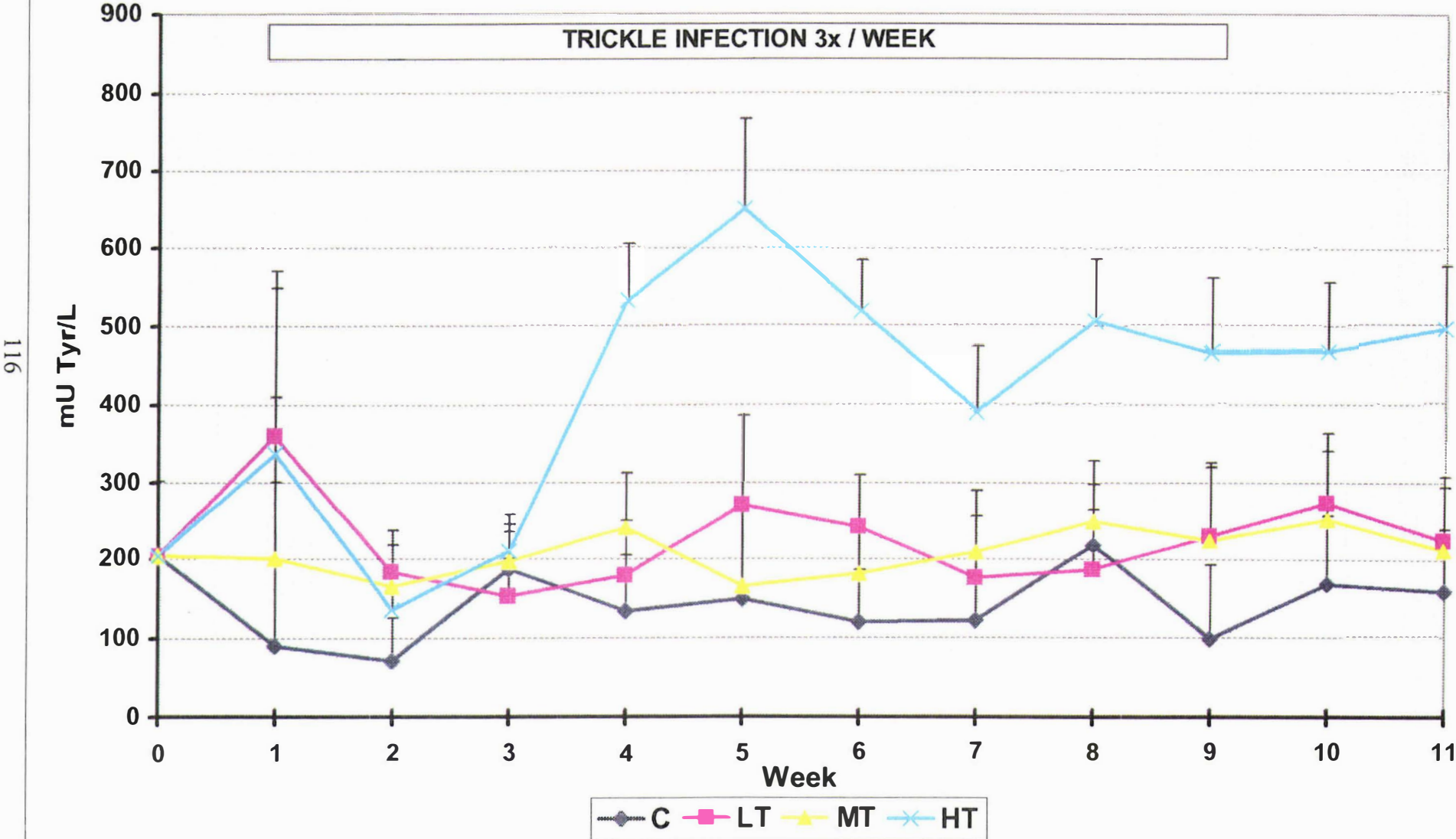
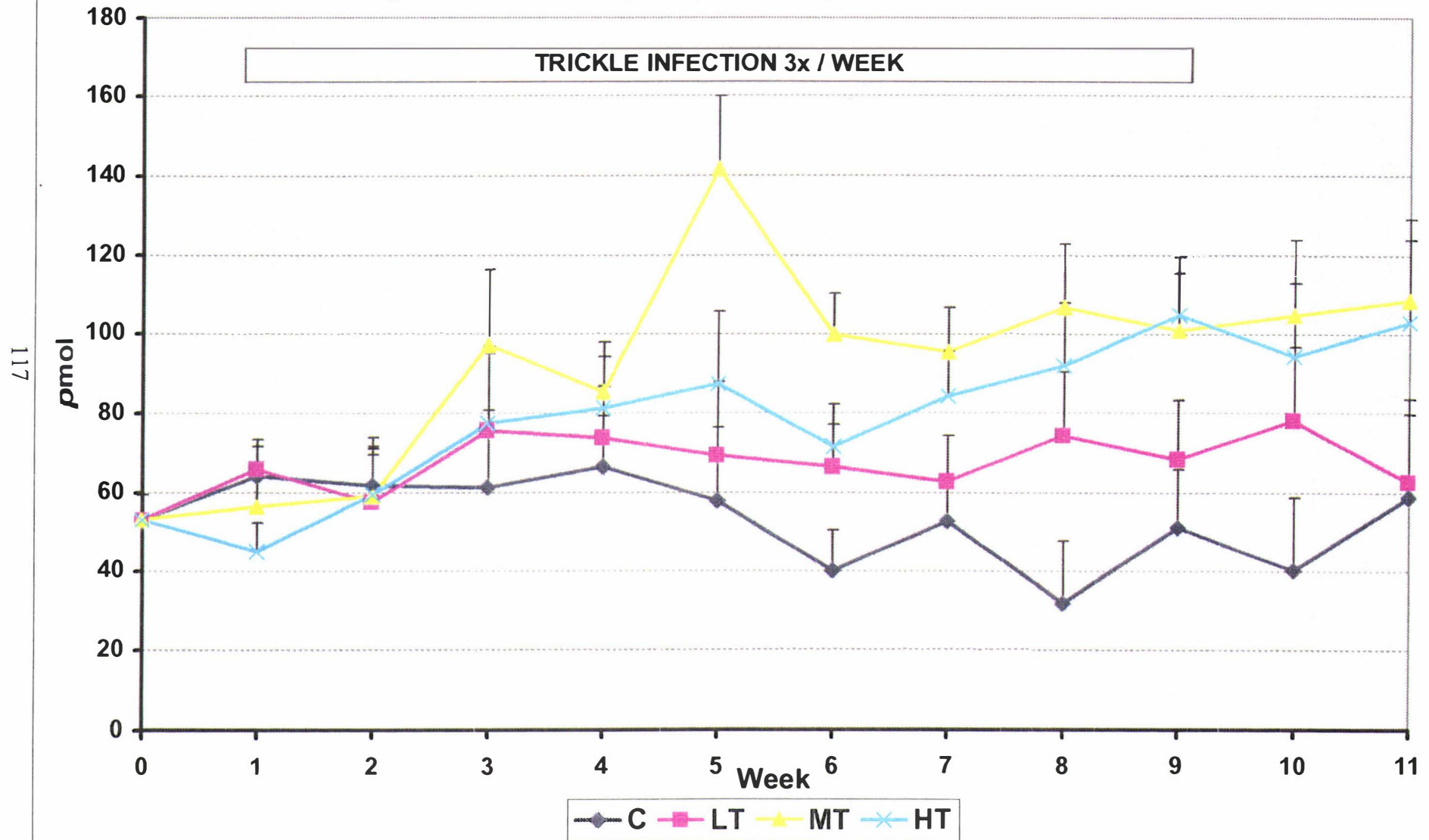


Figure 4.7. Mean (+SE) serum gastrin concentration



#### 4.4.2.4. Haematology

There were no significant treatment effects on total leucocyte (WBC) counts and total erythrocyte (RBC) counts (Fig. 4.8.). However, there was a trend towards higher RBC counts in the HT group compared with the C group during weeks 3, 4, 8, 11 ( $P=0.09$ ). Differential leucocyte counts are presented in Figure 4.9. including lymphocytes, neutrophils and monocytes. Mean ( $\pm$  range) basophils are presented in Appendix 4.1. Eosinophils are presented in Figure 4.10. There were no treatment effects on numbers of lymphocytes, monocytes and basophils. Total neutrophils were lower for the HT group compared with the LT group during week 2 ( $P=0.07$ ), with the reverse true in week 8 ( $P<0.05$ ). A significant time\*infection rate interaction ( $P<0.01$ ) was found for eosinophils, with the eosinophils in all infected groups increasing with time compared with the C group. Total eosinophil counts were greater in the HT than in the C group during weeks 8-9 ( $P<0.01$ ), 10 ( $P=0.06$ ), and 11 ( $P<0.05$ ); higher in the MT than in the C group during weeks 5 ( $P=0.07$ ), 8 ( $P<0.05$ ), 9 ( $P<0.01$ ), 10 ( $P=0.06$ ) and 11 ( $P<0.01$ ); and higher in the LT than in the C group during weeks 5 ( $P=0.07$ ) and 8 ( $P=0.06$ ).

Blood haemoglobin concentrations (HB) and packed cell volumes (PCV) are presented in Appendix 4.1. Mean HB and PCV of the HT group were greater than the C group ( $P=0.05$ ) during week 3 only.

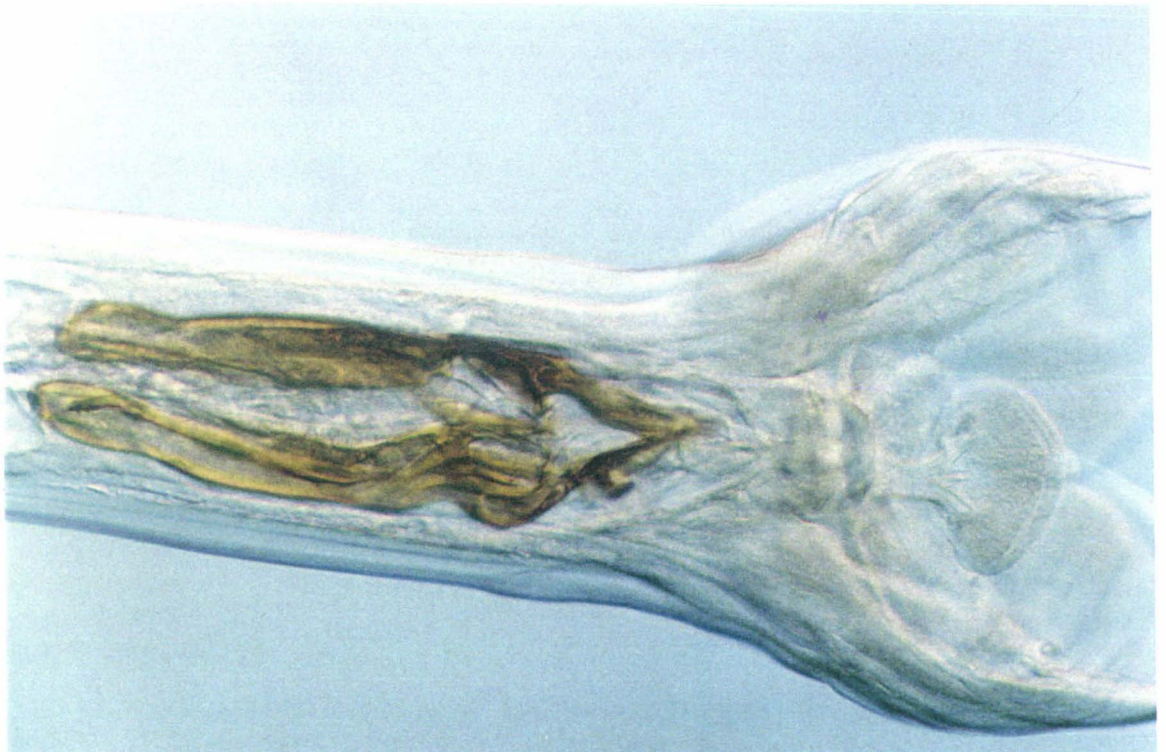


Plate 4.2. Spicules of *Spiculopteragia asymmetrica* from the abomasum.

Figure 4.8. Mean (+SE) total leucocyte (WBC) and erythrocyte (RBC) counts

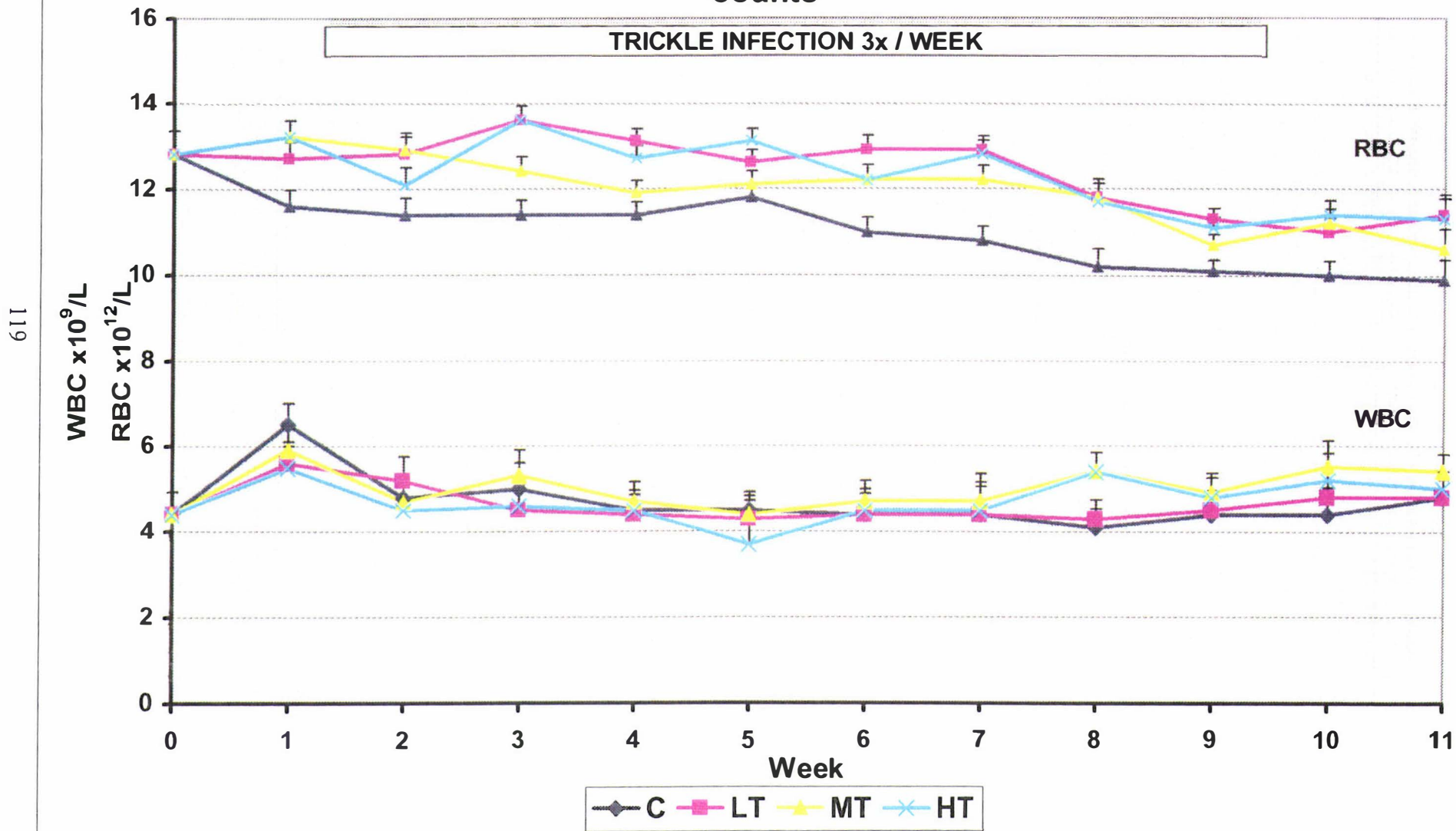


Figure 4.9. Mean (+SE) lymphocyte, neutrophil and monocyte counts

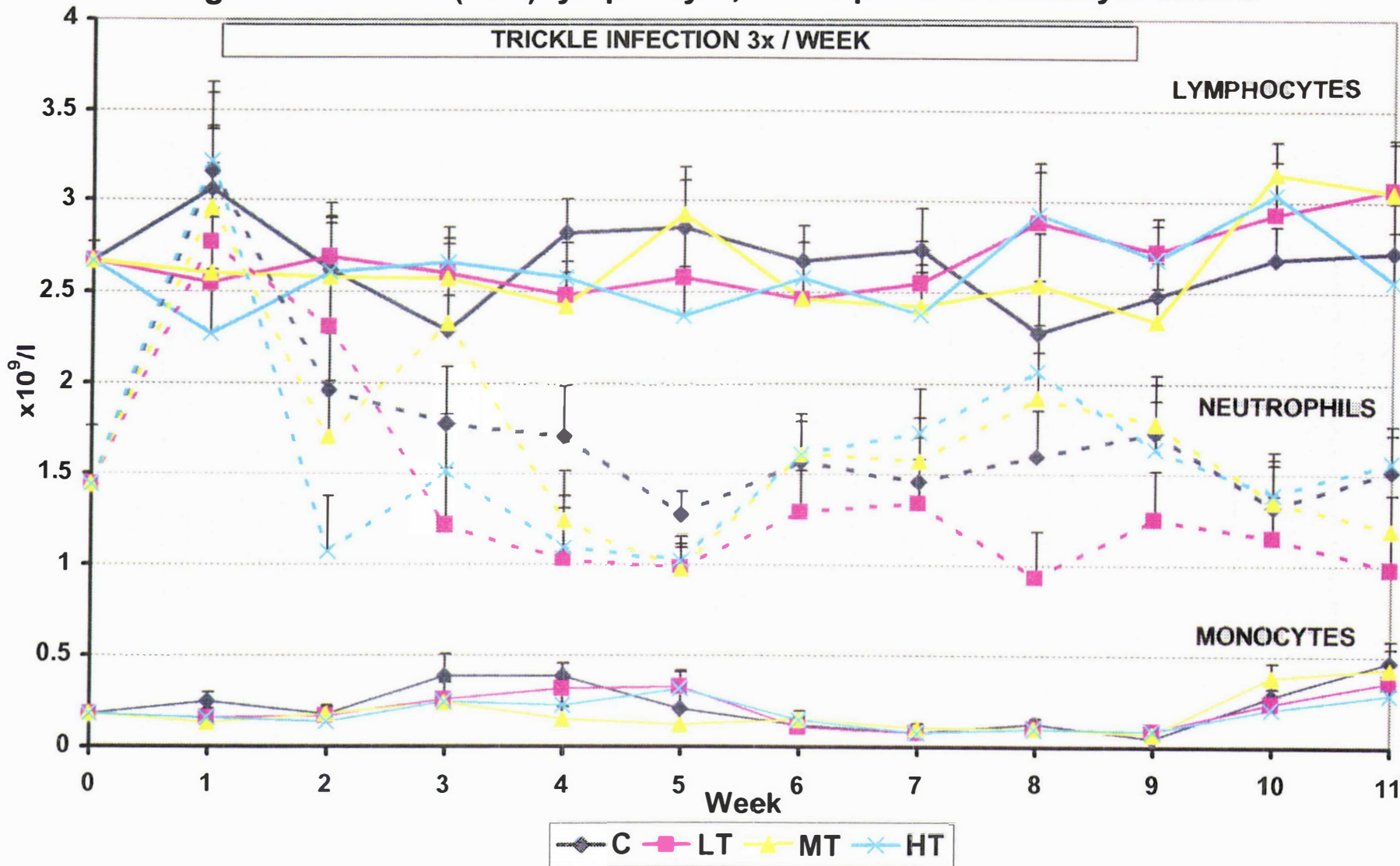
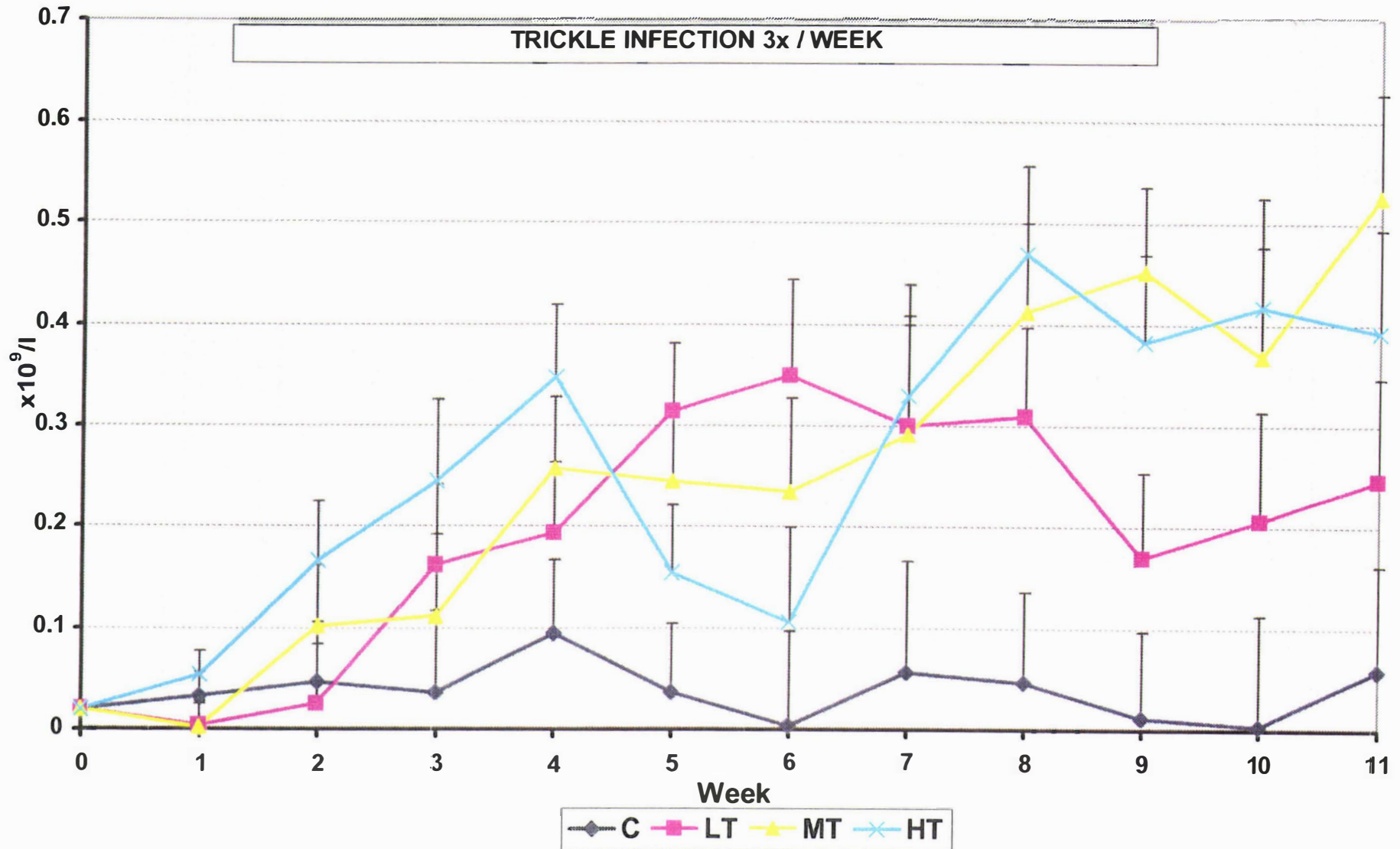


Figure 4.10. Mean (+SE) peripheral eosinophil counts





#### 4.4.2.5. Nematode counts

Table 4.3. shows the numbers of lung and GI nematodes recovered post-mortem. The mean number of lungworm recovered from the HT group was twice that of either the MT or LT groups ( $P=0.10$ ). Numbers of immature lungworm recovered were small. Total numbers of lungworm recovered ranged from 0.6 to 1.1% of infective larvae given for HT to LT groups, respectively.

Ninety-seven to 99% of GI nematodes recovered across all treatment groups were from the abomasum. The HT group had nearly twice the number of GI nematodes present compared to the LT group ( $P=0.10$ ). Table 4.4. presents the nematode species identified as a proportion of the total number of male nematodes counted in each organ across all treatments, with *Spiculopteria asymmetrica* the most common species found in the abomasum. The proportions of different nematode species recovered were similar for all treatment groups, although as infection rate increased, there was a trend towards a higher ratio of *Ostertagia*-type to *T. axei* nematodes recovered from the abomasum ( $P=0.10$ ). There were no significant differences between treatment groups in the low numbers of small and large intestinal nematodes recovered. Total numbers of GI worms recovered were low, reflecting an 'apparent take' of infective larvae of between 1.4-3% for high-low treatment groups respectively.

Plates 4.2, 4.3, 4.4, 4.5. and 4.6. show the male reproductive apparatus (spicules) of *Spiculopteria asymmetrica*, *Skrjabinagia kolchida*, *Ostertagia leptospicularis*, *Ostertagia circumcincta* and *Cooperia punctata*.

Table 4.3. Mean ( $\pm$  SE) numbers of lung and gastrointestinal nematodes recovered from deer artificially infected at four different levels of infection with infective-stage nematode larvae.

Infection Level	CONTROL	LOW	MEDIUM	HIGH	S.E.
					D.F.=16
<b>Nematodes/Organ</b>					
<b>Lung</b>					
Adult lungworm	0	28	29	62	16.1
Immature lungworm	0	2	2	5	0.9
Total lungworm	0	30	31	67	16.4
<b>Gastro-intestinal</b>					
Abomasal nematodes	0	408	584	765	191.7
Small intestinal nematodes	0	0	15	13	6.3
Large intestinal nematodes	0	4	6	5	1.7
Total GI nematodes	0	412	605	783	193.6
Ratio Abomasal					
● <i>stertagia</i> -type: <i>T.axei</i>	-	1.6:1	2.9:1	3.3:1	

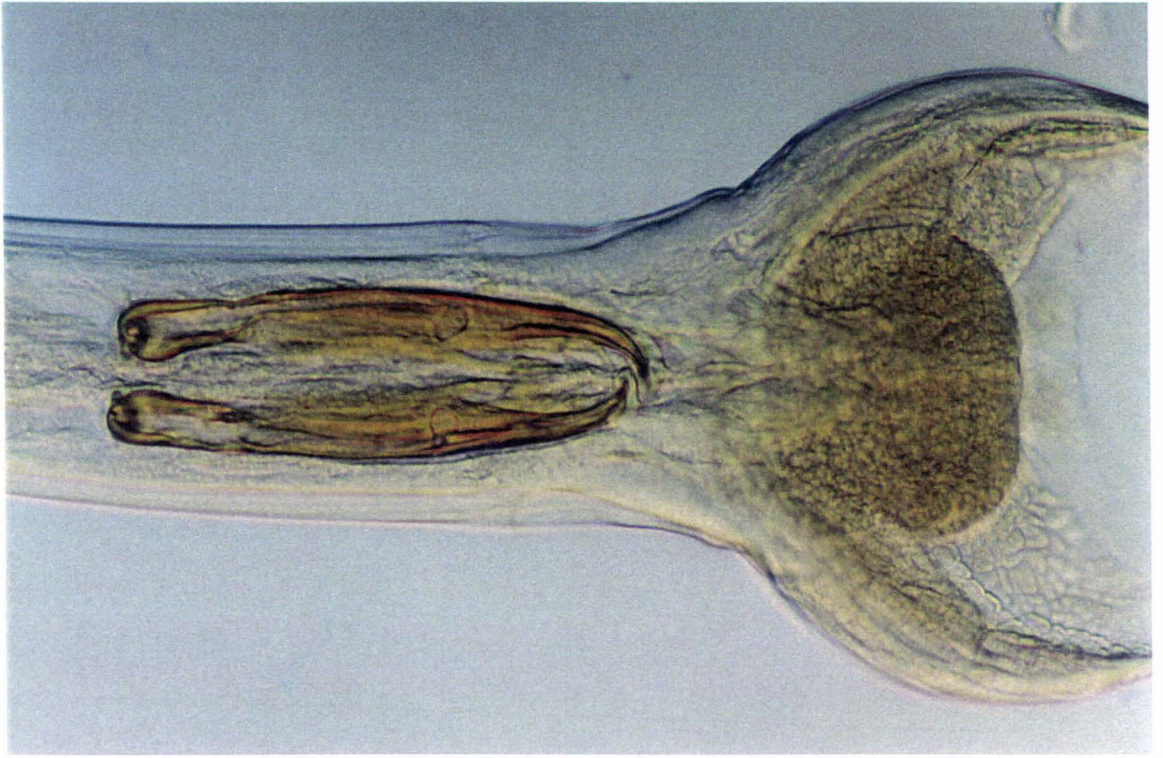


Plate 4.3. Spicules of *Skrjabinagia kolchida* from the abomasum.

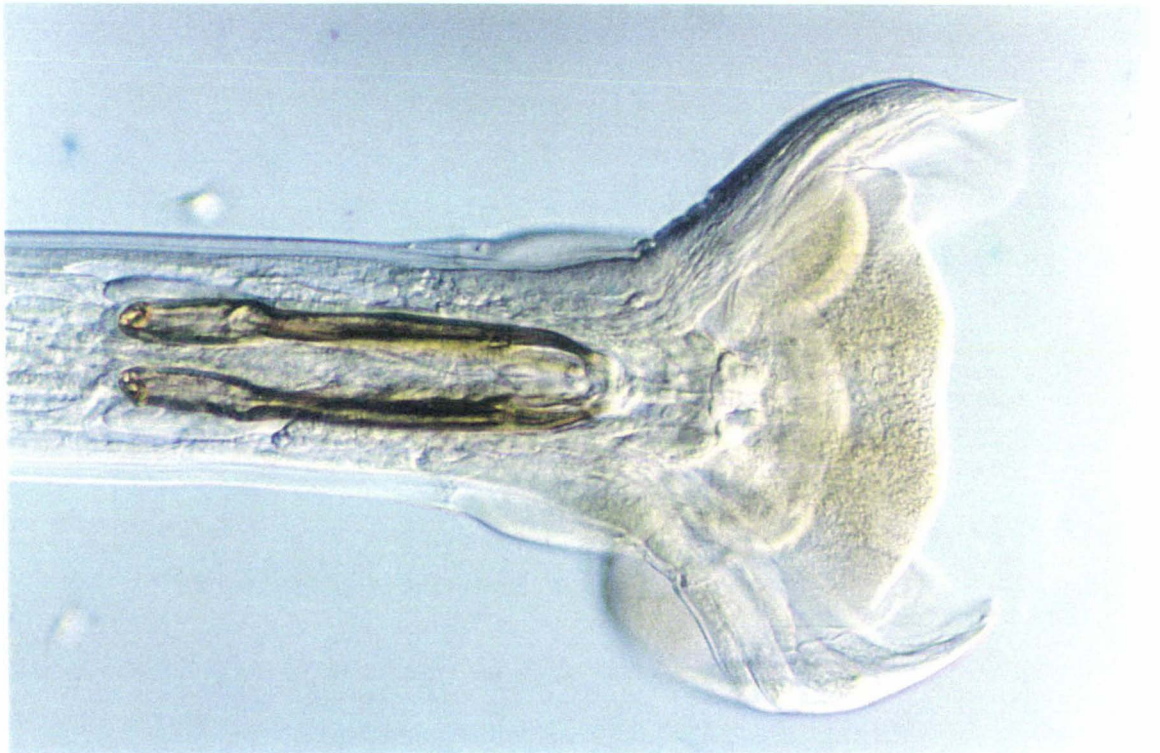


Plate 4.4. Spicules of *Ostertagia leptospicularis* (a morph of *S.kolchida*) from the abomasum.

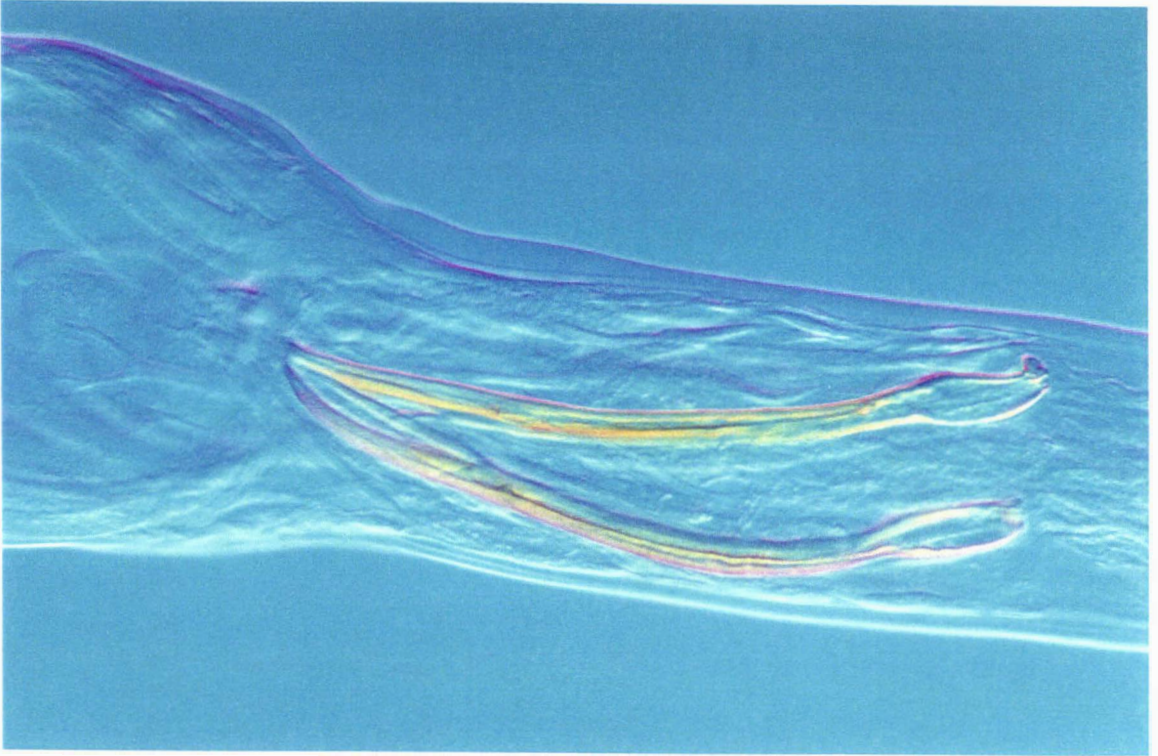


Plate 4.5. Spicules of *Ostertagia circumcincta* from the abomasum.



Plate 4.6. Spicules of *Cooperia punctata* from the small intestine.

**Table 4.4. Species of nematodes identified from deer artificially infected with infective stage nematode larvae, expressed as a proportion (%) of the total number identifiable nematodes counted\*.**

<b>Nematode Species/Organ</b>	<b>%</b>
<b>Abomasum (male nematodes only)</b>	
<i>Spiculopteragia assymetrica</i>	52.8
<i>Spiculopteragia spiculoptera</i>	3.6
<i>Ostertagia leptospicularis/ Skrjabinagia kolchida</i> <sup>#</sup>	28.6
<i>Ostertagia circumcincta</i>	0.1
<i>Trichostrongylus axei</i>	14.9
<i>Haemonchus contortus</i>	<0.1
<b>Small Intestine (male nematodes only)</b>	
<i>Trichostrongylus colubriformis</i>	28.0
<i>Trichostrongylus vitrinus</i>	43.0
<i>Cooperia mcmasteri</i>	29.0
<i>Capillaria</i> sp.	<1.0
<b>Large Intestine (all nematodes)</b>	
<i>Oesophagostomum venulosum</i>	99.0
<i>Trichuris</i> sp.	1.0
<b>Lung (all nematodes)</b>	
<i>Dictyocaulus</i> sp.	100

\*n=361 abomasum; n=7 small intestine; n=89 large intestine; n=799 lung. <sup>#</sup>Morphs

#### 4.4.3. Rearing Method Comparison

Initial liveweight, liveweight gain, VFI, total numbers of lung, abomasal and large intestinal nematodes recovered post-mortem (Table 4.5.) were not significantly different between naturally-(NR) and artificially-reared (AR) groups given the medium dose rate of infective larvae. The NR deer had more nematodes present in the small intestine than the AR deer ( $P < 0.01$ ), although total numbers of nematodes recovered from the small intestine were low. The NR group had a lower proportion of *Spiculopteragia asymmetrica* and a greater proportion of *Ostertagia leptospicularis* ( $P < 0.05$ ) than the AR group.

**Table 4.5. Liveweight, VFI and numbers of lung and gastrointestinal nematodes (mean  $\pm$  S.E.) recovered from artificially- and naturally-reared deer.**

	Rearing method...	
	ARTIFICIAL	NATURAL
<b>Initial liveweight (kg)</b>	46.0 $\pm$ 3.81	47.8 $\pm$ 2.05
<b>Liveweight gain (g/d)</b>	61 $\pm$ 0.1	44 $\pm$ 0.1
<b>Mean VFI/LW<sup>0.75</sup> (g/d)</b>	72 $\pm$ 0.3	66 $\pm$ 0.3
<b>Nematodes/Organ</b>		
<b>Lung</b>		
Adult lungworm	29 $\pm$ 15.4	29 $\pm$ 19.4
Immature lungworm	2 $\pm$ 0.8	2 $\pm$ 1.1
Total lungworm	31 $\pm$ 15.8	31 $\pm$ 20.0
<b>Gastro-intestinal</b>		
Abomasal nematodes	584 $\pm$ 233.9	402 $\pm$ 295.8
Small intestinal nematodes	15 $\pm$ 5.8	75 $\pm$ 7.3
Large intestinal nematodes	6 $\pm$ 1.2	3 $\pm$ 1.5
Total GI nematodes	605 $\pm$ 238.4	480 $\pm$ 301.6
Ratio abomasal		
<i>Ostertagia</i> -type : <i>T. axei</i>	2.9:1	30:1

Mean FEC of NR and AR deer are presented in Figure 4.11. Lungworm and GI nematode infections became patent during week 4 for both treatment groups. Mean FEC peaked over weeks 5-7 for the NR deer, and peaked during week 6 for the AR deer, with FEC for both treatments fluctuating at lower levels from weeks 8-12. Mean FEC were significantly greater for the NR group than the AR group during weeks 10 and 12 ( $P<0.01$ ).

Mean FLC of NR and AR deer are presented in Figure 4.12. There was a significant time\*treatment interaction for FLC ( $P<0.001$ ), explained by both primary and secondary peaks in FLC occurring later and being higher for the NR deer compared with the AR deer. Mean FLC were significantly lower for the NR group than the AR group during week 4 ( $P<0.05$ ). Mean FLC of the NR group was higher than the AR group

Figure 4.11. Mean (+SE) faecal egg counts of artificially (AR) and naturally (NR) reared deer

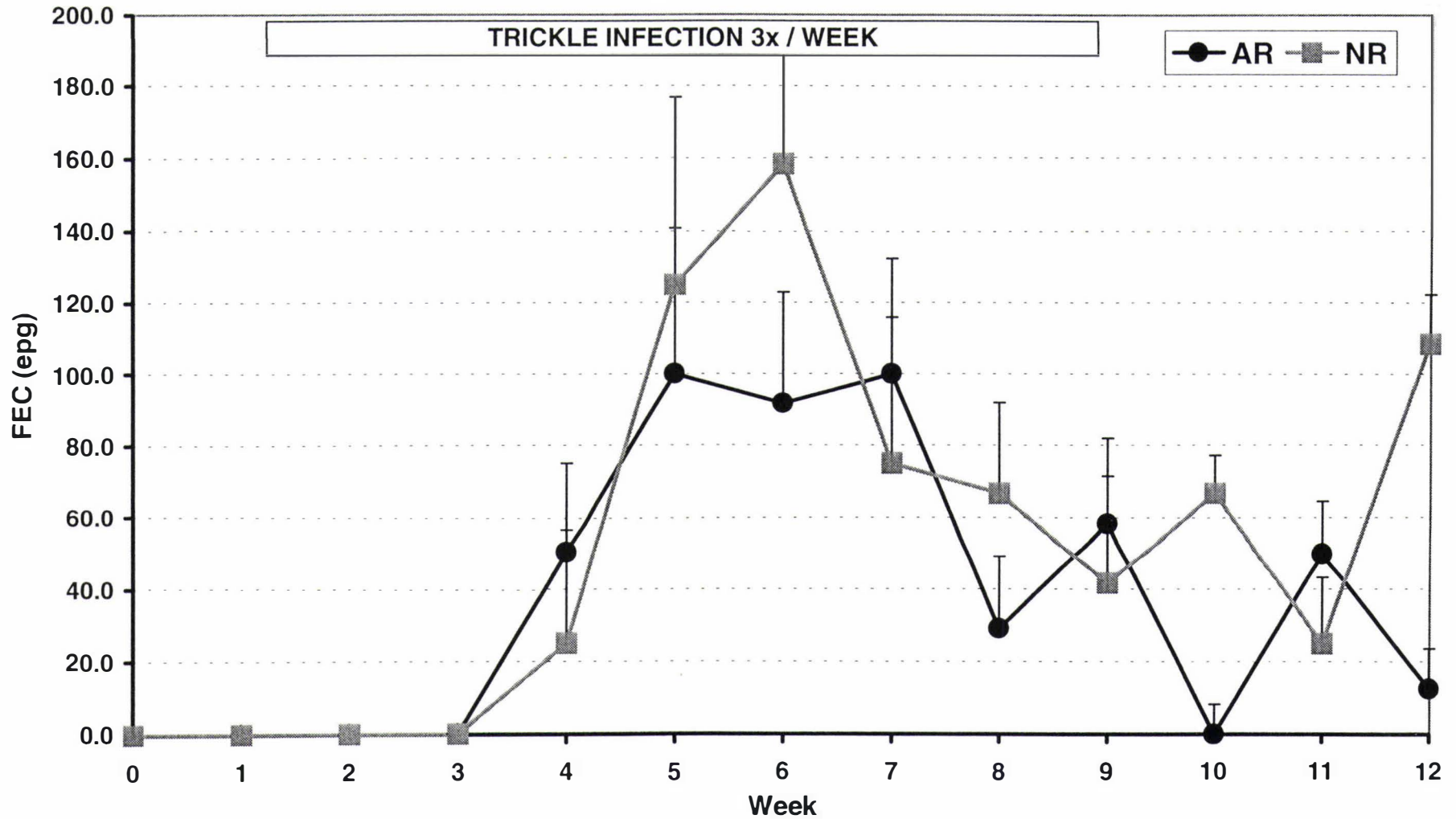
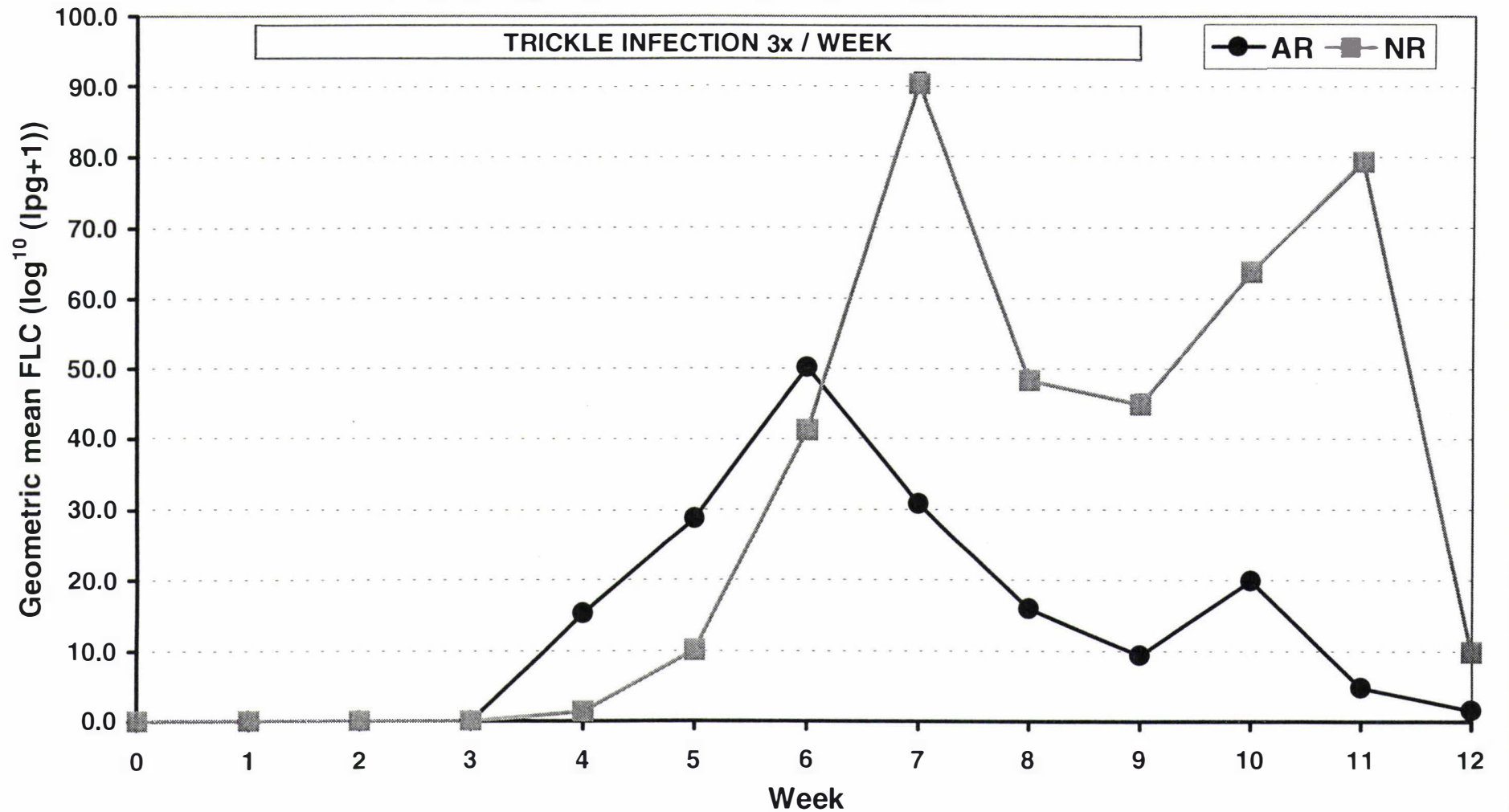




Figure 4.12. Geometric mean (+SE) faecal lungworm larvae counts of artificially (AR) and naturally (NR) reared deer



during weeks 11 ( $P<0.01$ ) and 12 ( $P<0.05$ ).

Serum total protein concentrations shown in Figure 4.13. were similar for both rearing-method groups. The NR group had a lower SA concentration compared with the AR group during weeks 2 and 6 ( $P<0.05$ ). There were no significant differences in SG between treatment groups. Serum pepsinogen concentrations shown in Figure 4.14. were greater for the NR group compared with the AR group from week 1-11, with this difference reaching significance during weeks 7 and 11 ( $P<0.05$ ). The standard error associated with SPC became lower in the latter part of the trial. Figure 4.15. shows the SGC of the NR deer tended to be lower than in the AR deer but this only reached significance during week 5 ( $P<0.05$ ).

Figure 4.16. shows the total numbers of leucocytes (WBC) and erythrocytes (RBC) present in the blood. Naturally-reared deer had a significantly lower WBC during weeks 3 and 8 ( $P<0.05$ ) than AR deer. The NR deer had a significantly greater RBC during week 1 ( $P<0.05$ ) compared with AR deer and there was a trend towards greater RBC for the NR deer during weeks 2 and 6-8 ( $P=0.08$ ). Differential lymphocyte, neutrophil and monocyte counts are presented in Figure 4.17. There were no significant differences in the total numbers of lymphocytes between rearing methods. The total numbers of neutrophils tended to be lower for the NR compared with the AR group, especially in weeks 3 ( $P=0.06$ ) and weeks 8-9 ( $P<0.05$ ). The total numbers of monocytes tended to be higher for the NR compared with the AR group, with this difference reaching significance during week 5 ( $P<0.05$ ) and 10 ( $P<0.01$ ). There was no difference in eosinophil and basophil counts between treatment groups (Appendix 4.2.).

Blood haemoglobin concentrations and PCV are presented in Appendix 4.2. Mean HB and PCV were significantly greater for the NR group compared to the AR group during week 9 ( $P<0.01$ ), but there were no other differences in HB or PCV between treatment groups at any other point during the experiment.

Figure 4.13. Mean (+SE) serum total protein, albumin and globulin concentration of artificially (AR) and naturally (NR) reared deer

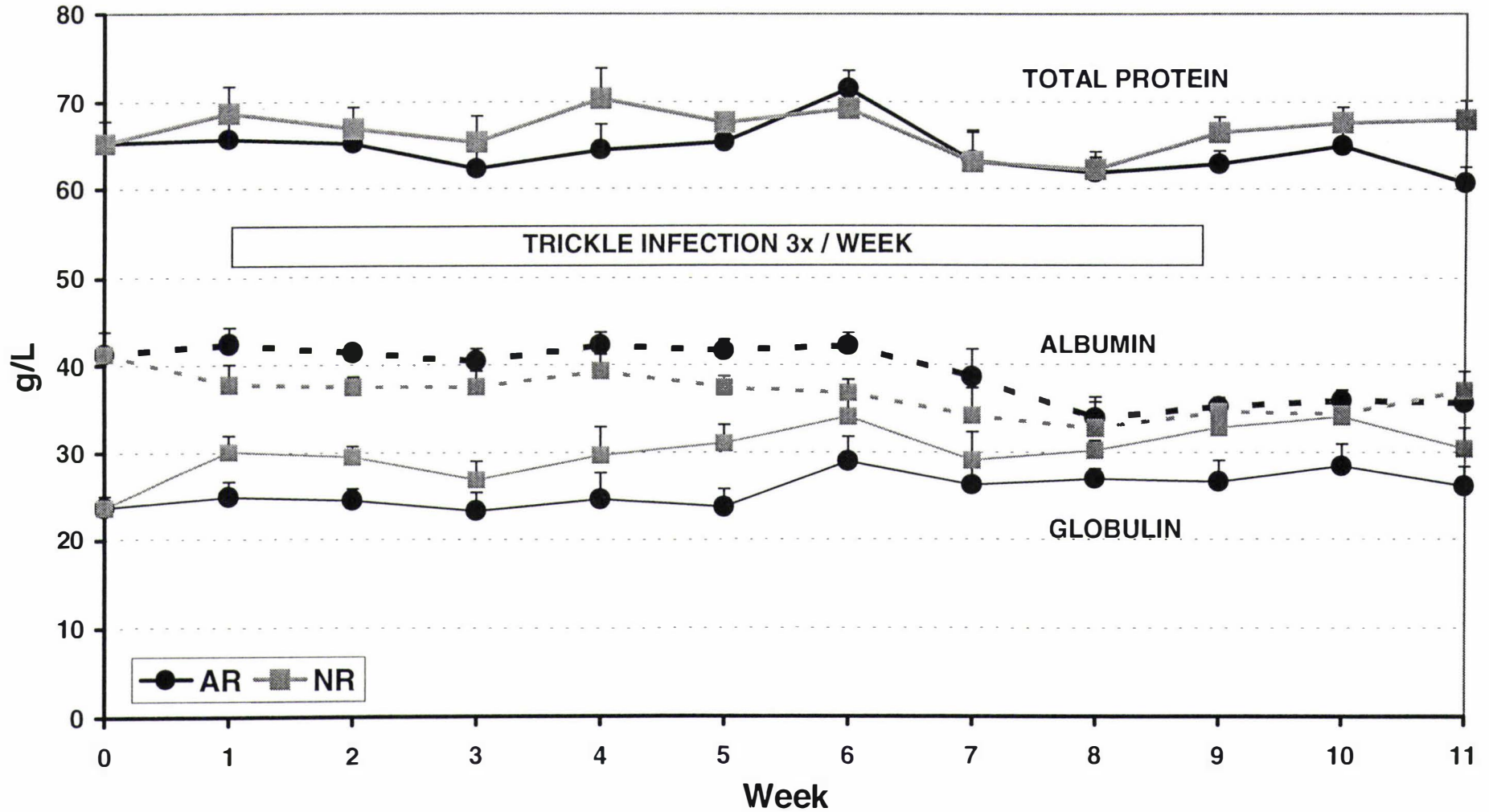


Figure 4.14. Mean (+SE) serum pepsinogen concentration of artificially (AR) and naturally (NR) reared deer

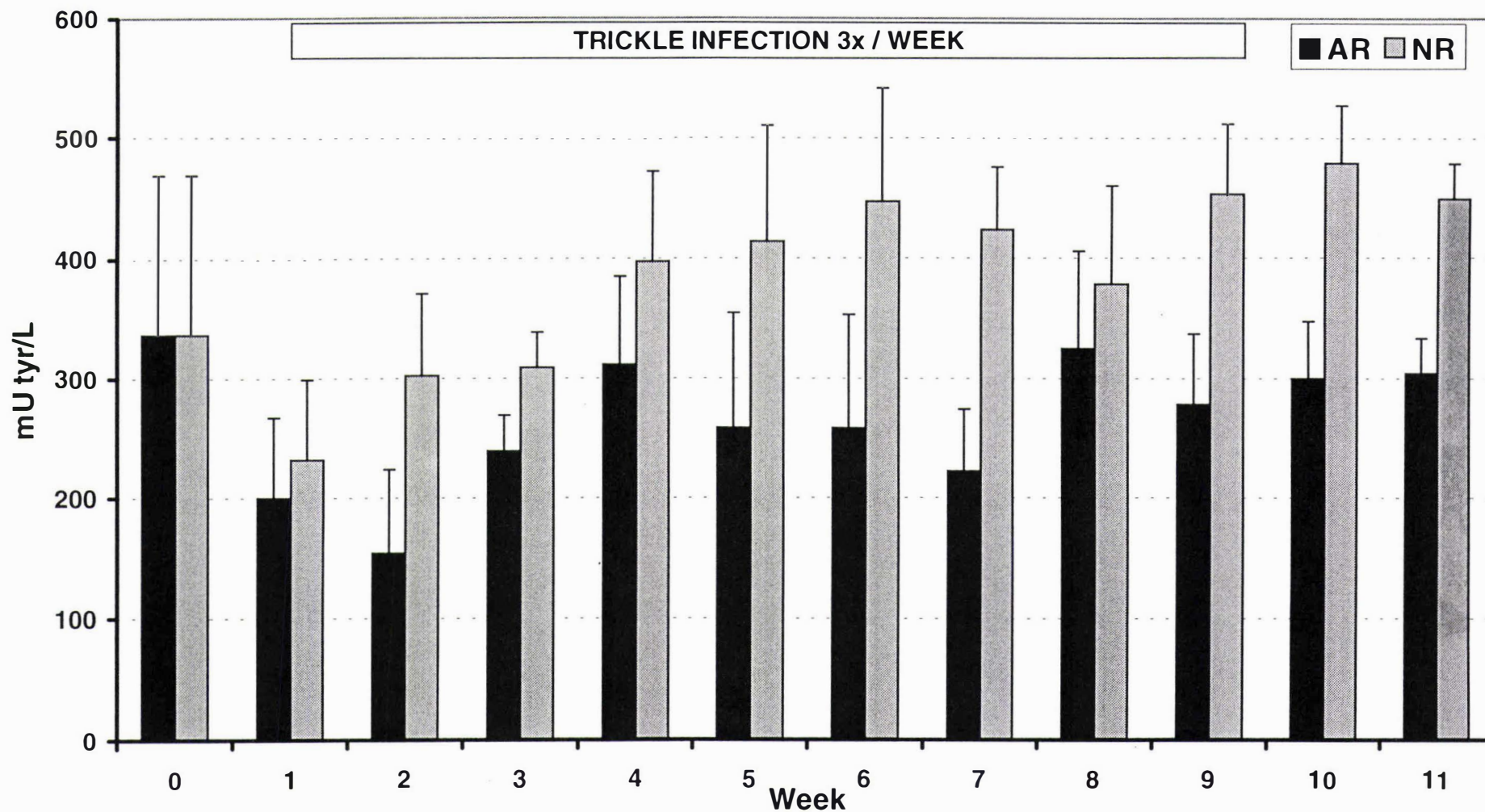


Figure 4.15. Mean (+SE) serum gastrin concentration of artificially (AR) and naturally (NR) reared deer

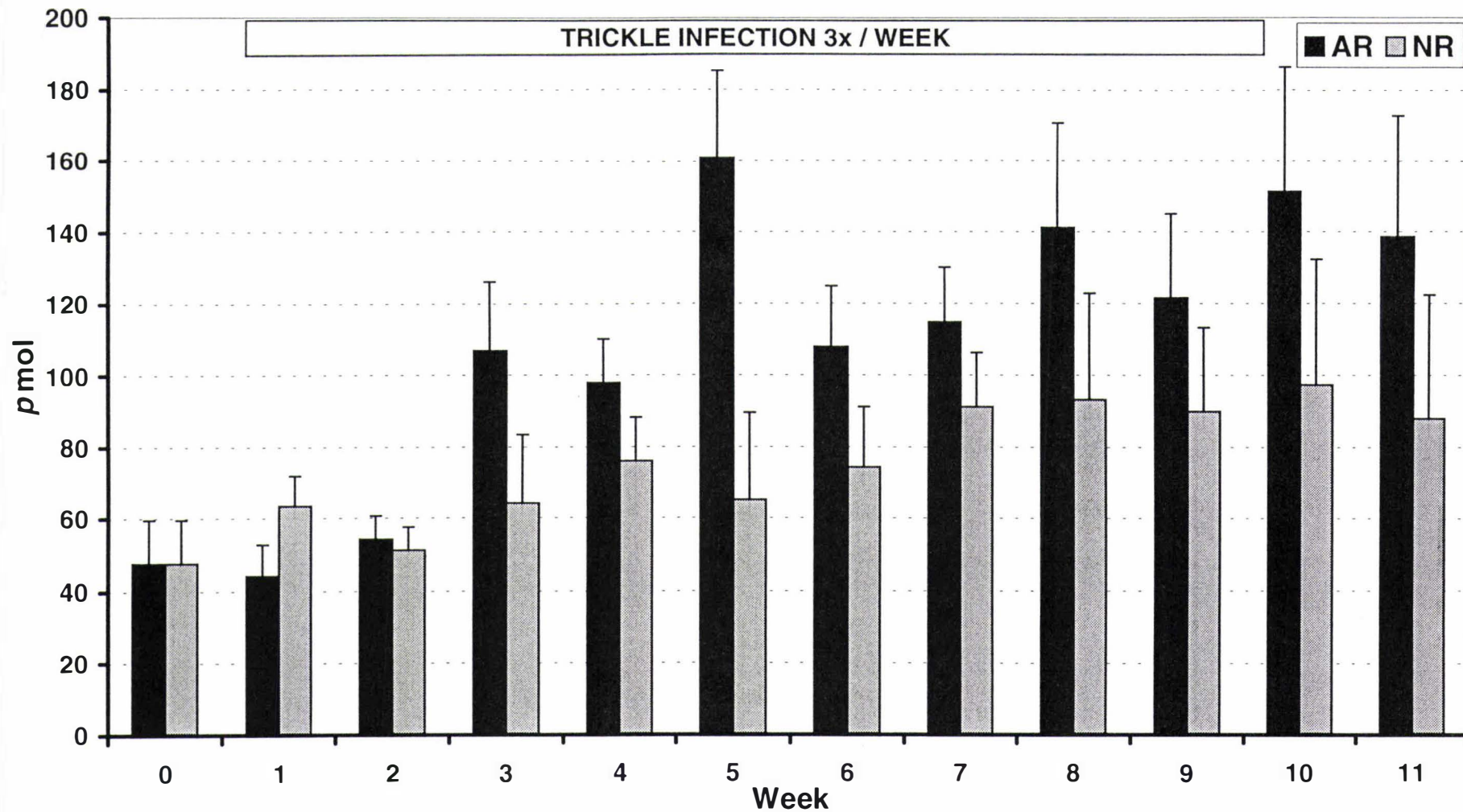


Figure 4.16. Mean (+SE) total leucocyte (WBC) and erythrocyte (RBC) counts of artificially (AR) and naturally (NR) reared deer

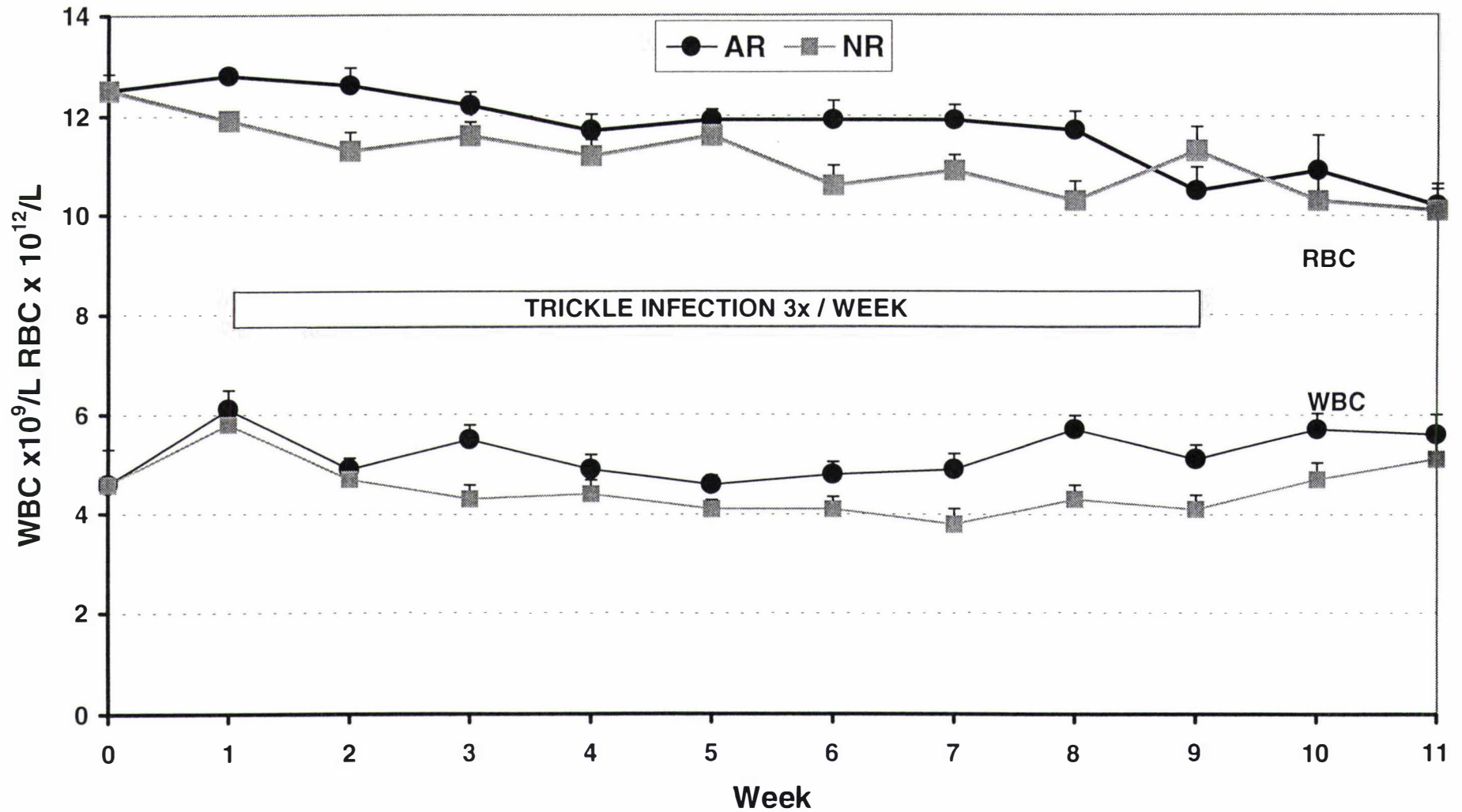
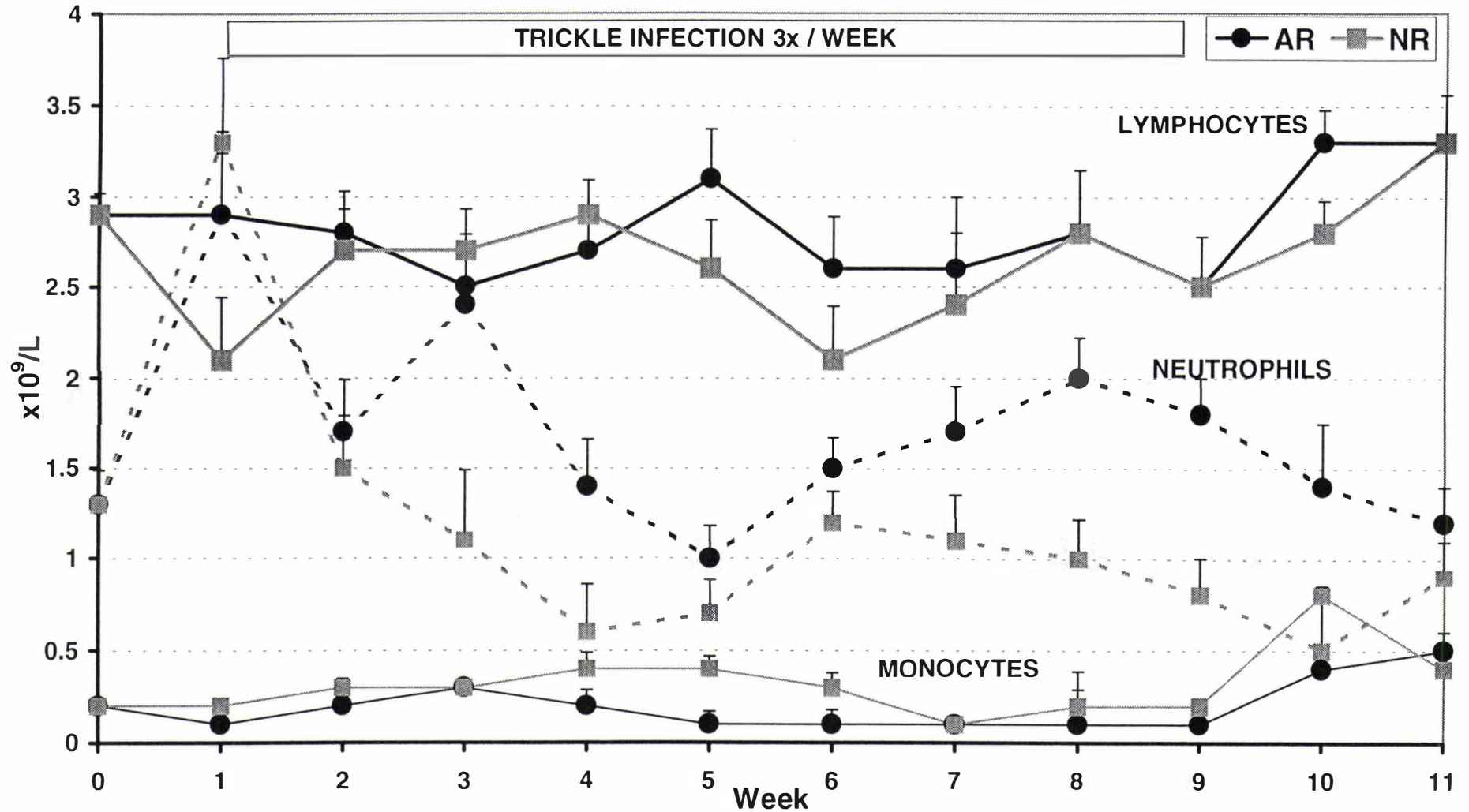


Figure 4.17. Mean (+SE) lymphocyte, neutrophil and monocyte counts of artificially (AR) and naturally (NR) reared deer



## 4.5. DISCUSSION

This is the first report of a deer-specific parasitological model. It is also the first report of experimental infection of red deer with both lungworm and GI nematodes concurrently.

The primary objective of this study, to develop a model of sub-clinical parasitism in deer, was achieved. This model has also shown that low lungworm and GI nematode burdens (Table 4.3.) reduced both liveweight gain and VFI. These effects increased with time, probably due to the combined effect of parasites in the lungs, abomasum, small and large intestines. Partitioning of the relative importance of each parasite group could be achieved by use of individual worm species cultures for infection. The infections resulted in elevated serum pepsinogen and gastrin, reduced serum albumin, elevated serum globulin, elevated peripheral eosinophil concentration and slight haemoconcentration and these changes are consistent with known effects of GI nematodes in sheep (Coop *et al.*, 1977) and cattle (Fox, 1993). Animal performance (VFI and liveweight), GI nematode burdens and FLC were related to infection rate. Relationships between lungworm burdens, FEC, serum biochemistry and haematology were less consistently related to infection rate.

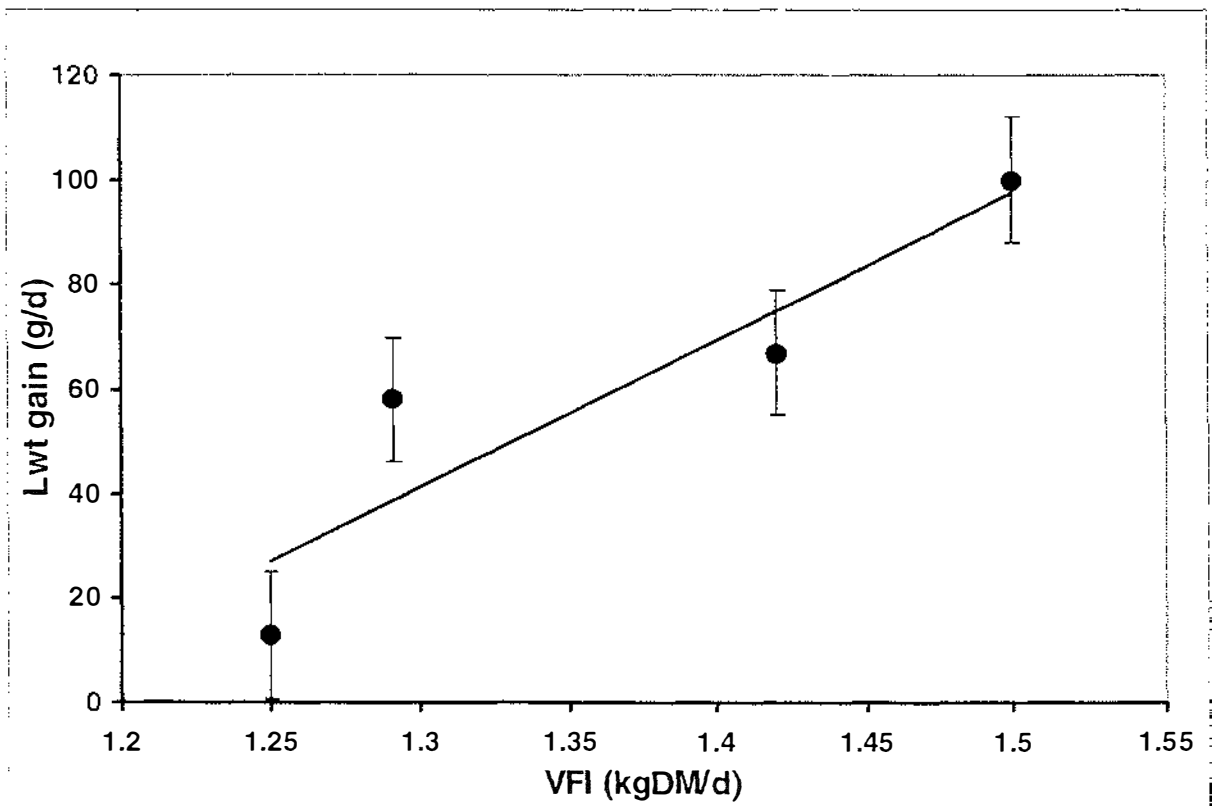
Corrigan *et al.*, (1982) found daily challenge of weaner red deer with 500L<sub>3</sub> *D. viviparus* per kilogram liveweight for 17 days reduced VFI and caused liveweight loss of up to 99g/d. This level of infection was greater than the 100-400 L<sub>3</sub>, 3x per week used in the current study, and produced clinical disease, whereas only sub-clinical effects were produced in this study. Nematode counts were not included in the report by Corrigan *et al.*, (1982). Johnston *et al.*, (1984) infected weaner deer with 30,000 *O.circumcincta*, *O.ostertagi* or *H.contortus* of sheep origin and recorded reduced VFI in the animals infected with *H.contortus* but not *Ostertagia* spp. during the seven weeks following infection. A number of parasites are known to be more pathogenic when they occur concurrently with others at different sites in sheep (Sykes *et al.*, 1988; Catchpole and Harris, 1989; Heath and Connan, 1991) and cattle (Davis *et al.*, 1959; Parkins *et al.*, 1990) and concurrent infections of *Dictyocaulus* and the mixed GI nematode species used in the present study may be more pathogenic than the single species infections used in other deer studies.

The 22% reduction in VFI and 9% reduction in final liveweight of the HT group compared with control deer at the point of slaughter represents a typical sub-clinical response to internal parasitism (Sykes, 1994). The reduction in growth could be due to



the infections reducing VFI, reducing digestive efficiency or reducing the efficiency with which absorbed nutrients are used by the body. This can be studied further through examining linear regression relationships between VFI and liveweight gain. The relationship in Figure 4.18. shows that reductions in liveweight gain were associated with reductions in VFI ( $P=0.088$ ). Digestive efficiency, including nitrogen retention, apparent digestibility of organic matter and flow of nitrogen from the abomasum to terminal ileum of a sub-set of deer used in this experiment are presented in Chapter 5. The other main factor thought to contribute to reduced liveweight gain is reduced efficiency of utilization of protein caused by diversion of protein synthesis away from body growth towards the repair, replacement and reaction to gut damage, or to loss of host tissues associated with parasitism (Poppi *et al.*, 1986, 1990; Bown *et al.*, 1991; MacRae, 1993; Sykes, 1994). This explanation is more likely than reduced efficiency of ME utilisation *per se* (MacRae *et al.*, 1982). However, research into efficiency of food utilisation of parasitised deer is required to confirm this.

**Figure 4.18. Mean daily VFI vs mean daily liveweight gain for each group ( $\pm$  SE) following the start of infection.**



The nematode burdens at slaughter ( $67 \pm 16.4$  lungworm and  $783 \pm 193.6$  GI nematodes; mean  $\pm$ SE) resulting from the highest rate of infection in this study are considered low when compared with counts observed in clinically affected deer of the same age (Wilson, pers comm). Other reports in the literature of mean untransformed counts obtained from slaughtered farmed red deer under one year of age range from 257 (Corrigan *et al.*, 1986) to 872 (Mackintosh and Mason, 1985) for lungworm and 700 (Connan, 1997) to 4538 (Mackintosh *et al.*, 1993) for abomasal nematodes. The numbers of adult nematodes recovered post-mortem were variable within and between treatments in this study. It is likely that given the 14-week period of infection, immunity had developed in most individuals, resulting in expulsion of worms and reduced fecundity of female nematodes. This is consistent with the pattern of faecal egg and larval counts (Figs 4.3. & 4.4.). A few adult GI nematodes (<5/animal) were recovered from faeces collected for FEC from some animals in the HT and MT groups during weeks 10 and 11, but it is not known if reduced nematode fecundity occurred at this time. Dobson *et al.*, (1990a) infected sheep at different rates with *T.colubriformis* for 20 weeks and found that a decline in establishment of L<sub>3</sub> and rejection of adult nematodes began at weeks 7-9 for the higher infection rates and weeks 12-14 for the low infection rate. A similar effect may have occurred in the present study, so it is recommended that future deer studies investigating establishment of internal parasites by trickle infection according to the method presented in this study conclude at about seven weeks. Dobson *et al.*, (1990a) also reported reduced fecundity of nematodes associated with the development of host immunity, but this needs to be investigated in deer.

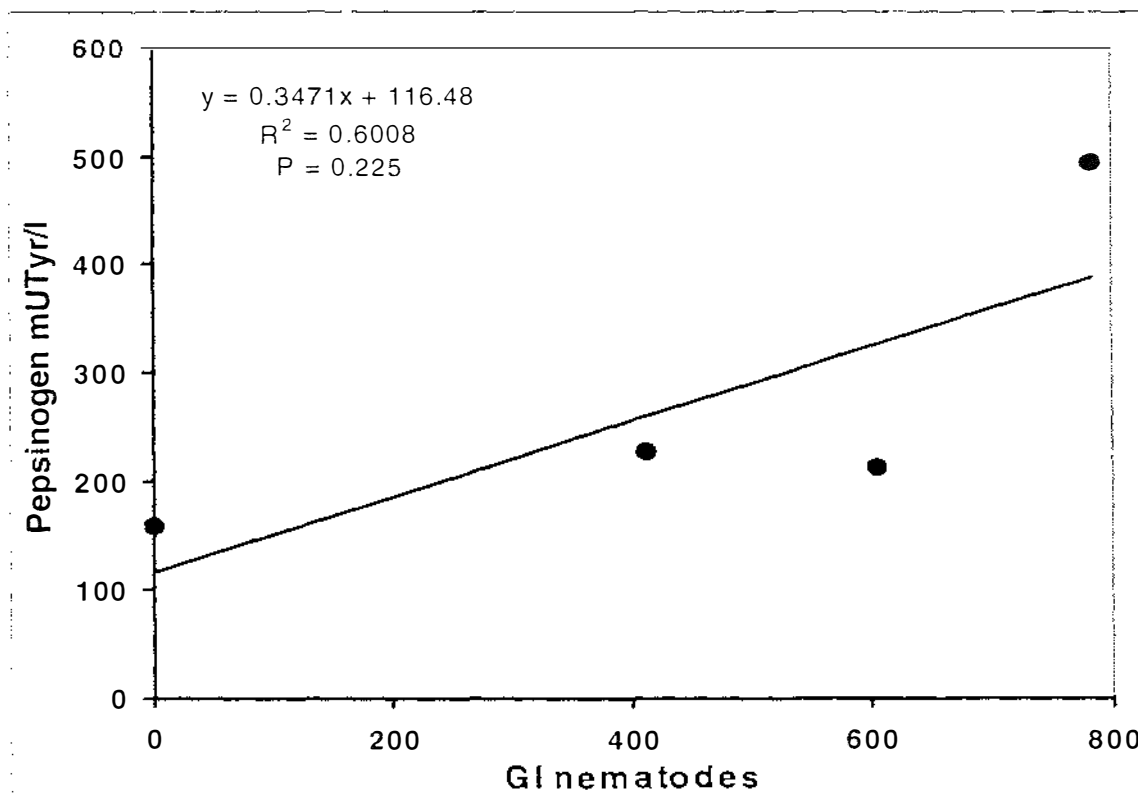
By the time of slaughter, both FEC and FLC were declining as eosinophil counts were rising, further supporting the hypothesis that animals in this study were developing immunity to infection (Buddle *et al.*, 1992). This may explain the low proportion of the total number of L<sub>3</sub> administered that were recovered at slaughter ('apparent take'), which for lungworm ranged from 1.1% for the LT group to 0.6% for the MT and HT groups and which for GI nematodes was 3%, 2.2% and 1.4% for the LT, MT and HT groups, respectively. However, it should be noted that the 'apparent take' of larvae administered to the NR animal euthanased in week 5 of infection was 1.6% for lungworm and 2% for GI nematodes. This was not much greater than for animals euthanased 6-7 weeks later. Christensen *et al.*, (1997) found that the 'take' of L<sub>3</sub> *Oe. dentatum* in pigs administered by trickle infection inversely ranked with infection rate. In the present study, as infection rate increased the 'apparent take' of L<sub>3</sub> decreased, particularly for GI nematodes.

The similarity in liveweight, VFI and nematode counts obtained from the comparison of naturally-reared (NR) and artificially-reared (AR) deer suggest that NR, anthelmintic-treated, pasture-reared deer calves would be suitable for use in some types of parasite control studies, particularly in relation to nutrition and responses to treatment. This finding led to the decision to use NR calves in the study described in Chapter 6. However, the few significant differences obtained in serum biochemistry and haematology results suggest that NR deer may not be appropriate experimental animals for use in some types of immunological studies, where parasite naive animals are essential. Advantages of using NR over AR animals in parasite control studies are reduced rearing cost and labour inputs and eliminating the need for specialist artificial rearing facilities. Furthermore, higher losses are associated with artificial rearing of deer calves and there are welfare implications involved. The suitability of NR deer calves may be enhanced if calves and their dams were grazed on a newly established forage crop such as chicory, further reducing the likelihood of parasite infection (see Chapter 3).

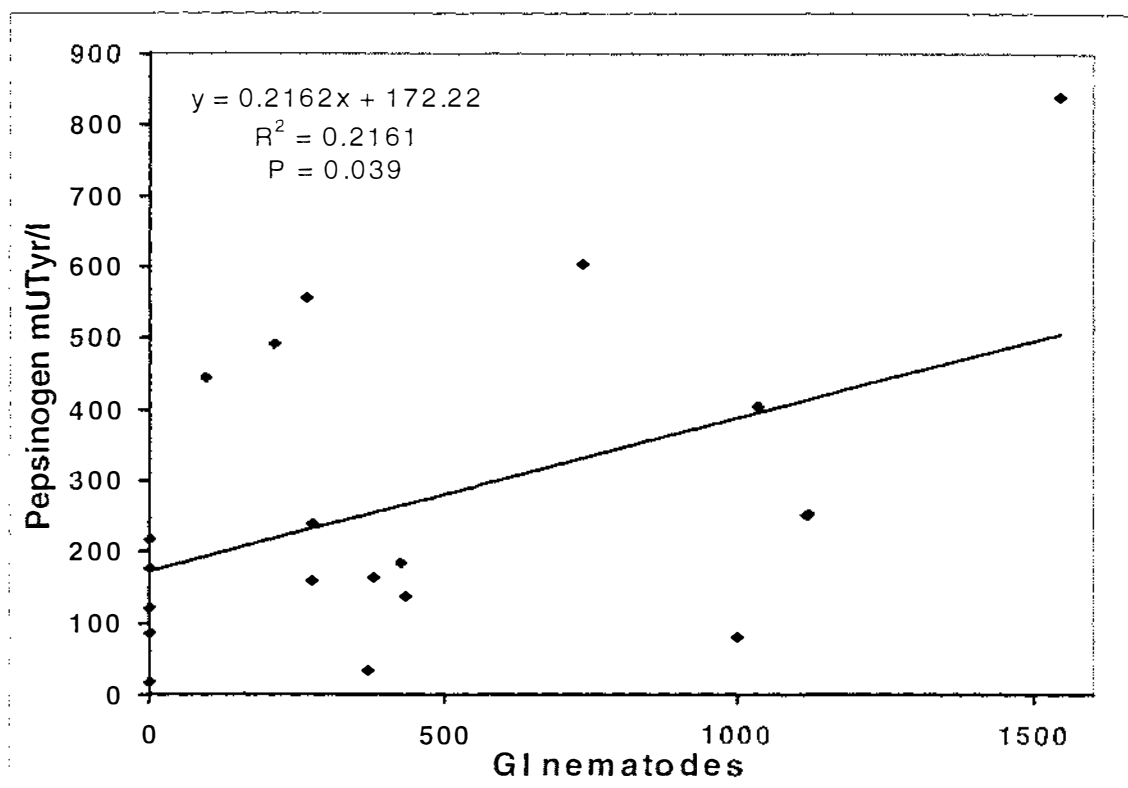
Haematological and blood biochemical changes which reflect gastric dysfunction are well documented in sheep infected with *O.circumcincta* (Holmes and McLean, 1971; McLeay *et al.*, 1973; Anderson *et al.*, 1976a,b; Coop *et al.*, 1977; Titchen and Anderson, 1977; Anderson *et al.*, 1981; Coop *et al.*, 1982; Anderson *et al.*, 1988). Similar changes occur in cattle infected with *O.ostertagi* (Anderson *et al.*, 1965; Fox *et al.*, 1987; Fox, 1993). These include decreased serum albumin concentrations (often with elevated serum globulin concentration), elevated serum gastrin and pepsinogen concentration, haemoconcentration, and eosinophilia. In the present study, RBC, HB and PCV data together indicates some haemoconcentration, especially in the HT group. This, combined with the reduced serum albumin and increased SPC in the HT group, suggests damage to the gastric mucosal barrier and increased permeability allowing leakage of plasma and albumin into the gut (Holmes and McLean, 1971) and perhaps the lungs. Inactivated pepsinogen can then diffuse into the blood due to “leak lesions” (Jennings *et al.*, 1966), although this cannot be the only mechanism by which serum pepsinogen concentration is increased by abomasal parasites (Anderson *et al.*, 1985; Lawton *et al.*, 1996). However, the degree of abomasal damage cannot be assessed without analyses of histological samples. The exact mechanisms by which such GI damage causes inappetance are unknown (Symons, 1985; Poppi *et al.*, 1990) but these effects are also likely to reduce the efficiency with which protein is deposited during growth. Further research is required in order to understand this more fully in deer.

The data collected in this study may also have relevance to diagnosis of parasitism in young deer. Audige *et al.*, (1998) found a significant negative relationship between farm mean (but not individual animal) serum pepsinogen concentration, and late spring growth rates of weaner deer. Figure 4.19. shows that in this study the relationship between pre-euthanasia group mean SPC and GI nematodes counts was not significant ( $P=0.23$ ). However, Figure 4.20. shows a significant relationship between individual animal pre-euthanasia SPC and GI nematode counts ( $P<0.05$ ) and the regression coefficient suggests that 22% of the increase in individual animal SPC can be explained by the increase in GI nematode counts. *Ostertagia*-type (Connan, 1991) and *H.contortus* infections (Johnston *et al.*, 1984) have been associated with elevated serum pepsinogen concentration (SPC) in deer. Other authors evaluating the use of anthelmintics for control of internal parasites in deer have also reported significant differences in serum albumin and SPC between treatments (Wagner *et al.*, 1993; Waldrup *et al.*, 1994). There was no relationship between pre-euthanasia individual animal serum albumin concentration and GI nematode counts in this study ( $R^2 = 0.0005$ ;  $y = 0.0002x + 37.33$ ;  $P=0.92$ ).

**Figure 4.19. Group mean pre-euthanasia serum pepsinogen concentration vs group mean GI nematode counts.**



**Figure 4.20. Pre-euthanasia individual serum pepsinogen concentration vs GI nematode count.**



The mean plus two standard deviations of the SPC for the control group over the duration of the experiment can be used to define the upper limits of the normal range ( $u + 2s = 290\text{mUTyr/l}$ ). The group mean SPC for the HT deer was greater than the upper limits of the normal range from weeks 4-11, confirming an elevation of SPC during this time. Audige *et al.*, (1998) reported geometric means of pasture grazed farmed weaner deer SPC ranging from 139-531mU, with individual deer up to 2609mU. The SPC recorded in controlled indoor experiments involving parasite-naïve deer, as in this study, therefore cannot be directly related to field data. The use of SPC, either in individual deer, or as a herd mean parameter, as a diagnostic tool for sub-clinical and/or clinical parasitism needs further evaluation under both controlled indoor and field conditions.

The lower limits of the normal range (mean minus 2 standard deviations) of serum albumin concentration (SA) in this study was 34.3g/l. As the group mean SA for the HT and MT groups was lower than the lower limits of the normal range during weeks 7 and 8, it is apparent that these groups exhibited reduced SA during this time. However, the SA values measured in the current study are high compared to other reports of group

mean SA from weaner red deer on pasture (25.2-28.7g/l Wagner *et al.*, 1993; 27.1-34.6g/l Waldrup *et al.*, 1994; 28.3-39.0 Audige 1995). Thus while a relationship has been observed here, direct comparison with absolute values from other studies could be misleading.

Eosinophils are effector cells of immunity and hypersensitivity reactions and are a predominant feature of GI inflammation, especially that associated with GI parasitism (Butterworth, 1984). For peripheral eosinophil counts the upper limit of the normal range in this study was  $0.13 \times 10^9/l$ , which was fairly low compared with data of Audige (1995) where mean eosinophil counts of  $0.12-0.21 \times 10^9/l$  were observed for farmed weaner red deer. Mean eosinophil counts of all infected treatments exceeded the upper limits of the normal range. These levels are abnormal and are thought to be associated with the development of host immunity (Buddle *et al.*, 1992). In this study a significant relationship was found between individual animal pre-euthanasia total eosinophil count and both GI nematode (Figure 4.21.) and lungworm counts (Figure 4.22.) ( $P < 0.05$ ).

**Figure 4.21. Pre-euthanasia peripheral eosinophil count vs GI nematode count.**

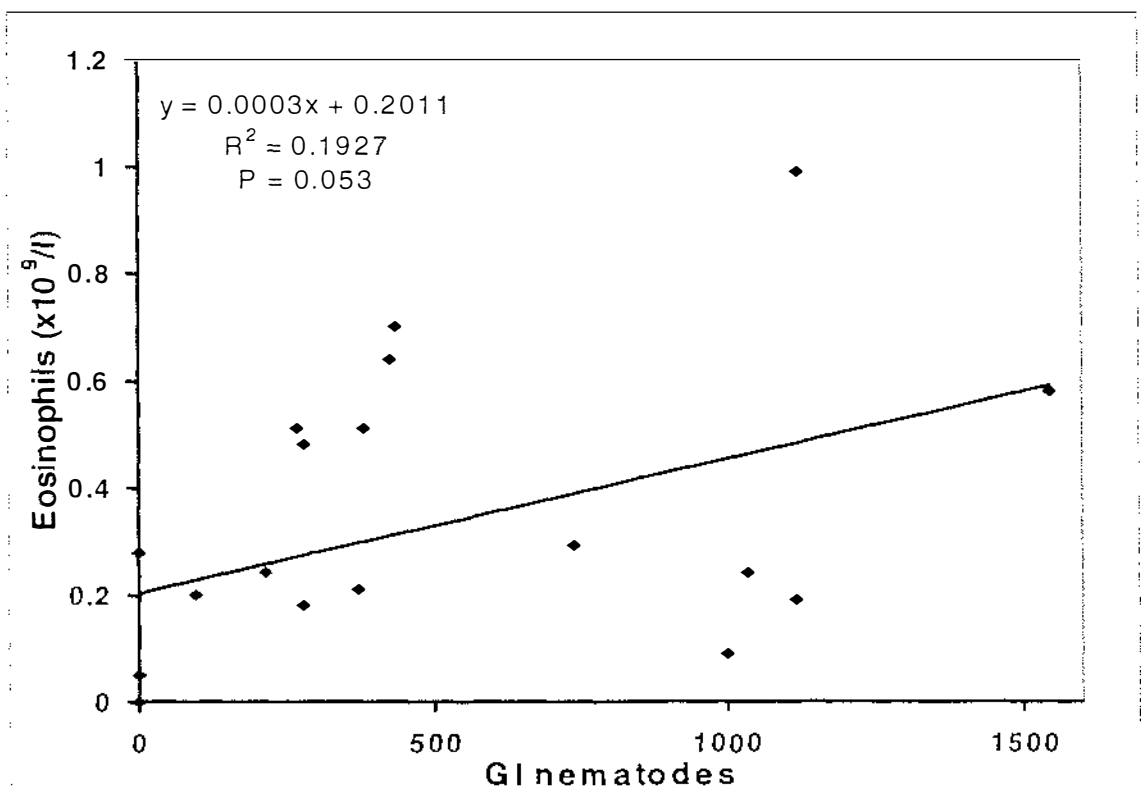
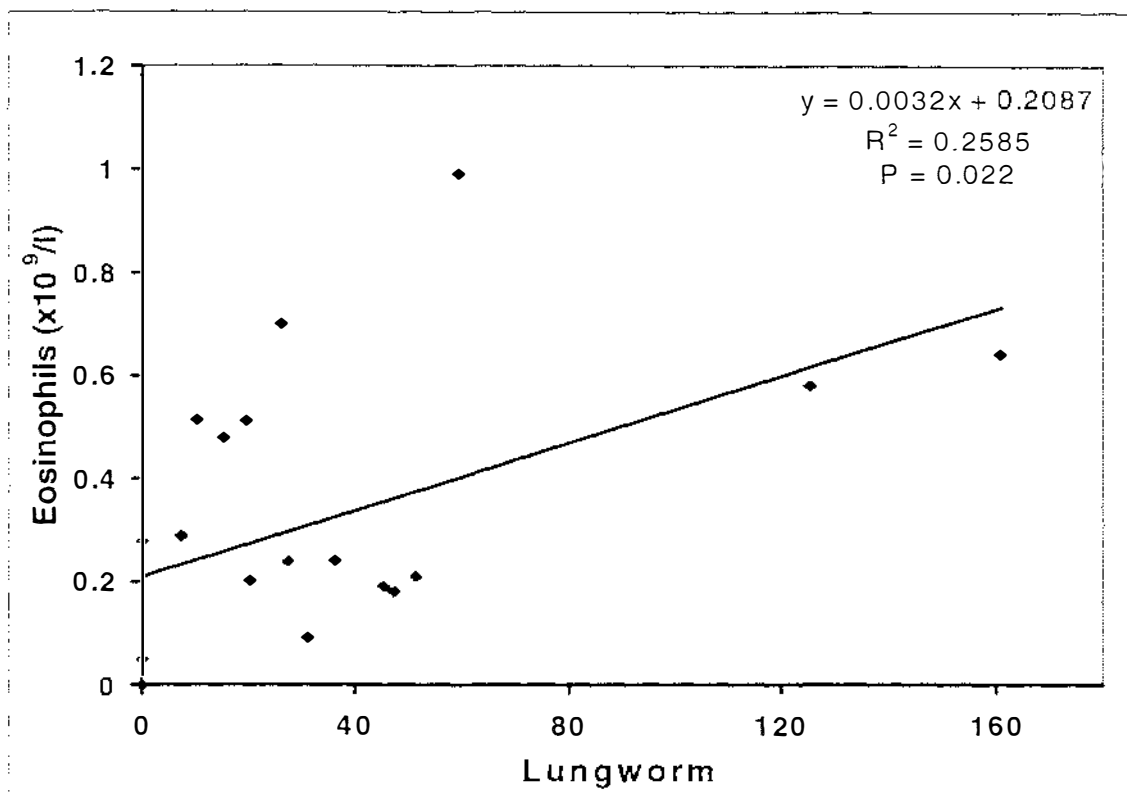


Figure 4.22. Pre-euthanasia peripheral eosinophil count vs lungworm count.

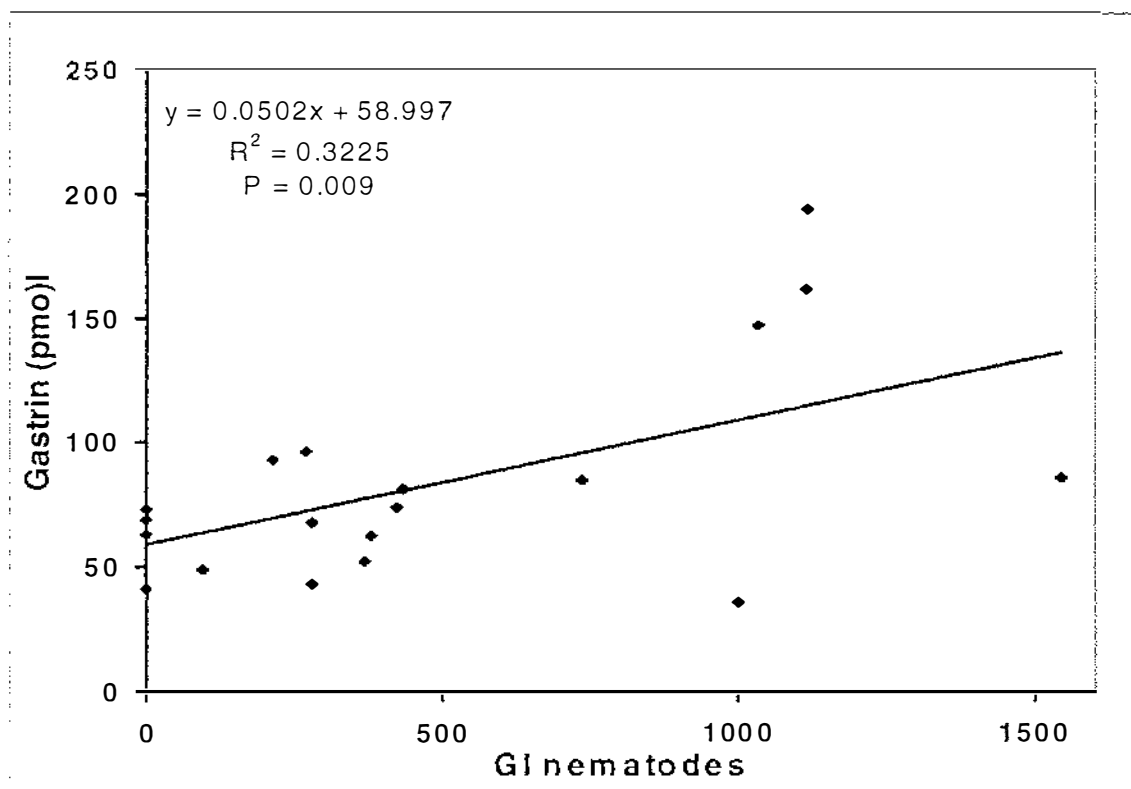


The regression coefficients, respectively, suggest that 19% and 26% of the variation in individual animal pre-euthanasia total eosinophil count is explained by the numbers of GI nematodes and lungworm recovered from each animal. Partitioning of the effect on peripheral eosinophil counts of GI nematodes and lungworm could be determined by infection of deer with individual parasite species. Further research is required to investigate the use of peripheral eosinophil counts as a diagnostic indicator of parasitism in deer under controlled indoor and field conditions.

The trend towards increased serum gastrin concentration (SGC) of parasitised deer compared with control deer in this study warrants further investigation. The regression coefficient (Figure 4.23.) suggests that 32% of the increase in individual animal pre-euthanasia SGC was explained by GI nematode counts ( $P < 0.01$ ). While SGC has been associated with abomasal parasitism in sheep (Titchen and Anderson, 1977; Anderson *et al.*, 1981) and cattle (Fox *et al.*, 1987, 1993) this is the first report of a relationship between SGC and parasitism in deer. Elevated SGC may contribute to inappetance (Fox *et al.*, 1989a,b), and it is possible that in this study the trend towards increased serum gastrin in HT/MT deer may have contributed to the reduced VFI of these groups. Further study of these relationships and the diagnostic significance of SGC in

parasitised deer is warranted.

**Figure 4.23. Pre-euthanasia individual serum gastrin concentration vs GI nematode count.**



Despite significant reductions in liveweight and VFI in this study, both FEC and FLC were low. Group mean FEC remained below 200epg and when comparing infected groups, FEC were not related to infection rate. This is similar to the report of Johnston *et al.*, (1984) where group mean FEC reached a maximum of 300epg in infected deer. However, in that study deer were infected with sheep-origin nematode species, not deer-origin species as in the current study. In Chapter 3, group mean FEC of deer calves on pasture prior to anthelmintic treatment reached a maximum of only 125epg. Audige *et al.*, (1998) reported that 12.6-33.6% of farmed red deer calves prior to first anthelmintic treatment had  $FEC \geq 200$ epg which, from the results of this study, indicate that 12.6-33.6% of those calves were likely to have reduced liveweight and VFI. Audige (1995) showed that liveweight at weaning was higher in deer calves which had received anthelmintic treatment prior to weaning.

The group geometric mean FLC in this study was related to infection rate but remained below 100 lpg. The highest individual FLC was 569 lpg. Despite these low



FLC's, some soft coughing was observed in a few deer (<10 total) from all infected groups during feeding, from week 5 onwards. No coughing was observed in control deer. In Chapter 3, group mean FLC of deer calves on pasture before anthelmintic treatment reached a maximum of 115 lpg, although clinical disease was observed in individual animals with FLC<10 lpg, probably because of a high number of immature parasites. These clinically-infected deer on pasture were observed to cough heavily, sometimes for extended periods (up to five minutes), following exercise. Audige *et al.*, (1998) reported a group geometric mean FLC of farmed deer calves prior to first anthelmintic treatment that had a positive FLC, of between 15.2-54.7 lpg, with the highest individual FLC of 1807 lpg. The relationship between FEC/FLC and nematode burdens in this study could not be accurately determined due to the low proportion of animals with FEC/FLC >0 at the last sampling.

This study has described development of a model for infection of deer with *Dictyocaulus* and GI nematodes, which has also helped to define the effects on weaner deer of sub-clinical concurrent lungworm and mixed GI nematode infection. While the HT level of infection used in this study produced several sub-clinical effects, it is proposed that to use this model to evaluate methods of parasite control, the dose rate be increased to improve the probability of achieving statistically significant results. An increase of 25% to 2500 GI nematode and 500 lungworm L<sub>3</sub>/dose, 3x per week is suggested. Variations of this model could be used for a multiplicity of purposes such as: the investigation of pathology and physiology of parasitism in deer including effects of nutrition; further evaluation of the diagnostic significance for deer of the serum constituents and haematological aspects highlighted in this study; investigation of the relationship between worm burdens, FEC/FLC and clinical/sub-clinical effects; immunological studies; anthelmintic resistance studies; and evaluation of both natural and chemical parasite control strategies. Many of these factors will be critical for parasite control on commercial deer farms as issues of sustainability, low chemical usage, organic production and anthelmintic resistance become more significant.

## CHAPTER 5.

# Apparent digestibility and nitrogen retention of weaner deer fed lucerne chaff and infected with lungworm (*Dictyocaulus* sp.) and gastrointestinal nematodes.

SIMONE O. HOSKIN<sup>1</sup>, T.N. BARRY<sup>1</sup>, G.C. WAGHORN<sup>2</sup>, P.R. WILSON<sup>3</sup>, W.A.G. CHARLESTON<sup>3</sup>

<sup>1</sup> Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand

<sup>2</sup> AgResearch, Palmerston North, New Zealand

<sup>3</sup> Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand

### 5.1. ABSTRACT

The effects of trickle infection with lungworm (*Dictyocaulus viviparus*) and mixed gastrointestinal nematode larvae upon apparent digestibility of chaffed lucerne hay and nitrogen (N) retention were studied with 12 weaned red deer calves in metabolism cages. Digesta flow at the abomasum and terminal ileum at slaughter was determined with those, plus an additional eight housed deer calves, using Cr<sub>2</sub>O<sub>3</sub> released from intra-ruminal slow-release capsules as a marker. The deer were trickle infected at three sub-clinical levels (low, medium and high) for nine weeks prior to the start of this experiment, with control deer uninfected (Chapter 4).

There were no significant differences between treatments in apparent digestibility of dry matter (DM), organic matter (OM), gross energy (GE), nor any of the components of plant fibre. Nitrogen retention (g/d) of the group infected at the high rate was lower than for the control group, with the low and medium infection rate groups being intermediate. This difference in N-retention was found to be largely due to differences between treatments in N intake ( $r^2=0.61$ ). There was no difference between treatments in

flow of N at the abomasum, either in g/d or expressed as a proportion of N intake. Flow of N at the terminal ileum was lower for HT deer than control deer (g/d), but this difference was not apparent when N flow at the ileum was expressed as a proportion of N intake. This study highlights the importance of reduced feed intake in depressing N-retention in young deer infected at sub-clinical levels with lungworm and GI nematodes.

## 5.2. INTRODUCTION

The pathophysiology of gastrointestinal (GI) nematode infections and the effect of GI parasitism on nutrition of farmed ruminants has been extensively reviewed (Steel, 1974; Sykes, 1978; Holmes, 1985; Parkins and Holmes, 1989; Poppi *et al.*, 1990; Fox, 1993; Holmes, 1993; MacRae, 1993; Sykes 1994; Coop and Holmes, 1996; Knox and Steel, 1996). Reduced voluntary feed intake (VFI) and reduced N-retention are characteristic features of GI parasitism. Studies have suggested little effect of parasites on protein digestion and absorption *per se* (Steel, 1974; Rowe *et al.*, 1988; Bown *et al.*, 1991b). Increased flow of N at the terminal ileum, partly as a result of leakage of plasma protein, but also from increased exfoliation of gut epithelial cells and mucus secretion (Poppi *et al.*, 1986; Kimambo *et al.*, 1988a; Bown *et al.*, 1991b) leads to either increased urinary N loss (Parkins *et al.*, 1973), implying a reduction in the efficiency of utilisation of absorbed amino acids (Parkins and Holmes, 1989), and/or to increased faecal N loss. Both these factors contribute to reduced N-retention.

Other than reduced VFI, the reduced efficiency of utilisation of digested protein for growth is thought to be the major contributor to reduced animal production associated with GI parasitism (MacRae, *et al.*, 1982). However, more minor effects such as reduced efficiency of energy utilisation due to both abomasal and intestinal infections (Sykes and Coop, 1977; Sykes *et al.*, 1991; MacRae *et al.*, 1982) and small reductions in apparent gross energy digestion have been recorded (Sykes and Coop, 1977, MacRae *et al.*, 1982, Armour *et al.*, 1987). Gastrointestinal parasite infections in calves have in some cases reduced apparent digestibility of dry matter (DM), organic matter (OM) and crude fibre (Armour *et al.*, 1987), but not in others (Randall and Gibbs, 1981).

Infection of bovine calves with lungworm (*Dictyocaulus viviparus*) was found to reduce N retention via increased urinary-N output, suggesting a reduced efficiency of N utilisation (Kroonen *et al.*, 1986). The same study showed slightly reduced metabolism of energy, but digestibility of DM and N were not affected by lungworm infection.

The objective of this study was to investigate the effects of different levels of

concurrent lungworm and mixed GI nematode infection in weaner deer upon apparent digestibility, N-retention and the flow of N at the abomasum and terminal ileum.

## 5.3. MATERIALS AND METHODS

### 5.3.3. Experimental Design

Table 5.1. Experimental design

Treatment	N	Larval Dose Rate*		Worm Count at Slaughter	
		<i>Dictyocaulus</i>	GI nematodes	Lung	GI Total
<b>1. Apparent Digestibility and Nitrogen Balance</b>					
C	3	0	0	0	0
LT	3	100	500	26	546
MT	3	200	1000	38	844
HT	3	400	2000	81	957
<b>2. Digesta Flow</b>					
C	5	0	0	0	0
LT	5	100	500	30	412
MT	5	200	1000	31	605
HT	5	400	2000	67	783

\*Given 3x/week for 9 weeks prior to trial commencement.

C = control, LT = low treatment, MT = medium treatment, HT = high treatment.

The experimental design is summarised in Table 5.1. The effects of trickle infection with lungworm and gastrointestinal nematode larvae upon apparent digestibility of lucerne hay and N-retention were studied with 12 weaned red deer calves maintained indoors in metabolism cages. Digesta flow was determined with the same 12 deer plus an additional eight deer housed indoors in modified sheep cages. Deer used in this study are those used in the study presented in Chapter 4. The internal parasites administered were three levels of lungworm (*Dictyocaulus viviparus*) plus gastrointestinal (GI) nematode (majority deer specific abomasal *Ostertagia*-type, eg. *Spiculopteragia* sp.) L<sub>3</sub> stage infective larvae, given as low (LT), medium (MT) and high (HT) rates of infection. A fourth control group (C) was uninfected. The infective larvae were given as trickle infections 3x/week for nine weeks before the nutritional measurements described here commenced. Infective larvae were not given during the period of nutritional

measurements, but existing nematodes were allowed to mature over this period. Worm counts following euthanasia of the treatment groups are presented in Table 5.1. (from Chapter 4). Of the total number of GI worms, 98% were abomasal nematodes. The proportion of specific nematode species recovered is presented in Chapter 4 (Table 4.4.).

Factors investigated included apparent digestibility, N-retention and flow of N at the abomasum and terminal ileum. The experiment was carried out at Massey University for 17 days from 24 July 1995 to 10 August 1995. Faeces and urine outputs were measured by direct collection from the 12 deer in metabolism cages, whilst abomasal and ileal N flows were estimated using Cr<sub>2</sub>O<sub>3</sub> as a marker, administered from intra-ruminal slow-release capsules.

### **5.3.2. Animals and Housing**

Rearing methods and pre-trial treatment of the animals are presented in Chapter 4. (see section 4.1.3.2.). Twelve deer calves (eight months of age; six stags, six hinds) that were housed in deer metabolism crates were used, plus an additional eight deer (four hinds, four stags) that were housed in modified sheep cages.

All deer were fed chaffed lucerne hay *ad libitum* at 0900hrs and had free access to water and a multi-mineral salt block during the digestibility and N balance period (seven days). This was followed by a pre-slaughter period (6-10 days). During the final 48hrs prior to slaughter they were fed equal sized meals at 0200hrs, 0600hrs, 0900hrs, 1200hrs, 1500 hrs, 1800hrs, and 2200hrs. This was to produce the steady state conditions which are essential for measurements of digesta flow at slaughter. An intra-ruminal chromium (Cr) slow-release capsule (CRDC, Cr<sub>2</sub>O<sub>3</sub> matrix, Captec Ltd, Auckland, New Zealand ) was administered to each deer (Parker *et al.*, 1989) 20 days prior to slaughter. Before administration, the distance from the base of the capsule to the top of the plunger was measured.

Details of parasitology are described in Chapter 4.

### **5.3.3. Apparent Digestibility and Nitrogen Balance**

Feed offered and refused was weighed, and faeces quantitatively collected daily, weighed and pooled frozen per animal (-20°C). Daily samples (200g) of feed offered and residual feed (per animal) were taken in triplicate for DM analysis (100°C; 18hrs) and in duplicate to be pooled per animal and stored at -20°C. Duplicate subsamples of pooled feed and residue were then taken for freeze-drying, grinding and chemical analysis.

Pooled faeces (per animal) was homogenised and triplicate samples were taken for DM analysis (100°C; 48hrs) and duplicate samples were taken for freeze-drying, grinding and chemical analysis.

Each animal's urine was quantitatively collected into buckets containing sulphuric acid (H<sub>2</sub>SO<sub>4</sub>; 25%, AnaLar, BDH Laboratory Supplies, Poole, UK), to keep the pH below 3.5 to prevent volatilisation of ammonia, which were stirred four times daily. Total daily urine production per animal was measured by volume (ml) and weight (g) and duplicate samples (100ml per deer) taken and pooled frozen (-20°C).

#### 5.3.4. Digesta Flow

Five deer, balanced for treatment groups were euthanased on each day from the 7th - 10th August 1995 at the post-mortem room of the Institute of Veterinary, Animal, and Biomedical Sciences, Massey University. The deer were euthanased and organs removed and processed as described in Chapter 4. The chromium capsule was removed from the rumen and the distance from the base of the capsule to the top of the plunger measured.

The abomasum was opened along the greater curvature and total contents were removed, weighed and a 100g subsample taken for nutritional analysis, which was immediately frozen (-20°C) for freeze-drying, grinding and chemical analysis. The length of the distal section of the ileum was measured, and milked-out to obtain a 100g sample for nutritional analysis which was immediately frozen (-20°C) for freeze drying, grinding and chemical analysis.

The Cr release rate for each animal was calculated by measuring the distance the CRDC plunger traveled during the experiment (mm/day). For the batch of capsules used, 222.1mgCr was released per mm of plunger travel (Kusmartono *et al.*, 1996a). Digesta flow ( $F$ ) was calculated as described in Equation 5.1.

**Eqn. 5.1.**

$$F = CrRR \div DCrC$$

Where:

$F$  = Digesta Flow (gOM/day)

$CrRR$  = Chromium release rate (mg/day)

$DCrC$  = Digesta chromium concentration (mg/gOM)

### **5.3.5. Dietary Chemical Analyses**

All samples of feed on offer, feed refused, faeces and abomasal and ileal digesta were freeze dried and then ground to pass a 1mm sieve (Wiley Mill, USA). Urine was thawed immediately prior to total N determination. Dry matter was determined by oven heating to 100°C for 16h, total N (N) by the Kjeldahl technique (Kjeltec Auto 1030 Analyser, Tectator, Sweden), gross energy by adiabatic bomb calorimetry (Gallenkamp Autobomb, UK) and organic matter (OM) by ashing overnight at 555°C. Water-soluble carbohydrates and pectin were determined following the procedure of Bailey (1967), whilst neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin were determined by the detergent system of Van Soest (1994). Cell wall data are presented as hemicellulose (NDF-ADF), cellulose (ADF-lignin) and lignin. Chromium analysis of abomasal and ileal digesta was done following the method of Costigan and Ellis (1987).

### **5.3.6. Statistical Analyses**

One-way analysis of variance was used to examine the effect of nematode larval infection rate on apparent digestibility, N-retention and digesta N flow (SAS 6.11; 1996, SAS Institute Inc. USA). Differences between means were considered statistically significant if  $P < 0.05$ , but differences between means of up to  $P = 0.10$  were considered in the text as trends. Linear regression was used to examine the relationship between N-intake and N-retention (Microsoft Excel; 1996, Microsoft Corporation, USA). One animal was omitted from the analysis of digesta flow due to low values associated with parapox virus infection that was diagnosed at post-mortem examination.

## **5.4. RESULTS**

The chemical composition of the chaffed lucerne hay fed is presented in Table 5.2.

There were no significant differences between treatments in apparent digestibility of DM, OM, energy and any of the components of plant fibre (Table 5.3.).

**Table 5.2. Chemical composition (%DM ± SE) of chaffed lucerne hay fed.**

	CHAFFED LUCERNE HAY	S.E.
	n=28	
Total N	3.24	0.051
OM	90.4	0.07
	n=4	
Water soluble CHO (a)	5.1	0.27
Pectin (a)	5.0	0.18
Hemicellulose (b)	13.3	0.67
Cellulose (b)	26.2	0.86
Lignin	7.4	0.13
Ratio (a/b)	0.26	0.021
NDF	46.9	1.59
ADF	33.6	0.97
GE (MJ/kg DM)	18.5	0.04

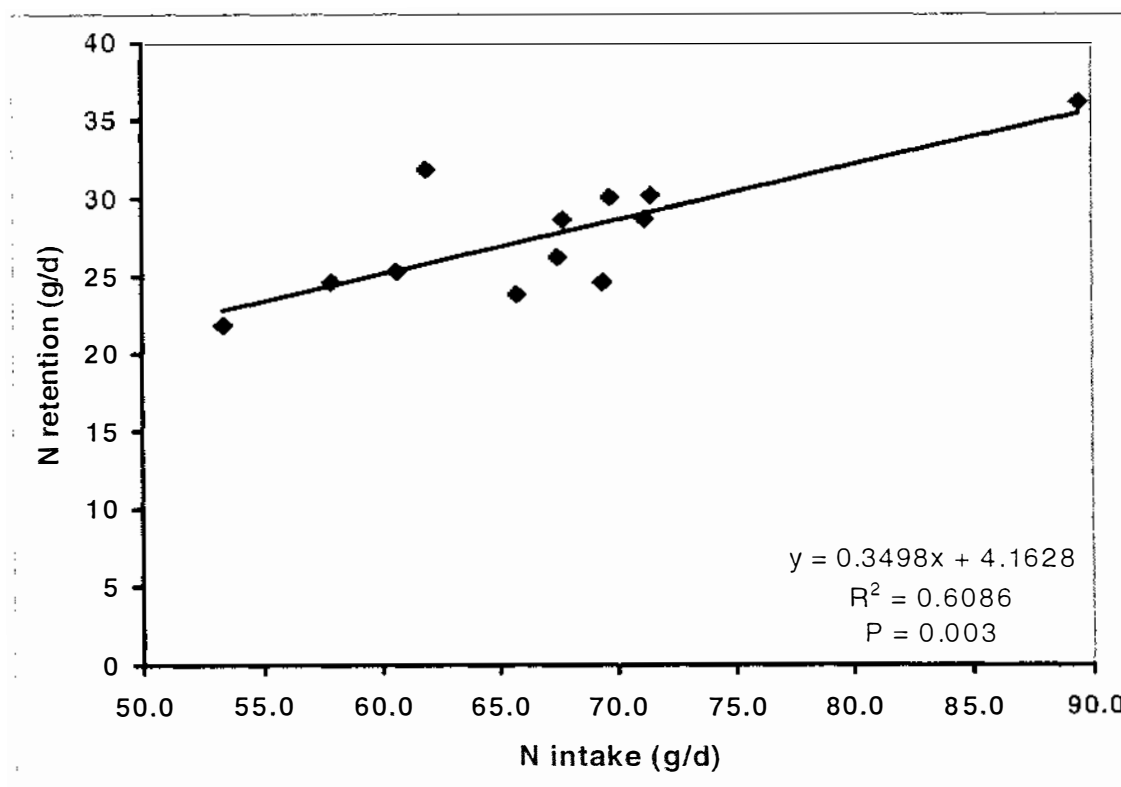
**Table 5.3. Apparent digestibility (%) of DM, OM, gross energy, ADF, NDF, cellulose, hemicellulose, and lignin in deer fed lucerne chaff and infected with zero (C), low (LT), medium (MT) and high (HT) levels of nematode larvae.**

	C	LT	MT	HT	S.E.
	n=3	n=3	n=3	n=3	D.F. = 8
<b>Intake (kgDM/d)</b>	1.92	1.48	1.72	1.44	0.117
<b>Apparent Digestibility</b>					
<b>DM</b>	54.4	54.9	55.0	54.4	0.73
<b>OM</b>	54.6	55.3	55.4	54.6	0.87
<b>Energy</b>	52.6	54.1	54.3	53.3	0.74
<b>NDF</b>	27.1	27.7	27.8	21.8	2.96
<b>ADF</b>	19.5	20.0	20.9	17.5	2.46
<b>Cellulose</b>	33.5	34.6	34.4	29.9	2.35
<b>Hemicellulose</b>	44.7	44.6	43.6	42.1	2.46
<b>Lignin</b>	-23.2	-32.0	-27.3	-53.3	10.27



Nitrogen intake and N-retention, expressed both in g/d and as a percentage of total N intake, and apparent N digestibility of the 12 deer in metabolism cages are presented in Table 5.4. There was no difference in apparent N digestibility between treatments. Excretion of N in urine and faeces (g/d) tended to be lower for infected groups than for the control group, but when expressed as a percentage of N-intake the values were similar for all groups. Nitrogen retention (g/d) of the HT group was lower than the control group ( $P=0.05$ ), with the other groups being intermediate. However, when N-retention was expressed as a percentage of N-intake there were no differences between treatment groups. Figure 5.1. shows a positive relationship between N-intake and N-retention, with 61% of the variation in N retention (g/d) being explained by changes in N intake ( $P=0.003$ ).

**Figure 5.1. Nitrogen intake vs nitrogen retention.**



Flow of N at the abomasum and terminal ileum in g/d and as a percentage of total N intake are also presented in Table 5.4. There were no significant differences between treatment groups in abomasal flow of N, either in g/d or as a percentage of N-intake

( $P>0.20$ ). The flow of N to the terminal ileum in g/d was less for the LT ( $P=0.09$ ) and HT ( $P<0.05$ ) groups compared with control deer. However, when expressed as a percentage of N-intake, the flow of N at the ileum did not differ significantly between treatment groups.

**Table 5.4. Apparent N digestibility, N-retention, and flow of N measured at the abomasum and terminal ileum, in g/d and expressed as a percentage of N intake, in deer fed lucerne chaff and infected with zero (C), low (LT), medium (MT) and high (HT) levels of nematode larvae.**

	C	LT	MT	HT	S.E.
<b>1. Apparent digestibility (%), faeces and urine N and N-retention (g/d):</b>					
n*	3	3	3	3	
N eaten	76.2	61.7	69.4	61.4	4.30
Faeces N	19.6	15.6	17.6	15.7	1.01
Urine N	25.0	18.2	25.3	21.2	2.10
N-digestibility	74.3	74.5	74.6	74.5	0.63
N-retention	31.7	27.9	26.5	24.6	2.02
<b>2. Flow of N (g/d):</b>					
n#	4	5	5	5	
N eaten	59.3	46.0	51.3	46.9	3.80
Abomasal N flow	29.9	23.8	27.5	23.8	3.08
Ileal N flow	19.5	13.4	16.1	11.7	1.41
<b>3. N-retention and flow as a proportion of N eaten (%):</b>					
Abomasal N flow (A)	50.0	51.6	53.3	50.4	3.91
Ileal N flow (B)	33.3	29.2	31.4	25.0	2.24
Faeces N	25.7	25.5	25.4	25.5	0.63
Urine N	32.7	29.4	36.4	34.4	1.82
N-retention	41.7	45.1	38.2	40.1	2.05

\*D.F. = 8

#D.F. = 15

## 5.5. DISCUSSION

This study is the first report concerning the effect of parasitism in young deer on apparent digestibility of chaffed lucerne hay, N retention and flow of N at the abomasum and terminal ileum. It has shown that sub-clinical parasitism reduced N retention (g/d), largely due to its effect in depressing VFI and hence N intake, but did not affect apparent digestibility of any of the dietary components measured or abomasal and ileal N flow as a proportion of N intake. Reduced feed intake is a primary effect of GI parasitism in sheep and cattle (Sykes and Coop, 1976, 1977; Holmes, 1985; Fox *et al.*, 1989a; Parkins and Holmes, 1989; Fox, 1993; Sykes 1994;) and is associated with *D.viviparus* infection of calves (Kroonen *et al.*, 1986). This study, together with data in Chapters 3 and 4, has shown that reduced feed intake is a primary effect of concurrent lungworm and GI nematode infection of young farmed deer.

Apparent digestibility, N retention and measurements of digesta flow are confounded by differences in VFI in this study. Although reduced VFI has a marked effect on the productivity of parasitised ruminants, studies using pair-feeding techniques have shown that inappetance is not the only consequence of infection. Decreased N-retention and increased flow of N at the terminal ileum (Poppi *et al.*, 1986; Kimambo *et al.*, 1988a; Bown *et al.*, 1991b) occur independently of VFI. In this study there was some evidence to suggest that parasitism *per se* may have affected N-retention, as 39% of the variation in N-retention was not explained by changes in N-intake (Fig. 5.1.). However, there was no evidence to suggest that parasitism had any effect on the flow of N at the terminal ileum, other than via effects on N-intake. Further research is required using pair-fed deer with in-dwelling gut cannulae to investigate the effect of GI parasitism on N retention and flow of N at the abomasum and terminal ileum, independently of effects on VFI.

That there were no significant differences in apparent digestibility in this study is in accordance with reports indicating small, if any, effects of parasitism on apparent digestibility in other ruminant species (Steel, 1974; Rowe *et al.*, 1988; Bown *et al.*, 1991b; Sykes *et al.*, 1991; Sykes, 1994).

The measurements in this study were made following nine weeks of trickle infection with both GI nematode and lungworm larvae. There is evidence to suggest that the effects of parasitism on N metabolism in sheep trickle-infected with *T.colubriformis* (small intestinal parasite) vary with time (Kimambo *et al.*, 1988a; Poppi *et al.*, 1986). The nutritional penalty associated with the development of resistance is thought to be greater

than that associated with the primary infection (Poppi *et al.*, 1986). It is not known whether this also occurs in deer infected with predominantly abomasal *Ostertagia*-type parasites and/or lungworm. Research is needed to investigate this aspect.

In this study abomasal flow of N was approximately 50% of N intake indicating a large loss of N as ammonia absorption from the rumen. This is supported by the equation of Hogan *et al.*, (1970), who showed that high losses of dietary N across the rumen-reticulum occur with dried forages in which dietary N concentration exceeds 3% of the digestible organic matter (DOM), with the N/DOM ratio obtained in this study being approximately 6%. However, flow of N at the abomasum may have been underestimated by the use of Cr<sub>2</sub>O<sub>3</sub> as a single marker (Faichney, 1972; Beever *et al.*, 1978). Cr<sub>2</sub>O<sub>3</sub> flows independently of either solid or liquid phases of the digesta (Faichney, 1975), making it an ideal marker for the measurement of digesta flow at the terminal ileum, where solid and liquid digesta phases flow at similar rates (Barry *et al.*, 1985). In the abomasum the two phases flow independently of one another and in order to accurately measure abomasal flow the use of two markers, one for each phase, is recommended. However, use of Cr<sub>2</sub>O<sub>3</sub> should not have invalidated relative comparisons in abomasal N flow between the four treatment groups in this study.

This study has shown that reduction in VFI of sub-clinically parasitised deer was the main factor responsible for reduced N-retention, following nine weeks of concurrent trickle-infection with lungworm and GI nematode larvae. Further research is required to investigate the effects of concurrent and separate infections of lungworm and GI nematodes in deer upon N retention and flow of N through the digestive tract, independently of VFI, using both shorter and longer periods of trickle infection than used in the current study.

## CHAPTER 6.

# Effect of forage legumes containing different concentrations of condensed tannins on establishment of internal parasites in young red deer.

SIMONE O. HOSKIN<sup>1</sup>, T.N. BARRY<sup>1</sup>, P.R. WILSON<sup>2</sup>, W.A.G. CHARLESTON<sup>2</sup> & G.C. WAGHORN<sup>3</sup>

<sup>1</sup> Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand

<sup>2</sup> Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand

<sup>3</sup> AgResearch, Palmerston North, New Zealand

For submission to Journal of Agricultural Science, Cambridge

### 6.1. ABSTRACT

Thirty red deer calves were naturally reared on pasture and regularly treated with anthelmintic to prevent establishment of internal parasites. The calves were weaned, individually housed, allocated to one of three legume forage diets (each n=10) and trickle-infected 3x/week with gastrointestinal (GI) nematodes and lungworm (*Dictyocaulus viviparus*) larvae for seven weeks, prior to slaughter at nine weeks. The freshly cut legumes fed *ad-libitum* were lucerne (*Medicago sativa*; 0.1% CT), birdsfoot trefoil (*Lotus corniculatus*; 1.9% CT) and sulla (*Hedysarum coronarium*; 3.5% CT). The forages were otherwise of similar chemical composition.

There was a significant linear relationship between CT concentration of the diet and establishment of abomasal nematodes, particularly *T. axei*, with reduced establishment of these parasites at higher CT-concentration. Deer fed sulla had reduced faecal lungworm larval counts, higher liveweight gain, carcass weight and dressing out percentage, serum total protein and albumin concentration and lower serum gastrin

concentration, compared with lucerne-fed deer. Deer fed CT-containing forages had lower blood haemoglobin concentration. There were no significant differences in mean VFI between treatment groups during the period of infection, suggesting that the increased liveweight gain of deer fed CT-containing forages was due to an increased efficiency of utilisation of digested nutrients caused by CT counteracting protein losses normally associated with parasite infections. The reduced establishment of abomasal nematodes and reduced faecal lungworm larval count in deer fed sulla containing a high concentration of CT may be due to a direct effect of free CT on nematodes in the GI tract.

## 6.2. INTRODUCTION

Farmed red deer in their first autumn and early winter are susceptible to internal parasites (Mason, 1985). Clinical and sub-clinical effects of both lungworm (*Dictyocaulus* sp.) and gastro-intestinal (GI) nematodes have been reported in farmed deer (Connan, 1991; Waldrup and Mackintosh, 1992; Wagner *et al.*, 1993; Waldrup *et al.*, 1994a,b; Audige *et al.*, 1998). Control of these parasites in young deer is by anthelmintic treatment (Mackintosh and Mason, 1985; Mason and Beatson, 1985; Bowie *et al.*, 1987; Andrews *et al.*, 1993; Mackintosh *et al.*, 1993), combined with grazing management (Wilson, 1984).

The increased incidence of anthelmintic resistance in farmed sheep, goats and cattle in New Zealand (NZ), combined with rising consumer concerns about chemical use on farms, has encouraged research into alternative strategies for the control of internal parasites (Niezen *et al.*, 1993d). Anthelmintic resistance has not yet been reported in farmed deer. The NZ deer industry promotes venison as a clean, green, natural, quality-assured product CERVENA® which should be produced using minimal chemical inputs. Research into natural means of parasite control is consistent with that strategy.

Condensed tannins (CT) present in forage legumes protect plant proteins against rumen degradation and increase the flow of amino acids to the small intestine (Waghorn *et al.*, 1987b; Waghorn *et al.*, 1994). At low concentrations (22-35gCT/kgDM), they increase the absorption of amino acids from the small intestine of sheep (Waghorn *et al.*, 1987a). Feeding forage legumes containing CT, such as sulla (*Hedysarum coronarium*) and lotus major (*Lotus pedunculatus*) has significantly increased growth of parasitised lambs (Niezen *et al.*, 1995; Robertson *et al.*, 1995), relative to feeding non CT-containing legumes, and sulla has been found to reduce established worm numbers in sheep (Robertson *et al.*, 1995). Bown *et al.*, (1991a) infused protein into the abomasum of

lambs trickle-infected with *T.colubriformis*, and this resulted in reduced worm numbers and improved lamb performance. Studies involving feeding diets containing by-pass protein to parasite-infected sheep showed a reduction in production losses attributed to parasitism (van Houtert *et al.*, 1995a), and enhanced immunity (Coop *et al.*, 1995; van Houtert *et al.*, 1995b; Smith *et al.*, 1996). Although the exact mechanisms are unknown, it is thought that the increased by-pass protein supply caused by the action of CT in forages helps counteract the protein losses caused by GI nematode infections (Poppi *et al.*, 1986, 1990; MacRae, 1993; Sykes, 1994). However, no research has been conducted into the effects of feeding by-pass protein, or forages containing CT on any aspects of internal parasitism in deer.

The objective of this study was to determine the effect of feeding three legume forages containing different concentrations of CT upon the establishment of both lungworm and GI nematodes in housed weaner deer.

## 6.3. MATERIALS AND METHODS

### 6.3.1. Experimental Design

The experimental design is summarised in Table 6.1. Parasite-free weaner deer were produced through regular (14-day interval) anthelmintic drenching of both hinds and suckling calves (as described in Chapter 4). The weaned calves were then housed indoors, individually fed and trickle-infected with infective L<sub>3</sub> stage larvae of lungworm (*Dictyocaulus viviparus* and gastrointestinal (GI) nematodes (majority *Ostertagia*-type) of deer origin over a five-week period. The forage diets evaluated were lucerne (0.1%CT), birdsfoot trefoil (1.9%CT) and sulla (3.5%CT).

**Table 6.1. Experimental design.**

Treatment	No. Deer		CT* content of forage % DM
	Stags	Hinds	
Lucerne (L)	3	7	0.1
Birdsfoot Trefoil (B)	3	7	1.9
Sulla (S)	4	6	3.5

\*CT= condensed tannin

Factors investigated included liveweight change, voluntary food intake (VFI), haematology and serum biochemistry, faecal gastrointestinal nematode egg and lungworm larval excretion, carcass weight and numbers of adult gastrointestinal and lung parasites counted at slaughter which represents the apparent establishment of these parasites. The trial was carried out at Massey University from 15 March 1996 to 30 May 1996, following an initial animal-handling phase of four weeks. Table 6.2. shows the sequence of experimental events including an indoor adjustment phase and VFI covariate period with all deer fed chicory (seven days); the three test legumes were fed in all other periods which included a pre-parasite infection covariate period with deer fed legume forages (nine days), a trickle parasite infection period (37 days), and a post-infection period (19-23 days) prior to slaughter. The post-infection period allowed all nematodes to mature, simplifying recovery and identification.

**Table 6.2. Sequence of experimental events.**

<b>Week*</b>	<b>Dates</b>	<b>Events</b>
<b>1995</b>		
-1	15-22/3	Adjustment phase, deer fed chicory, VFI measured for covariate
0	23/3-1/4	Deer weighed, allocated to 3 legume forages 23/3/96. VFI + liveweight measured, blood sampled for covariate, faeces sampled
1-5	2/4-7/5	Trickle infection 3x/week, deer weighed + VFI measured weekly, blood + faeces sampled weekly
6-7	8/5-26/5	Nematodes allowed to mature, weekly measurements last taken 21/5
8	27-30/5	Deer slaughtered

\*Reference to week is consistent with Figures 6.1.-6.9.

### **6.3.2. Animals and Housing**

Thirty weaned red deer calves were used that had been naturally-reared outdoors on their dams on three separate farms in the Manawatu District. These calves, together with their dams had been treated with anthelmintic (“Cydectin pour-on”, 0.5mg/kg liveweight moxidectin, Cyanamid of NZ Ltd), on three subsequent occasions, 10-14 days apart from 14/1/96-8/2/96, weaned on 8/2/96 (average liveweight  $\pm$ SD=38.4  $\pm$ 6.36kg), brought to Massey University Deer Research Unit (MUDRU) and given anthelmintic on



1/3 and 15/3/96 (“Oxfendazole”, 9.06mg/kg liveweight, Ancare NZ Ltd.). Once at the MUDRU calves were adjusted to grazing chicory from 15/2 – 30/2/96. They were then fed cut chicory from 1/3-14/3 in a small enclosure to quieten them, before being brought indoors and housed in specially designed deer metabolism crates (10 deer; Milne *et al.*, 1978) or in modified sheep pens (20 deer) on 15/3/96. All deer produced zero faecal parasite egg floats and larval counts on three occasions prior to commencement of the experiment.

On 26 February 1996 all calves were ear-tagged and vaccinated against clostridial infections (Clostridial 5 in 1; “Ultravac”, CSL Ltd,NZ) and yersiniosis (“Yersiniavax”; AgVax, AgResearch, NZ) by injection into the anterior of the neck, with booster vaccinations given 30 days later.

Animals were randomly assigned to treatment groups based on liveweight, sex and farm of origin, with 10 deer per treatment group. A seven-day initial period allowed the deer to adjust to being individually fed chicory and to handling procedures. All deer were fed fresh forage *ad-libitum* at 0900hrs and 1500hrs, and had free access to water. Residual feed was removed at 0900hrs daily and weighed. During the trial the deer were removed from their cages weekly for weighing and blood sampling. While housed indoors the deer were under constant daily lighting conditions of 14h light and 10h dark.

### **6.3.3. Forages**

The established, pure forages cut and fed were: chicory (*Cichorium intybus* L.) cv Grasslands Puna (C), sulla (*Hedysarum coronarium*) cv Aokau (S), birdsfoot trefoil (*Lotus corniculatus*) cv Grasslands Goldie (B) and lucerne (*Medicago sativa*) cv Grasslands Oranga (L). All contained <5%DM as weeds. Both the C and B were grown at Massey University while the S and L were cut and transported from AgResearch Grasslands Aorangi Research farm and AgResearch Grasslands Flock House Research Centre, respectively. All forages were cut four times per week at 0800hrs with a sickle-bar mower and immediately transported to refrigerated storage (4°C) adjacent to the deer pens. Maximum storage time was 36hrs). All forages were lush and predominantly vegetative, as a result of being mechanically topped prior to the trial commencing, although some flowers were evident in the last three weeks of the trial.

Following each cutting, triplicate forage samples (200g) were taken for dry matter (DM) analysis (100°C; 18hrs). Representative samples of feed refused for each forage were also taken for triplicate DM analysis (100°C; 18hrs; 4x weekly). Samples of feed on

offer (200g) were taken daily from each forage (including C fed during the adjustment phase) and pooled weekly and stored at -20°C. Voluntary feed intake (VFI; kgDM/deer/day) of the forages fed was recorded daily and calculated weekly.

#### **6.3.4. Culture and Administration of Infective Larvae**

Infective stage lungworm and gastrointestinal (GI) nematode larvae were cultured from the faeces of infected weaner deer using the procedures described in Chapter 4. The 30 experimental deer were infected for five weeks with infective stage (L<sub>3</sub>), deer-origin lungworm and GI nematode larvae by trickle infection on Monday, Wednesday and Friday of each week. The GI nematode larvae comprised 32% *Ostertagia*-type, 18% *Trichostrongylus*, 38% *Cooperia* and 12% *Oesophagostomum* (average of 10 counts of 100 larvae). The infection rate used was 500 *D. viviparus* and 2500 GI nematode larvae per dose, 3x/week.

Larvae were cultured from faeces collected from six infected donor red deer stag calves. These calves had been identified from the Massey University deer herd as excreting the highest numbers of lungworm larvae and GI nematode eggs in faeces. These calves were weaned on 1 February 1996, without anthelmintic treatment, and kept in a small paddock adjacent to the deer house. The three calves excreting the greatest number of eggs and larvae (max FLC=103 lpg; max FEC 350 epg) were removed from pasture and housed in a modified pen in the Massey University Large Animal Veterinary Hospital, injected with dexamethasone trimethylacetate three times per week (0.05mg/kg liveweight; "Opticortenol", Ciba-Geigy NZ Ltd.) and fed chaffed lucerne hay. Faeces were then collected for 4-6 weeks or until animals showed moderate clinical signs of parasitism, such as weight loss (max FLC 924 lpg; max FEC 1850 epg). At this point these animals were treated ("Ivomec" 0.4%w/v at 200µg/kg liveweight; Merck, Sharp and Dohme, NZ) and removed and another calf started on steroid treatment. Faecal collection was by collection bag and harness, five days per week, with bags changed every 24hrs at 0800hrs. Details on culture of infective larvae from faeces are given in Chapter 4.

#### **6.3.5. Blood Sampling, Serum Biochemistry and Haematology**

Blood samples were taken via jugular venipuncture once per week as described in Chapter 4. Four ml of the serum obtained following centrifuging was frozen (-20°C) for later pepsinogen and gastrin analysis, and 0.5ml was used fresh for determination of serum total protein, albumin and globulin concentrations using an automatic analyser (Hitachi

704, Boehringer-Mannheim, Mannheim, Germany). Serum pepsinogen levels were assayed according to the method described by Pomroy and Charleston (1989) and serum gastrin was determined by radio-immunoassay (Simpson *et al.*, 1993) based on the method of Hansky and Cain (1969).

Analysis of haematological characteristics was carried out on the fresh EDTA blood samples within three hours of sampling. White cell counts (WBC), red cell counts (RBC), haemoglobin concentration (HB) and packed cell volume (PCV) were determined using an automatic analyser (Cobas Minos-Vet, Roche, Montpellier, France). Blood smears were stained for differential leucocyte counts.

### **6.3.6. Slaughter**

Deer were balanced according to treatment for slaughter on 27 (n=6), 28 (n=9), 29 (n=9) and 30 May 1996 (n=6) at Feilding Deer Slaughter Premises (Venison Packers New Zealand Ltd). Immediately following veterinary inspection of viscera, ties were placed at the omasal/abomasal junction, pylorus/duodenal junction, the ileo-caecal junction and distal rectum, and GI tracts were sectioned into abomasum, small intestine and large intestine and placed separately in plastic bags. The intact respiratory tract, together with heart and pericardial sac were dissected from the remaining viscera and bagged for perfusion and incubation of the lungs to recover lungworm. Organs were then transported to the post-mortem room of the Institute of Veterinary, Animal and Biomedical Sciences, Massey University for processing within 3hrs of slaughter.

Hot carcasses were weighed (kg) and the soft tissue depth over the 12th rib, 16cm from the dorsal midline, an indirect measure of subcutaneous fat depth (carcass GR), was recorded for each animal.

Once at MU, the mesentery was removed from the entire digestive tract. The abomasa and small intestines were stored separately for each animal (-20°C). The lower part of the large intestine from the point at which faecal pellets form was discarded, and the remainder was stored (-20°C).

### **6.3.7. Parasitology**

Fresh faeces (10g) were collected weekly from trays placed beneath individual deer cages. The presence or absence of nematode eggs was detected by a coverslip flotation test where 2g faeces were mixed with 28ml saturated salt solution and poured into a universal container with a coverslip placed on top for at least five minutes to allow

eggs to float onto the coverslip (based on Thienpont *et al.*, 1979 ). If these were positive, FEC were determined using a modified McMaster method (Stafford *et al.*, 1994) where a count of one egg was equivalent to 50 eggs/g (epg) of faeces. If FEC were negative, but the faecal float positive, then the sample was considered to contain <50epg, which for purposes of data analysis was designated 25epg. Faecal lungworm larval counts (FLC) were determined using a modified Baermann technique (Hendriksen, 1965) as described in Chapter 4.

Perfusion and incubation of the lungs to recover lungworm and the recovery of nematodes from the abomasum, small and large intestine were carried out as described in Chapter 4. The method of preservation and counting of samples, and classification of nematodes is described in Chapter 4, the only difference being that 20 mature, *Ostertagia*-type nematodes from the abomasum of each animal were randomly selected for counting of eggs *in-utero* for this experiment.

#### **6.3.8. Dietary Chemical Analyses**

Weekly pooled samples of feed on offer were mixed and duplicate subsamples (200g) were taken for drying, grinding and chemical analysis. All samples of feed on offer were freeze-dried and ground to pass a 1mm sieve (Wiley Mill, USA). Organic matter (OM) content was determined by ashing overnight at 555°C. *In-vitro* organic matter digestibility (OMD) was determined by incubation with fungal cellulase and hemicellulase enzymes (Roughan and Holland, 1977) and total nitrogen (N) was determined by the Kjeldahl technique (Kjeltec Auto 1030 Analyser, Tectator, Sweden).

Pooled samples of feed on offer were analysed for extractable and bound CT, which were determined by the modified butanol/ HCl procedure of Terrill *et al.*, (1992a); extractable CT levels were also determined by the vanillin/ HCl procedure of Broadhurst and Jones (1978). Extractable CT values (%OM; butanol-HCl) were deducted from *in vitro* OMD determinations because extractable CT would be solubilised in the initial *in vitro* steps, but is known to be indigestible *in vivo* (Terrill *et al.*, 1994). Water-soluble carbohydrates and pectin were determined following the procedure of Bailey (1967), whilst neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin were determined by the detergent system of van Soest (1994). Cell wall data are presented as hemicellulose (NDF-ADF), cellulose (ADF-lignin) and lignin.

### 6.3.9. Statistical Analyses

Analysis of variance by repeated measures was used to examine the effects of time, time x forage and sex on VFI, liveweight, FEC, FLC and serum biochemical and haematological values (SAS 6.11; 1996, SAS Institute Inc. USA). One-way analysis of variance was used to assess the effect of forage fed and sex on worm numbers at slaughter and carcass weight. Differences between means were considered statistically significantly different if  $P < 0.05$ , but differences between means of  $P = 0.05-0.10$  were considered as trends. There were no significant effects of sex, or significant interactions found involving sex for all parameters measured ( $P > 0.10$ ) other than carcass weight. Sex was subsequently removed from the model as appropriate. Carcass GR was  $< 2\text{mm}$  for all animals so the data are not presented. Initial liveweight, VFI on chicory (prior to feeding of test forages) and blood data prior to parasite infection were used as covariates for subsequent analyses of liveweight, VFI and blood data respectively. Linear regression was used to examine the relationships between abomasal worm number, number of *T. axei*, FLC and CT concentration of the diet, and between FEC and FLC and number of GI and lung nematodes counted at slaughter, respectively (Microsoft Excel; 1996, Microsoft Corporation, USA).

## 6.4. RESULTS

Summary data are presented in Figures 6.1.-6.9. and Tables 6.3.-6.5. Means, maxima and minima of raw FEC, FLC, worm counts, leucocyte counts, erythrocyte counts, differential leucocyte counts and packed cell volume data are presented in Appendix 6.1.

### 6.4.1. Diet

Dry matter content and chemical composition of the forages fed are presented in Table 6.3. The three legume forages were similar in composition except for the concentrations of CT and total N and the ratio of readily fermentable:structural carbohydrates.

The DM content of sulla (S) fed was lower than for birdsfoot trefoil (B) and lucerne (L), and the DM content of B was lower than L ( $P<0.001$ ). The nitrogen content of S was lower than B ( $P<0.01$ ) and L ( $P<0.001$ ), and B had a lower N content than L ( $P<0.001$ ). There were no differences in OM, ADF, NDF cellulose and pectin concentrations between the three legume forage diets. All three legumes were of similar OMD, but OMD of S was slightly greater than B ( $P<0.05$ ). Sulla and L had a lower lignin content than B ( $P<0.001$ ). The ratio of readily fermentable:structural carbohydrate was much greater for S than L and B ( $P<0.001$ ), due to S having more than twice the water-soluble carbohydrate content and a lower hemicellulose content than B and L ( $P<0.01$ ). Birdsfoot trefoil had a lower hemicellulose content than L ( $P<0.05$ ).

**Table 6.3. Dry matter content (%) and chemical composition (%DM  $\pm$  S.E.) of fresh forage fed to weaner red deer housed indoors and fed chicory (adjustment period), lucerne, birdsfoot trefoil or sulla.**

	CHICORY	LUCERNE	BIRDSFOOT TREFOIL	SULLA
D.F. = 62	(n=5)	(n=21)	(n=21)	(n=21)
Dry Matter %	12.4 $\pm$ 0.44	17.2 $\pm$ 0.38	14.4 $\pm$ 0.38	10.6 $\pm$ 0.38
D.F. = 41	(n=3)	(n=14)	(n=14)	(n=14)
Total Nitrogen	3.2 $\pm$ 0.13	4.8 $\pm$ 0.122	3.6 $\pm$ 0.122	3.1 $\pm$ 0.12
Organic matter	84.5 $\pm$ 0.38	88.6 $\pm$ 0.43	88.2 $\pm$ 0.43	88.5 $\pm$ 0.43
OMD	75.7 $\pm$ 2.97	72.9 $\pm$ 0.71	71.0 $\pm$ 0.71	73.8 $\pm$ 0.71
D.F. = 11	(n=3)	(n=4)	(n=4)	(n=4)
NDF	27.9 $\pm$ 0.59	31.2 $\pm$ 1.22	33.1 $\pm$ 1.22	29.9 $\pm$ 1.22
ADF	21.4 $\pm$ 0.45	22.2 $\pm$ 0.92	25.5 $\pm$ 0.92	24.4 $\pm$ 0.92
Water soluble carbohydrate (a)	8.8 $\pm$ 0.56	6.9 $\pm$ 0.61	8.3 $\pm$ 0.61	17.2 $\pm$ 0.61
Pectin (a)	5.6 $\pm$ 0.44	5.1 $\pm$ 0.42	5.0 $\pm$ 0.42	5.9 $\pm$ 0.42
Hemicellulose (b)	6.5 $\pm$ 0.15	9.0 $\pm$ 0.33	7.7 $\pm$ 0.33	5.5 $\pm$ 0.33
Cellulose (b)	15.6 $\pm$ 0.17	17.1 $\pm$ 0.69	18.0 $\pm$ 0.69	19.1 $\pm$ 0.69
Ratio (a/b)	0.65 $\pm$ 0.04	0.46 $\pm$ 0.04	0.52 $\pm$ 0.04	0.94 $\pm$ 0.04
Lignin	5.8 $\pm$ 0.29	5.2 $\pm$ 0.29	7.5 $\pm$ 0.29	5.3 $\pm$ 0.29
Free CT †	0.17 $\pm$ 0.020	0.11 $\pm$ 0.021	0.89 $\pm$ 0.021	1.42 $\pm$ 0.021
Free CT ‡	0.03 $\pm$ 0.001	0.02 $\pm$ 0.086	1.06 $\pm$ 0.086	2.12 $\pm$ 0.086
Protein-bound CT ‡	0.21 $\pm$ 0.021	0.09 $\pm$ 0.056	0.71 $\pm$ 0.056	1.19 $\pm$ 0.056
Fibre-bound CT ‡	0.13 $\pm$ 0.032	0.01 $\pm$ 0.009	0.13 $\pm$ 0.009	0.17 $\pm$ 0.009
Total CT ‡	0.38 $\pm$ 0.046	0.11 $\pm$ 0.131	1.90 $\pm$ 0.131	3.49 $\pm$ 0.131

† Vanillin-HCl method; ‡ Butanol-HCl method

The total, extractable (butanol-HCl and vanillin-HCl) and protein-bound CT content of sulla (S) was greater than for birdsfoot trefoil (B) and lucerne (L), and was greater for B than for L ( $P<0.001$ ). The fibre-bound CT content of S was greater than that of B ( $P<0.01$ ) and L ( $P<0.001$ ), and was greater for B than for L ( $P<0.001$ ).

#### **6.4.2. Voluntary Feed Intake and Liveweight**

Weekly VFI and liveweights of deer fed the three forages are presented in Figures 6.1. and 6.2., respectively. Group mean VFI over the period of infection (weeks 1-7 combined) and group mean daily liveweight gain (LWG) during the same period are presented in Table 6.4. Mean VFI was similar for all forages, with a tendency for greater VFI of S compared with B ( $P=0.063$ ). Mean LWG during the infection period was greater for deer fed S than L ( $P<0.05$ ), with the B group intermediate. There was a tendency for LWG of deer fed S to be greater than deer fed B ( $P=0.071$ ).

Both weekly VFI and liveweight showed significant time\*forage interactions ( $P<0.001$ ). Voluntary feed intake of S and B fluctuated with time compared with L, with B having the highest VFI during weeks 2 and 3, and S having the highest VFI during weeks 5, 6 and 7. Voluntary feed intake of S was greater than L during weeks 5, 6 ( $P<0.001$ ) and 7 ( $P=0.09$ ) and was greater than B during weeks 1 ( $P<0.01$ ) and 7 ( $P<0.001$ ). Voluntary feed intake of B was greater than S and L during week 3 ( $P=0.06$ ). Liveweight of deer fed both S and B steadily increased with time and was greater than for deer fed L, with this difference also increasing with time. Liveweight of the S group was greater than the L group during weeks 5 ( $P<0.05$ ), 6 ( $P<0.01$ ) and 7 ( $P<0.05$ ).



Figure 6.1. Mean (+SE) voluntary feed intake of deer fed lucerne (L), birdsfoot trefoil (B) or sulla (S)

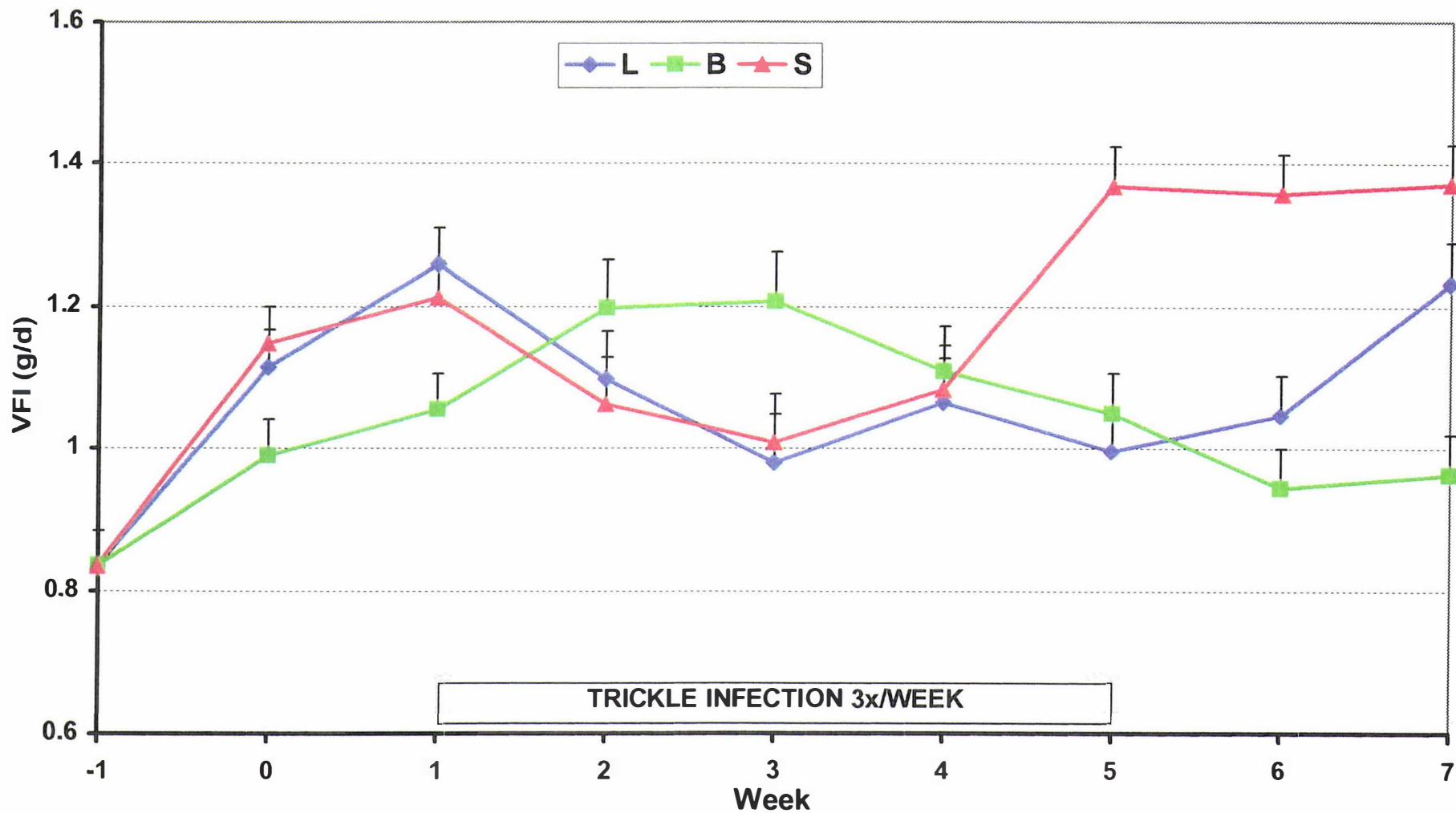
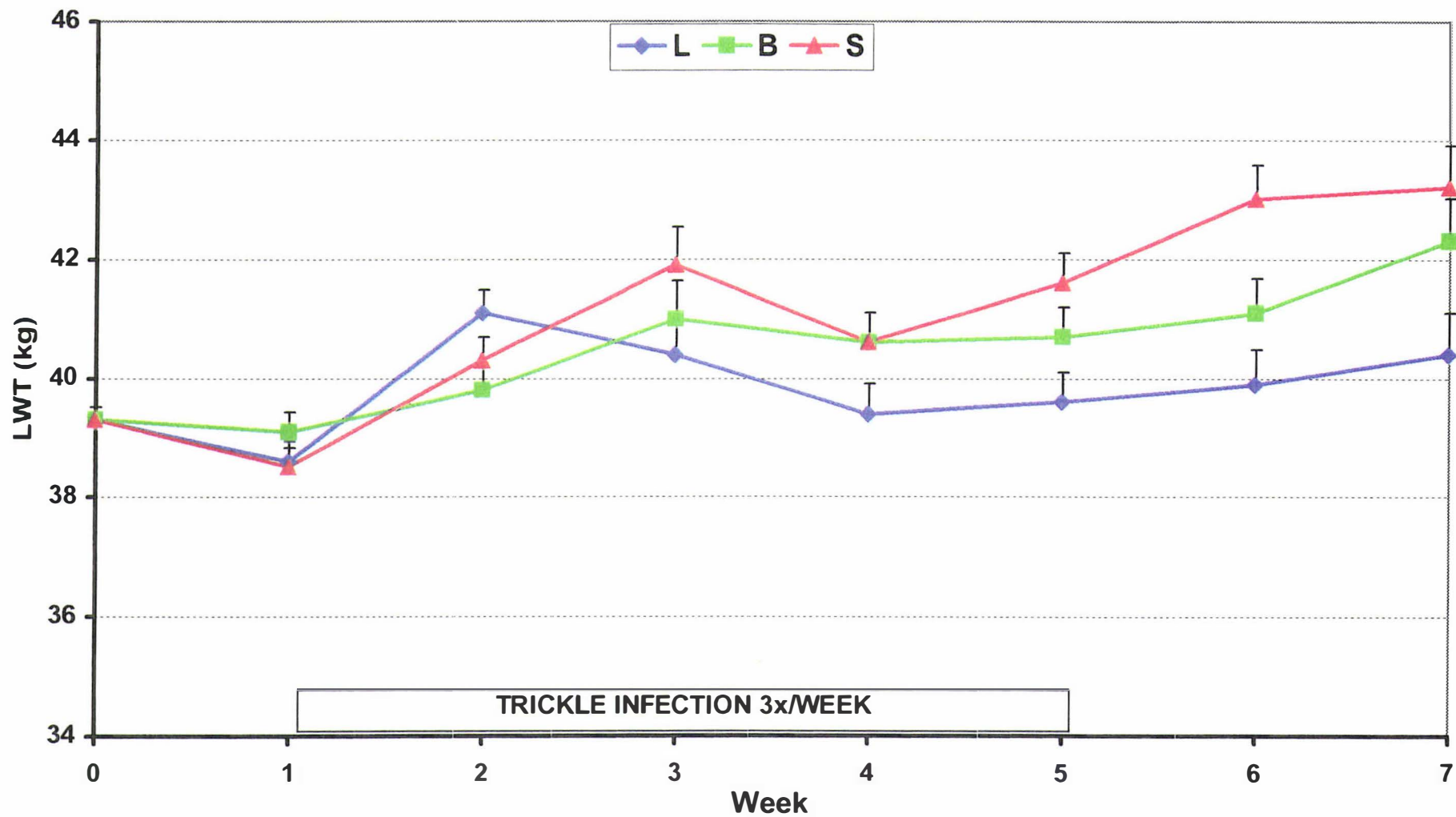


Figure 6.2. Mean (+SE) liveweight of deer fed lucerne (L), birdsfoot trefoil (B) or sulla (S)



### 6.4.3. Carcass Data

Mean carcass weights (CW) and carcass dressing out percentages (DR%) are presented in Table 6.4.

**Table 6.4. Group mean ( $\pm$ SE) VFI and LWG of deer fed lucerne, birdsfoot trefoil or sulla during infection with internal parasites (weeks 1-7), carcass weight (CW; with and without initial liveweight as a covariate) and carcass dressing out percentage (DR%) at slaughter.**

	LUCERNE	BIRDSFOOT TREFOIL	SULLA	S.E. DF = 27
VFI (kgDM/d)	1.15	1.08	1.22	0.051
LWG (g/d)	49.4	62.0	99.2	14.45
CW (kg) no covariate	23.6	23.0	25.4	1.30
CW (kg) with covariate	22.6	22.4	24.4	0.50
DR%	55.6	53.0	56.9	0.60

The mean CW of the S group was 2kg greater than both B and L groups, but this not statistically significant ( $P=0.15$ ) without the use of initial liveweight as a covariate. When initial liveweight was used as a covariate for CW, the CW of the S group was greater than both L ( $P<0.05$ ) and B ( $P<0.01$ ) groups. The carcass weight of stags was greater than hinds ( $P<0.01$ ; stags =  $25.9 \pm 0.81$ kg; hinds =  $21.9 \pm 1.16$ kg) when CW was analysed without initial liveweight as a covariate, but not when the covariate was used. The DR% of S ( $P<0.01$ ) and of L ( $P<0.05$ ) were both greater than that of B, with no difference in DR% between the S and B groups, and no effect of sex on DR%.

### 6.4.4. Faecal Egg and Larval Counts

Mean FEC and FLC are presented in Figures 6.3. and 6.4., respectively. Weekly mean, minima, maxima and number of animals with positive FEC/FLC are presented in Appendix 6.1. There were no significant differences in FEC between treatments at any time during the trial. Faecal egg counts for S and B groups peaked at week 6 and sharply declined, whereas at week 7 the mean FEC of the L group was still increasing. Lungworm infections became patent in the L and B groups in week 4, and in the S group

Figure 6.3. Mean (+SE) of faecal egg counts of deer fed lucerne (L), birdsfoot trefoil (B) or sulla (S)

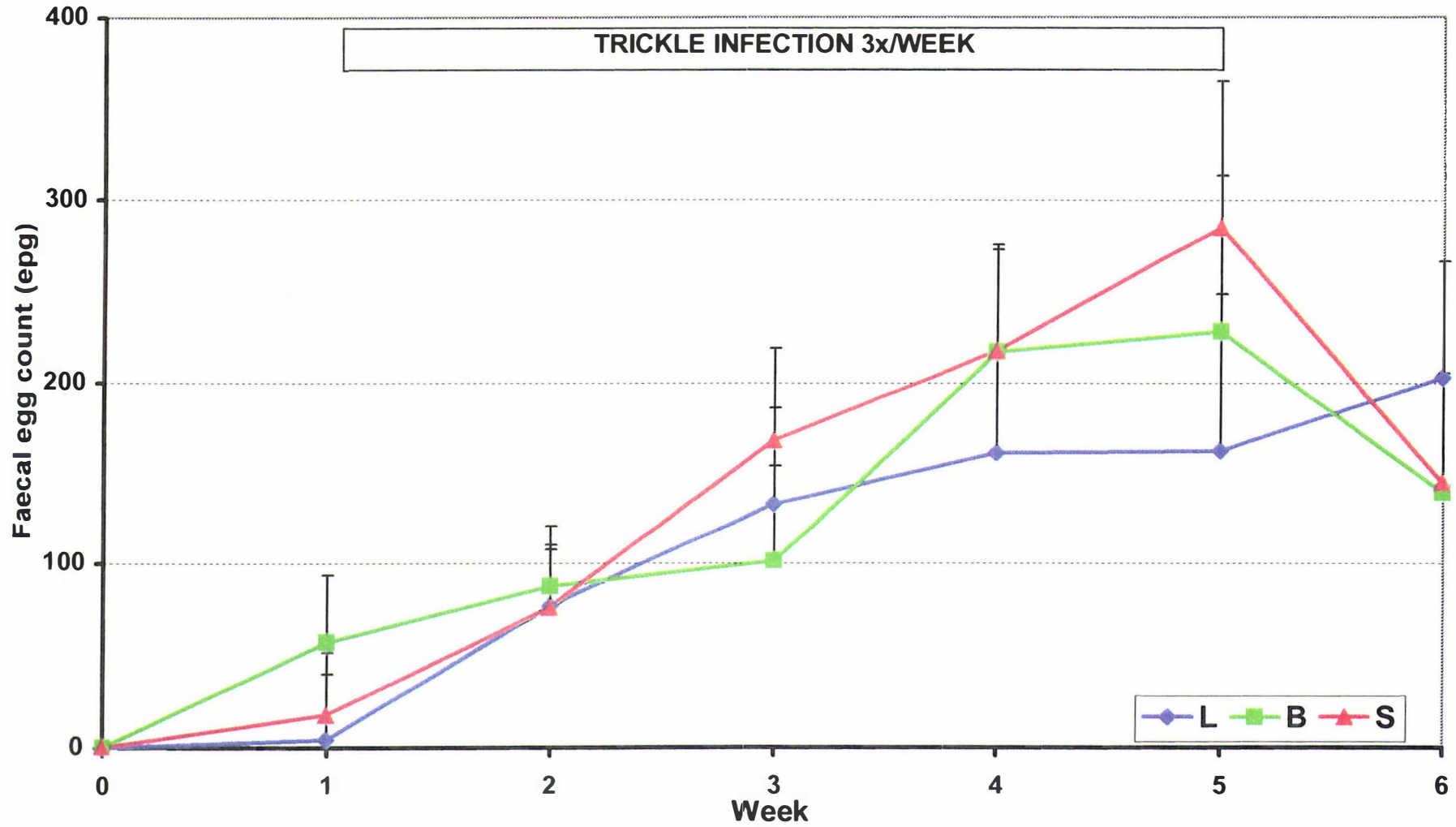
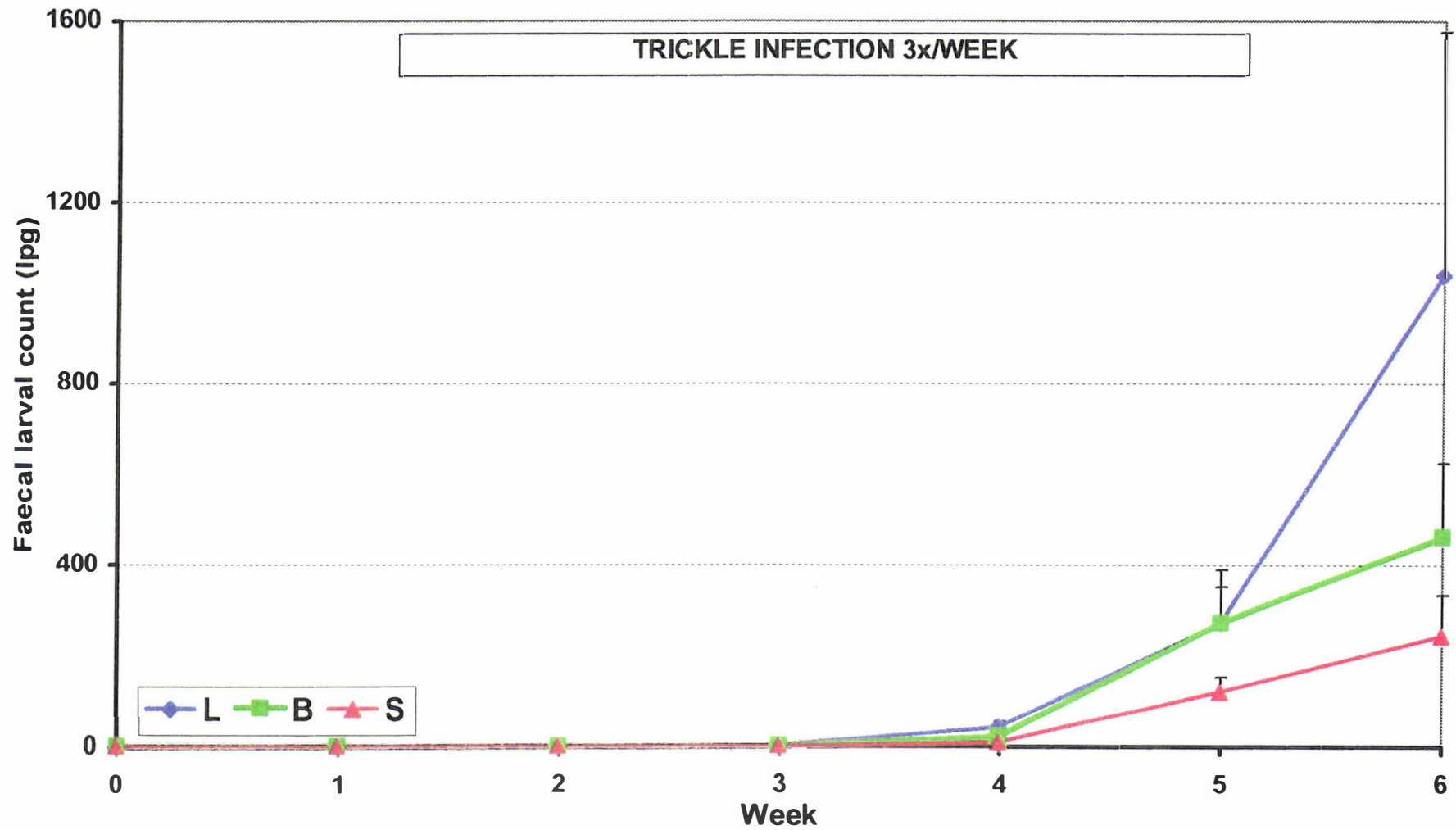


Figure 6.4. Mean (+SE) faecal lungworm larval counts of deer fed lucerne (L), birdsfoot trefoil (B) or sulla (S)



in week 5. Faecal lungworm larval counts were still increasing in all groups when animals were slaughtered. There was a tendency for lower FLC in the S group compared with the L group in weeks 5 and 6 ( $P=0.099$ ), with large individual animal variation in FLC recorded during those weeks.

#### **6.4.5. Serum Biochemistry**

Mean serum total protein (STP), albumin (SA) and globulin (SG) concentrations are presented in Figure 6.5. A time\*forage interaction was found for STP and SA ( $P<0.05$ ), explained by STP and SA of deer fed S becoming greater than for deer fed L, with this difference increasing with time. With few exceptions there were no significant differences between treatments in STP and SA. Deer fed S had a greater STP than deer fed L and B during week 6 ( $P=0.05$ ). Deer fed S had a greater SA concentration than deer fed B during week 6 ( $P=0.09$ ). There were no differences in SG between forages.

Mean serum pepsinogen concentrations (SPC) are presented in Figure 6.6. Although SPC tended to be higher in B-fed animals for much of the experiment, differences only approached significance on two occasions. Deer fed B had a higher SPC than deer fed S during week 3 ( $P=0.07$ ) and deer fed B had a higher SPC than deer fed L during week 5 ( $P=0.07$ ). Mean serum gastrin concentrations (SGC) are presented in Figure 6.7. Deer fed S had a consistently lower SGC than deer fed L during weeks 1 ( $P=0.07$ ), 2-3 ( $P<0.05$ ), 4-5 ( $P=0.06$ ) and 6 ( $P<0.05$ ). Deer fed S also had a significantly lower SGC than deer fed B during weeks 2, 3 and 6 ( $P<0.05$ ).

#### **6.4.6. Haematology**

There were no effects of forage fed on total leucocyte (WBC) and erythrocyte (RBC) counts or differential leucocyte counts (see Appendix 6.1.). The mean numbers of eosinophils circulating in peripheral blood (Figure 6.8.) did not differ significantly between treatments but increased slightly in all groups during infection from an initial mean of  $0.12 \pm 0.021 \times 10^9/l$  to a mean of  $0.23 \pm 0.051 \times 10^9/l$  during week 6.

Mean blood haemoglobin concentrations (HB; Figure 6.9.) was similar for all treatments except during weeks 3 and 4, when HB of both S and B groups was lower than for the L group ( $P<0.05$ ). A drop in HB across all treatments was observed from week 0 to week 4. A similar pattern was shown for packed cell volume (PCV), but the differences seen in weeks 3 and 4 were not significant (Appendix 6.1.).

Figure 6.5. Mean (+SE) serum total protein, albumin and globulin concentration of deer fed lucerne (L), birdsfoot trefoil (B) or sulla (S)

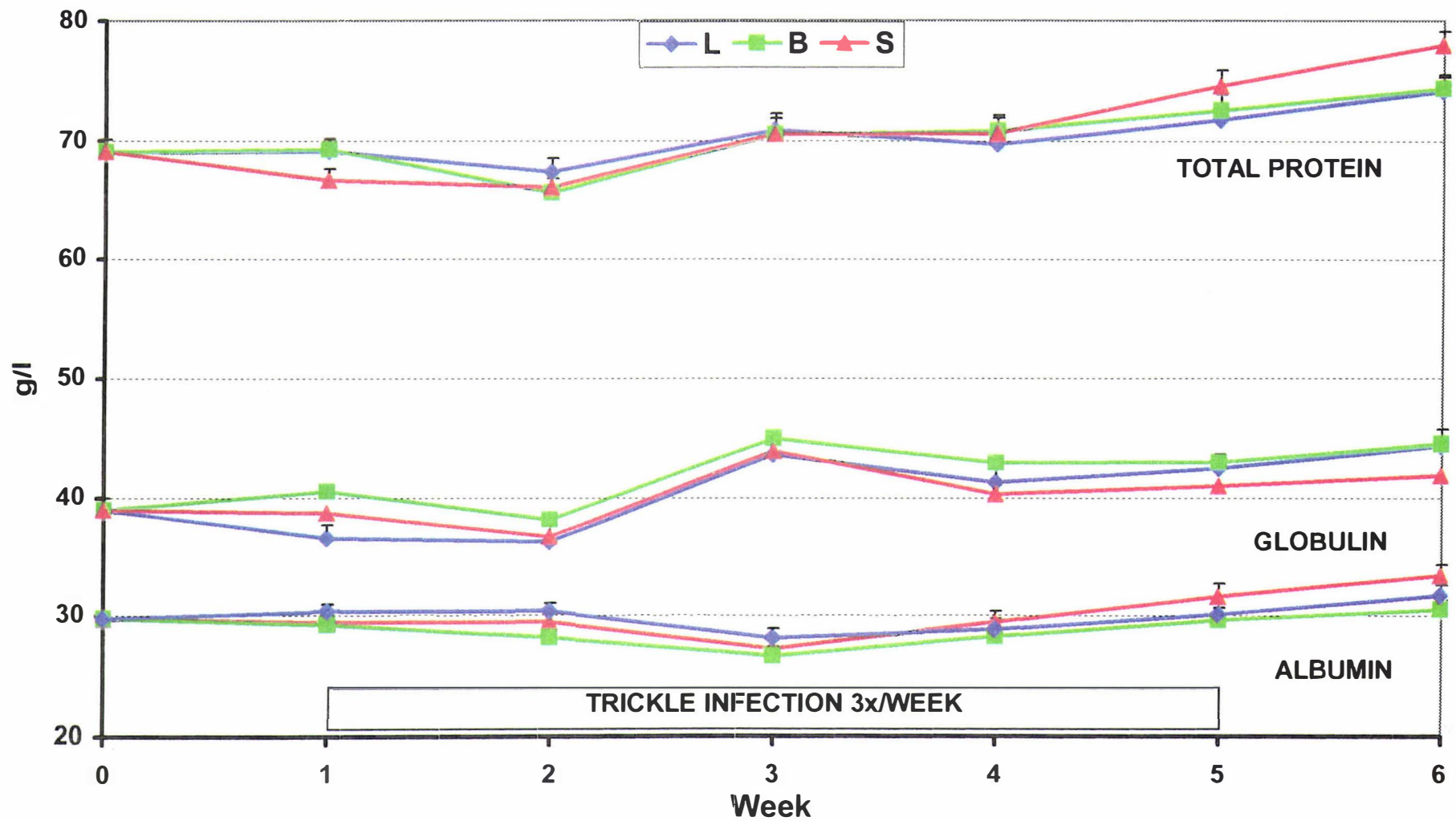


Figure 6.6. Mean (+SE) serum pepsinogen concentration of deer fed lucerne (L), birdsfoot trefoil (B) or sulla (S)

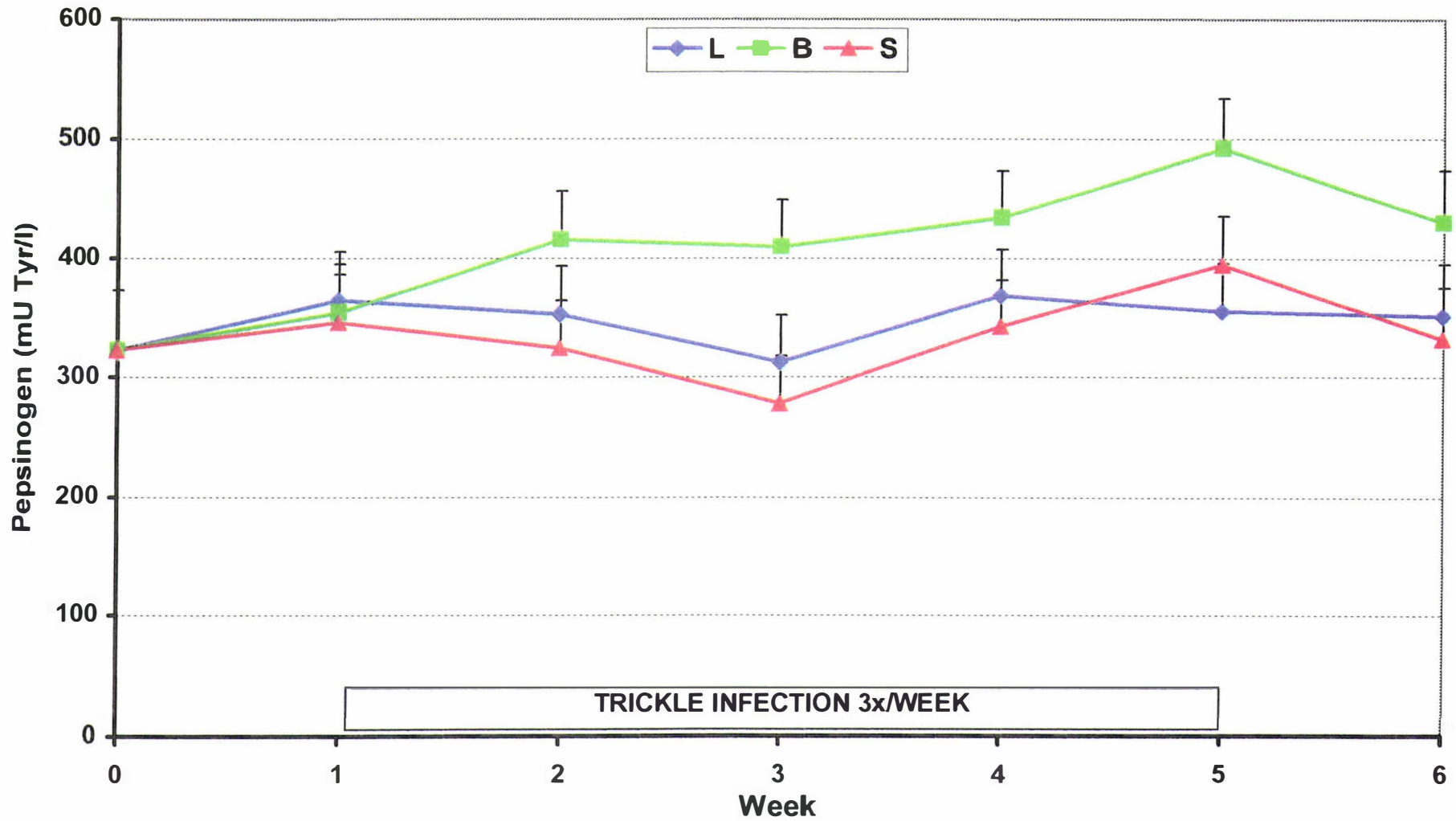




Figure 6.7. Mean (+SE) serum gastrin concentration of deer fed lucerne (L), birdsfoot trefoil (B) or sulla (S)

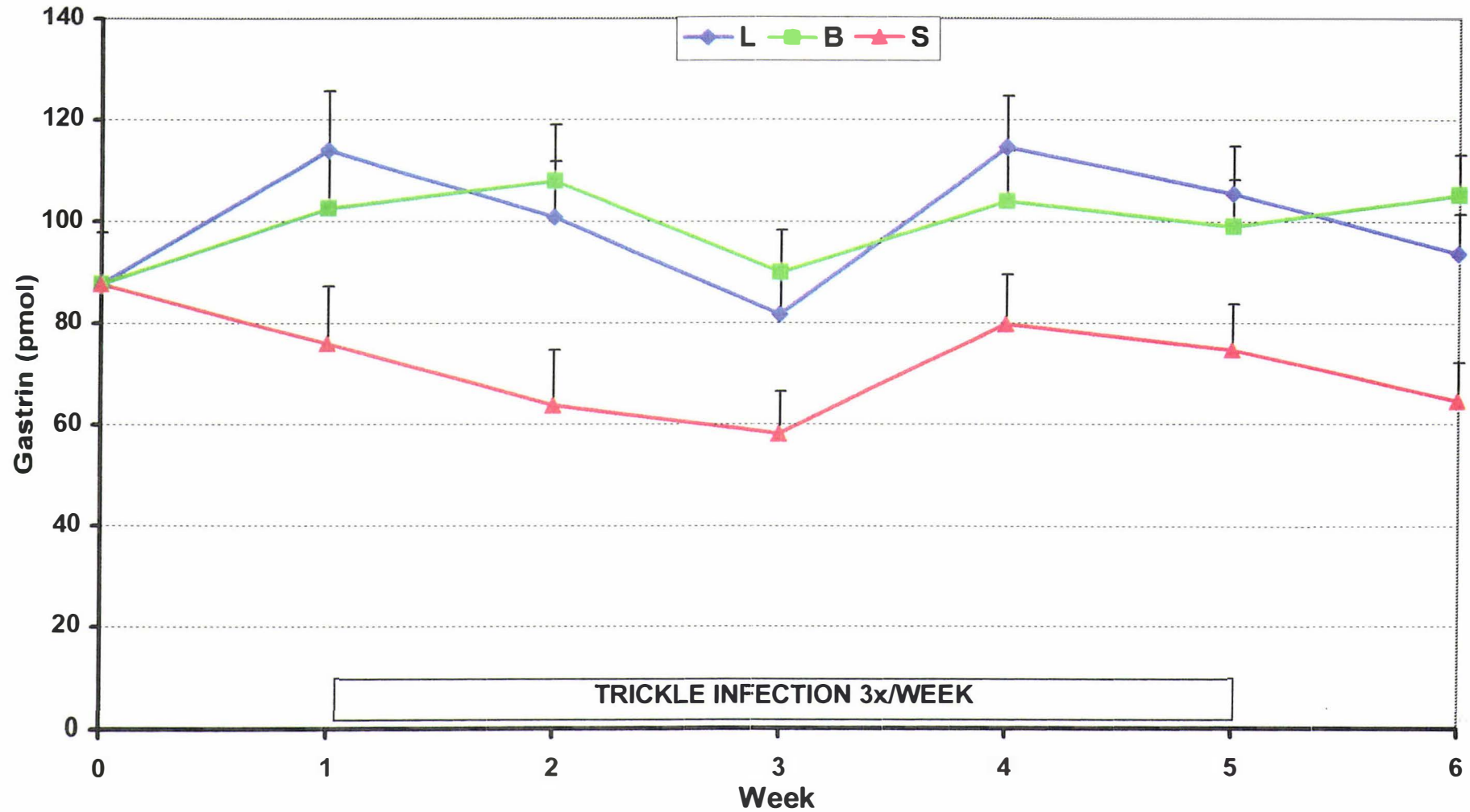


Figure 6.8. Mean (+SE) peripheral eosinophil counts of deer fed lucerne (L), birdsfoot trefoil (B) or sulla (S)

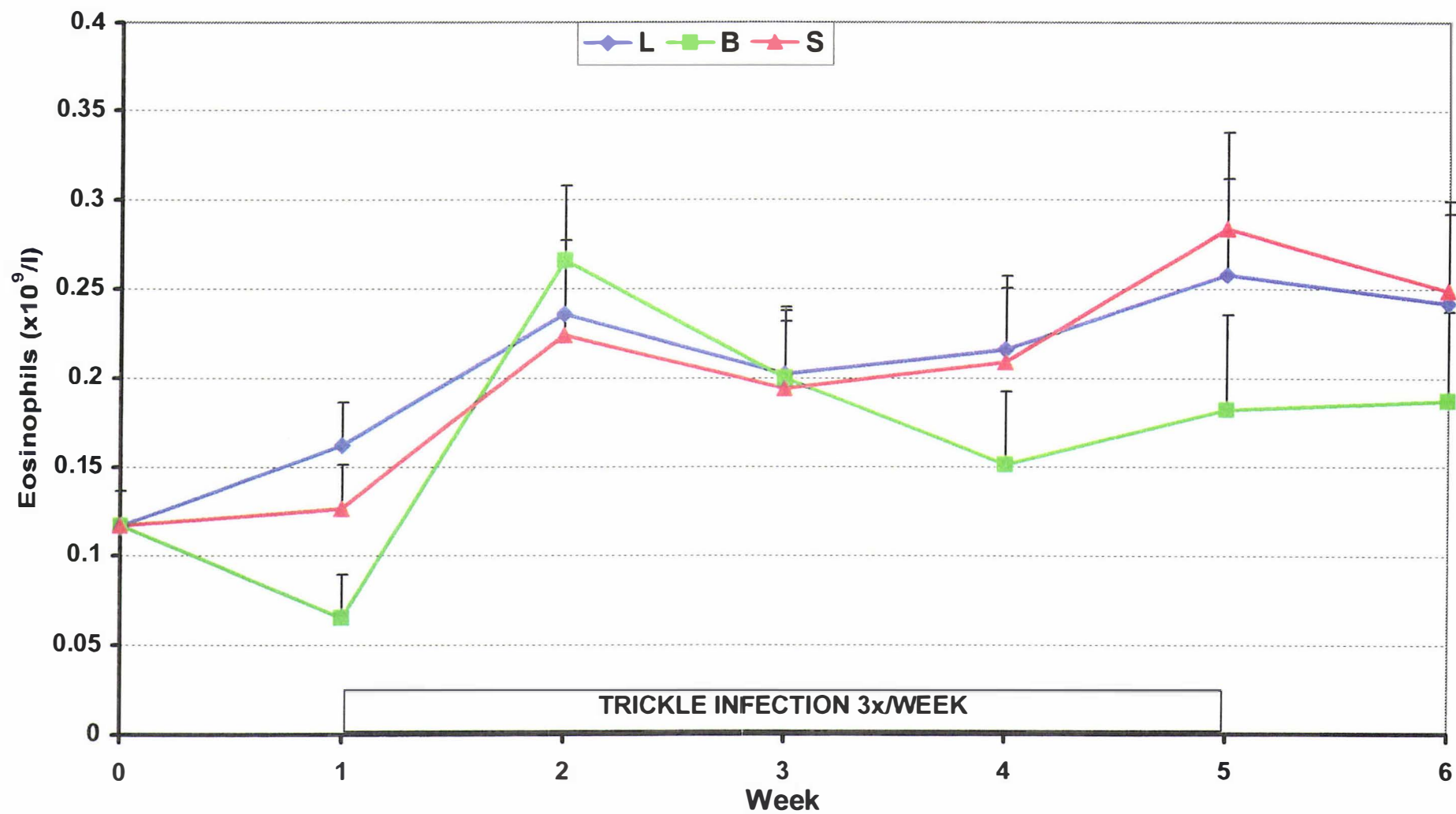
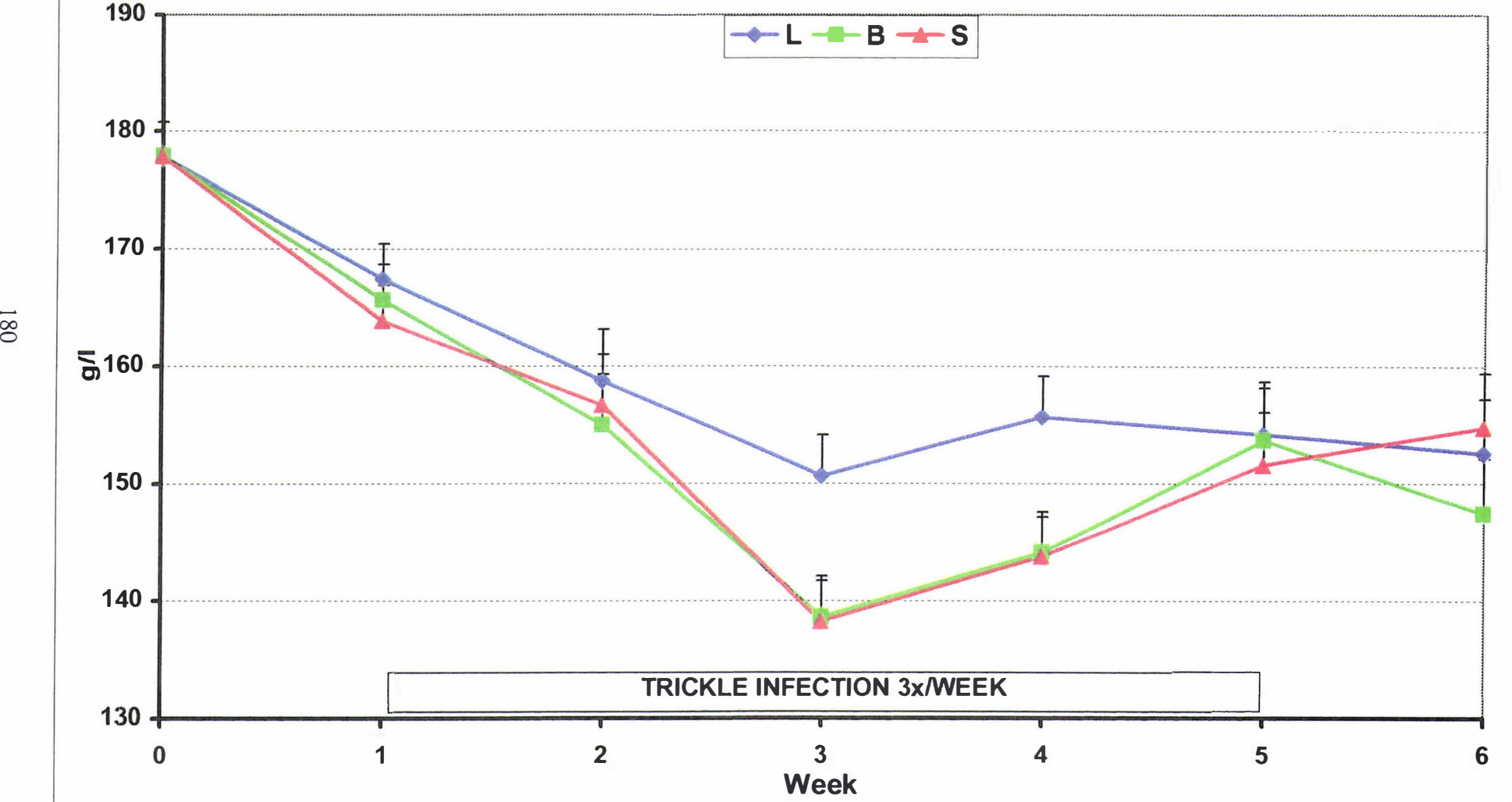


Figure 6.9. Mean (+SE) blood haemoglobin concentration of deer fed lucerne (L), birdsfoot trefoil (B) or sulla (S)



#### 6.4.7. Nematode Counts at Slaughter

Mean adult lung and GI nematode counts at slaughter are presented in Table 6.5. There were a few immature nematodes recovered (<3/animal) in four animals only, so numbers of immature nematodes recovered are not presented. There was no significant difference in lungworm numbers between treatment groups. The S group had a lower total number of GI (total of abomasal, small intestinal and large intestinal nematodes) and total abomasal nematodes (all abomasal species) than the L group ( $P<0.05$ ). This was due to lower numbers of both *Ostertagia*-type and *T.axei* in the deer fed sulla, with the S group having a significantly lower number of *T.axei* compared with the L group ( $P<0.01$ ) and B group ( $P=0.05$ ). The numbers of total GI and abomasal nematodes in the B group were intermediate and did not differ significantly from the other groups. There was no significant difference in the total number of males or females or ratio of male:female nematodes for both *Ostertagia*-type nematodes and *T.axei*, between groups. The fecundity of female *Ostertagia*-type nematodes as measured by the number of eggs per female worm did not differ between treatments. There was no significant difference in the numbers of small and large intestinal nematodes recovered between groups. The species and the proportions of species of GI nematodes found in deer in this study are very similar to those found in the study reported in Chapter 4, so are not presented here.

**Table 6.5. Lung and gastro-intestinal nematodes (mean  $\pm$ SE) recovered from weaner red deer fed lucerne, birdsfoot trefoil or sulla.**

	LUCERNE	BIRDSFOOT TREFOIL	SULLA	S.E. D.F. = 27
<b>LUNGS</b>				
Lungworm	211	280	254	47.7
<b>GASTROINTESTINAL TRACT</b>				
<b>Total GI Nematodes</b>	3409	2647	2389	330.5
<b>Abomasum</b>				
Total Nematodes*	3347	2555	2290	312.9
Total <i>Ostertagia</i> -types	2656	2126	2061	260.9
Total <i>T. axei</i>	684	422	226	91.3
Eggs/female <i>Ostertagia</i> -type	7.14	7.57	7.25	0.808
<b>Small Intestine</b>				
Total Nematodes	14	4	10	5.0
<b>Large Intestine</b>				
Total Nematodes	54	88	89	30.1

\*includes *Ostertagia*-types, *T. axei*. and others

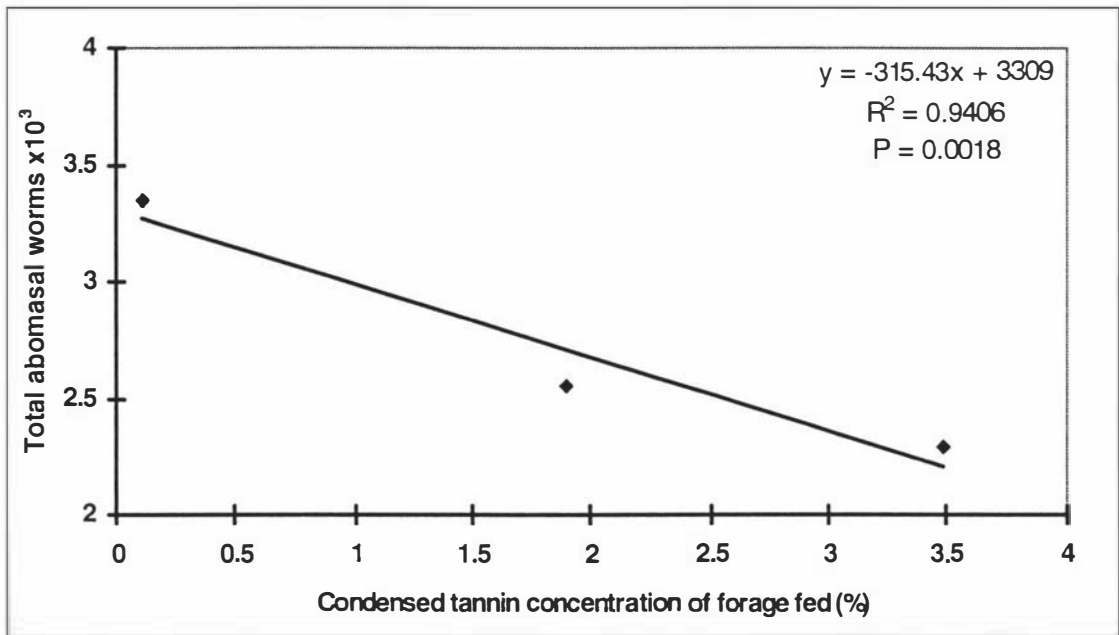
## 6.5. DISCUSSION

This is the first report linking GI parasite establishment in farmed deer with feeding CT-containing forages. This study has shown that feeding CT-containing forages to young deer trickle-infected with internal parasites apparently reduced establishment of abomasal nematodes and increased liveweight gain and carcass weight. As there were no significant differences in mean VFI between treatment groups during the period of infection, it is suggested that efficiency of food conversion was improved when feeding legumes containing CT. The three legume forages differed markedly in CT concentration, but otherwise were similar in chemical composition. This supports the hypothesis that the CT in these forages, acting either directly on the GI parasites themselves and/or indirectly, via nutritional effects in the host, were responsible for the effects observed.

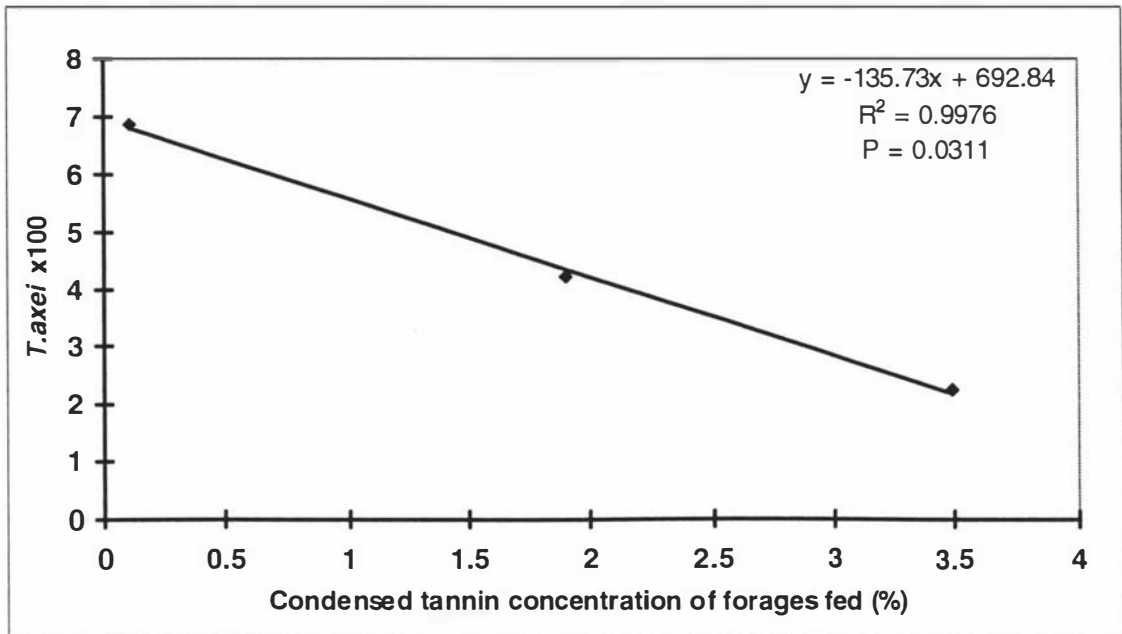
This can be studied further through examining linear regression relationships between parasite establishment and forage CT concentration. Data in Figures 6.10. and

6.11. show negative relationships between the CT content of the forages fed and the group mean total abomasal nematode number and group mean abomasal *T.axei* number, respectively. The regression coefficients suggest that 94% (P=0.002) of the variation in total abomasal nematode numbers and 99.8% (P=0.031) of the variation in number of *T.axei* could be explained by the CT content of the forages fed.

**Figure 6.10. Group mean number of abomasal worms at slaughter as a function of forage condensed tannin concentration.**



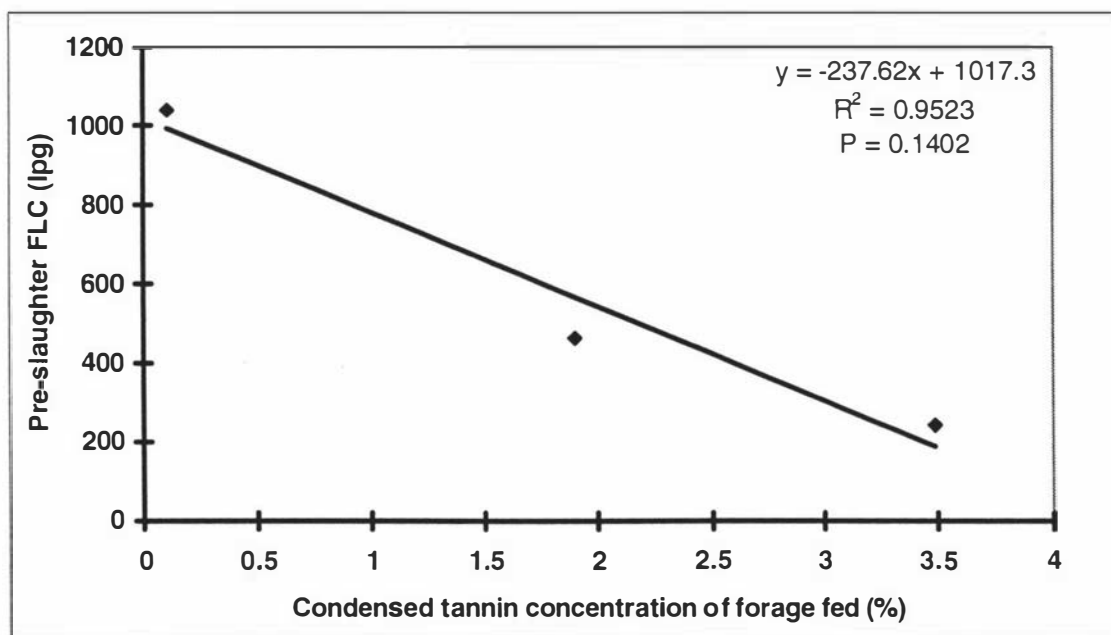
**Figure 6.11. Group mean number of abomasal *T.axei* at slaughter as a function of forage condensed tannin concentration**



A trend towards reduced FLC of deer fed sulla was found (Figure 6.4.), but no effect of forages containing CT was found for establishment of lungworm. Figure 6.12. shows a negative relationship between dietary CT concentration and group mean pre-slaughter FLC, with 95% of the variation in FLC being explained by dietary CT content. However, this relationship was not significant ( $P=0.140$ ), and further research is required to investigate whether this effect was real by using a greater number of diets differing in CT content or by feeding a non CT-containing diet to which varying amounts of extracted CT are added.

The relationships shown in Figures 6.10.-6.12. suggest that differences between forages in abomasal parasite establishment were probably due to differences in their CT concentration. However, the possibility remains that some other component in the forages fed in this study, that was not measured, was responsible for the effects seen. This could also be tested by feeding a non CT-containing forage to which varying amounts of extracted CT are added.

**Figure 6.12. Group mean pre-slaughter faecal larval count as a function of forage condensed tannin concentration.**



Condensed tannins react and form complexes with protein in a pH-dependant manner (Jones and Mangan, 1977) and these reactions are highly specific for different tannins as well as different proteins (Asquith and Butler, 1986). Condensed tannins found in forage legumes fed to deer could therefore interact with protein-based products

excreted by nematodes in the gut (McKellar *et al.*, 1985), or excreted by gut microorganisms in response to the presence of parasites (Lawton, 1995), and/or the protein surface of immature and adult GI nematodes, the outer sheath of ingested GI and lungworm infective-stage L<sub>3</sub> larvae in the gut, and/or the single sheath of lungworm L<sub>1</sub> that are coughed up from the lungs, swallowed and pass through the GI tract to be excreted in faeces. If one or more of these hypotheses are correct, it is suggested that interactions of CT with the surface of the parasite could reduce its motility, or affect the host response to parasitism by, for example, bonding of CT with products which are thought to be excreted by the parasite (McKellar *et al.*, 1985) and/or to microorganisms in the gut that may multiply in response to the presence of parasites (Lawton, 1995). The data show lower mean FLC associated with high dietary CT content (Figures 6.4. and 6.12.), but it is not known whether the CT reduced the number of lungworm larvae excreted by the animals, or temporarily or permanently inactivated (i.e. paralysed) the larvae and reduced their motility. The modified Baermann method for FLC used in this study is a measure of the number of motile L<sub>1</sub> larvae that can be recovered from faeces, so any factor reducing larval motility will reduce the FLC, while not necessarily reducing the actual number of larvae excreted in faeces. *In-vitro* studies involving the addition of different concentrations of CT extracted from fresh forages to internal parasite larvae cultured from deer faeces, at varying stages of development, under conditions of varying pH could be employed to investigate the hypothesis that forage CT can inactivate or paralyse nematode larvae present in the gut.

Lungworm infections of the S group apparently reached patency one week later than for L and B groups (as determined by the time when excretion of L<sub>1</sub> larvae in faeces first occurs). This may be due to sampling interval or that motility of lungworm L<sub>1</sub> larvae was reduced by CT, delaying the point at which the first L<sub>1</sub> larvae produced were recovered from faeces.

The action of CT may also reduce parasite establishment through improved protein nutrition, enabling the animal to better resist a parasite challenge. Condensed tannins have been found to increase the absorption of amino acids, especially essential amino acids from the small intestine of sheep (Waghorn *et al.*, 1987a,b; McNabb *et al.*, 1993) due to increased abomasal flux. The 'by-pass protein' effect of CT has been well documented in ruminants fed fresh forages containing low levels of CT (<4.0%) leading to increased animal production (Barry and Manley, 1984; Barry *et al.*, 1986; Waghorn *et al.*, 1987b; Waghorn *et al.*, 1990; Terrill *et al.*, 1992b; Hart and Sahlu, 1993; Wang *et al.*,



1994a, 1996a, b). Many long-term studies have shown that feeding high protein diets or diets containing bypass protein to animals infected with *Haemonchus* sp., *Ostertagia* spp., *Trichostrongylus* spp., or *Oesophagostomum* spp., enhances development of host immunity or resistance (Dobson and Bawden, 1974; Kambara *et al.*, 1993; van Houtert *et al.*, 1992, 1995b; Coop, *et al.*, 1995; Donaldson *et al.*, 1997) and reduces production losses due to parasitism (Abbott *et al.*, 1986a,b,, 1988; Bown *et al.*, 1991a ; Blackburn *et al.*, 1991; van Houtert *et al.*, 1995a; Smith *et al.*, 1996; Wallace *et al.*, 1996).

However, the general consensus from various short-term studies is that increased protein intake or increased protein supply to the small intestine does not influence initial parasite establishment in parasite-naive sheep infected with *H.contortus* (Abbott *et al.*, 1985a, b, 1986a, b), *T.colubriformis* (Bown *et al.*, 1991a; van Houtert *et al.*, 1995a; Kyriazakis *et al.*, 1996) and *Oe.columbianum* (Dobson and Bawden, 1974). So, although the CT present in forages in this study would increase bypass protein supply to the deer, it is proposed that the reduced establishment of abomasal nematodes in this short-term study may have been due to a direct effect of CT on larval or immature adult parasites, rather than via increased protein nutrition of the animal. Increased protein supply from CT-containing forages may be of greater benefit in studies of longer duration than used here, using deer with existing mature infections or reinfection of deer previously exposed to parasites, where the increased protein supply may increase the animals immunity. All studies reported investigating the effect of diet on parasite establishment have involved single species infections and there is a lack of information in the literature concerning the effect of nutrition on the establishment of cattle and sheep *Ostertagia* spp., and *Ostertagia*-type nematodes which are important GI nematodes of deer.

Reduced liveweight gain caused by parasitism can be due to reduced VFI, reduced dietary digestion and absorption, or reduced efficiency with which digested nutrients are utilized for growth. Parasitism of the gut is well known to increase protein losses in sheep (Poppi *et al.*, 1986; Kimambo *et al.*, 1988; Bown *et al.*, 1991b) and, in young deer infected with both GI and lung nematodes, reduced nitrogen retention (Chapter 5) and reduced liveweight gain and carcass weight (Chapter 3). The pathophysiological consequences of parasitism are more severe in animals on lower planes of protein nutrition, as protein synthesis is diverted away from body growth towards the repair, replacement and reaction to gut damage, or to endogenous loss of protein (Coop and Holmes, 1996). CT increase the efficiency with which digested nutrients are used for body synthetic reactions (Wang, 1995). It is likely that this may be one reason for the

increased liveweight gain while feeding sulla, through action of CT counteracting the protein losses caused by parasite infection. The failure of deer fed B to achieve significantly greater liveweight gain during the period of infection and carcass weight than deer fed lucerne (L) suggests that the 1.9% CT level in this forage was too low to cause significant effects.

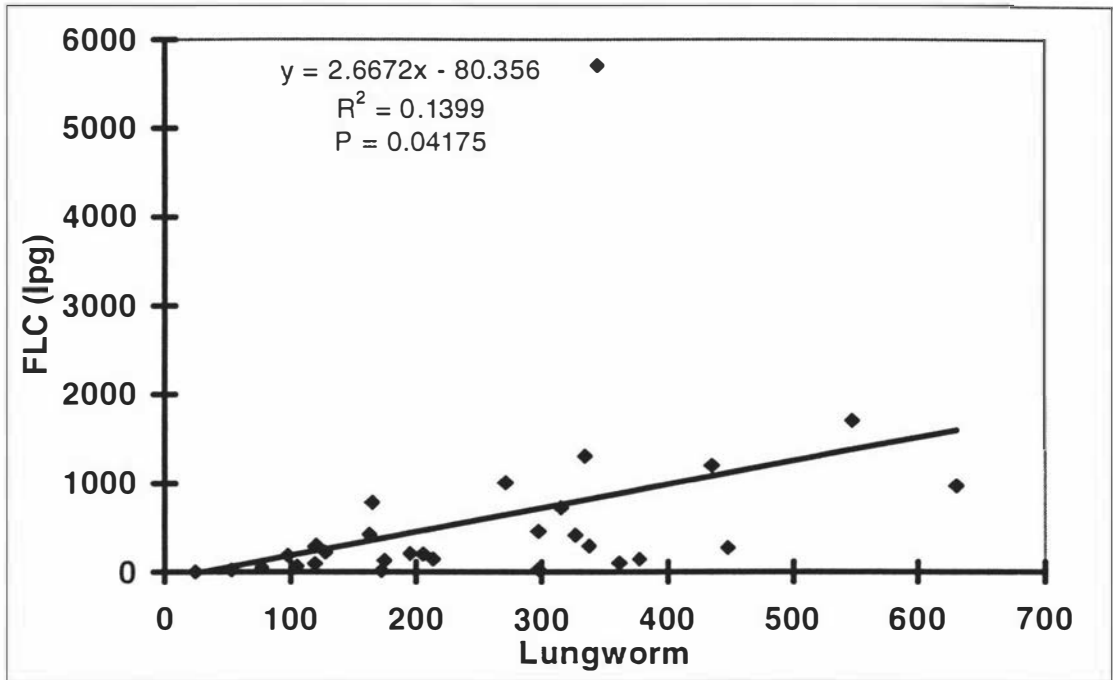
There was no significant difference in group mean VFI during over the period of infection (Table 6.4.) and weekly VFI was not significantly affected until the final two weeks following cessation of trickle infection. Thus reduced VFI was not the main contributor to differences between treatments in liveweight in this study. However, if this study had been of longer duration then VFI may have had a greater effect on liveweight gain, as was shown in Chapter 4. The increase in VFI of S-fed deer towards the end of the trial may have been partly due to the greater ratio of readily fermentable:structural carbohydrate for S than B and L, which would allow faster particle breakdown in the rumen and faster rumen outflow rate. This effect may have increased in magnitude if the trial had been of longer duration.

Deer in this study received a larval dose rate 25% greater than the high rate group in Chapter 4. The group mean lungworm counts were 3.4-4.5 times greater and the group mean GI nematode counts were 3.1-4.4 times greater in this study than in the high infection rate group in Chapter 4. The group mean 'apparent takes' of lungworm and GI nematode larvae in this study were 2.8-3.4% and 6.4-9.1%, respectively, compared with 0.6% and 1.4%, respectively, for the high rate group in Chapter 4. Despite the high apparent takes of lungworm and the greater FLC recorded in this experiment compared with the study reported in Chapter 4, deer in this experiment were not heard coughing. This may be due to deer in this study only having patent lungworm infections for 3-4 weeks, compared with 10 weeks in Chapter 4. The greater worm counts and 'apparent take' in this study were possibly due to the earlier slaughter of animals, relative to the cessation of infection. Parasite strain, year, animal and random effects may also have contributed.

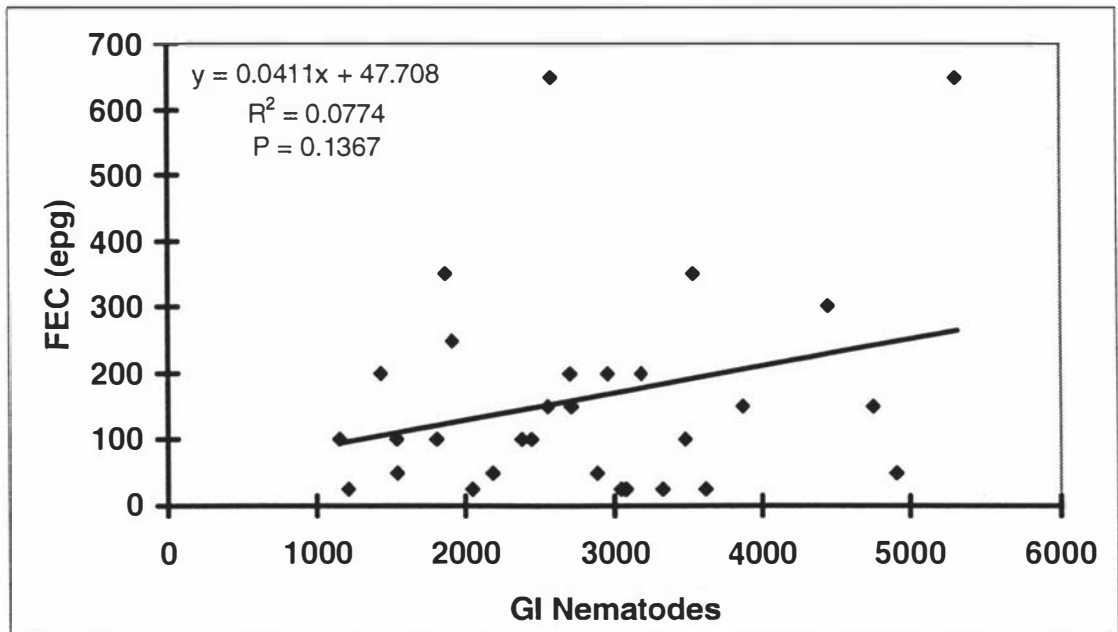
The results of this study also have relevance to diagnosis of parasite infections in young deer consuming fresh forage. Data in Figure 6.13. shows a significant relationship between pre-slaughter FLC and lungworm numbers ( $P=0.042$ ). The regression coefficient suggests that only 14% of the variation in FLC can be explained by the variation in lungworm number, with some of the remainder of the variation probably due to dietary effects. Figure 6.14. shows no significant relationship between pre-slaughter FEC and GI

nematode numbers ( $R^2=0.08$ ;  $P=0.137$ ). Anderson (1985) found a significant relationship between FEC and numbers of GI worms, but the range of worm counts was up to 30,000 compared with 5,500 in the current study. As in Chapter 4, there was great individual animal variation in FEC, FLC and worm number in this study.

**Figure 6.13. Lungworm recovered at slaughter vs pre-slaughter faecal larval count.**



**Figure 6.14. GI nematodes recovered at slaughter vs pre-slaughter faecal egg count.**



The higher serum total protein and albumin concentrations of the S group in the final two weeks of the experiment suggest that either GI tract damage in this group was less, due to the reduced nematode number, or deer in this group were better able to cope

with the effects of parasitism due to the effect of CT increasing the efficiency of utilisation of digested nutrients.

Serum gastrin concentrations (SGC) in this study were similar to those in Chapter 4. It is suggested that the lower SGC of the S group compared with the other two groups from weeks 1-6 is due to the lower number of worms present. However, chemical composition of the diet has been shown to have an influence on the gastrin response in humans (Blair *et al.*, 1975; Taylor *et al.*, 1982; Eysselein *et al.*, 1992), dogs (Strunz *et al.*, 1978; Delvalle & Yamada, 1990) and sheep (Van Bruchem, 1977) and it is possible that some aspect of dietary chemical composition affected SGC concentration of the deer fed different forages in this study. Fox (1993) suggested a negative relationship between SGC and VFI in cattle infected with *O.ostertagi*, but in this study there was no association between SGC and VFI in any of the three treatment groups.

Mean and individual serum pepsinogen concentrations (SPC) in this study did not appear to be related to worm counts. Why mean SPC of both L and S groups remained relatively constant, yet the group mean SPC of the B group was elevated from week 2-6, is unknown.

The drop in blood haemoglobin concentration and PCV in all treatment groups with time, especially during the first half of the experiment is thought to be due to a reduction in the stress response of animals to blood sampling procedures with time (Wilson and Pauli, 1983). However, it is not known why the significantly lower blood haemoglobin concentration of deer fed both CT containing forages compared with deer fed lucerne occurred during weeks 3 and 4.

This study has shown reduced establishment of abomasal nematodes and increased liveweight gain of deer fed a high CT-containing forage and a significant negative relationship between forage CT concentration and abomasal nematode establishment. Further research into the effect of feeding forage legumes containing CT on parasite infections in deer is warranted, both indoors and in the field. Also it is desirable to define whether CT in forage legumes have any direct effect on parasites *in-situ* in the GI tract, or whether the effects on parasite establishment and liveweight gain seen in this study are attributable to increased protein nutrition of the animals fed CT-containing forages. The relative contributions of these two effects in deer with sub-clinical parasitism needs to be resolved through future research.

# **CHAPTER 7.**

## **General Discussion**

### **7.1. INTRODUCTION**

This thesis covers fundamental aspects of internal parasitism in young deer and investigations into the use of forage crops, particularly those containing CT, as an aid in the control of internal parasites of farmed deer. It has highlighted the potential use of sulla as a forage crop particularly suited to deer production under a regime of minimal anthelmintic input.

Data in Chapter 3 confirmed that reduced VFI and liveweight gain occur in young deer with sub-clinical internal parasitism, and showed that the severity of the response of young deer to parasitism varies significantly according to forage species grazed. The model of sub-clinical internal parasitism developed (Chapter 4) is a tool that can be used to investigate many aspects of internal parasitism in deer. The initial use of this model showed that low sub-clinical levels of infection significantly reduced VFI, liveweight and nitrogen retention and significantly altered haematological and serum biochemical parameters of infected deer. This model was then used to demonstrate a significant negative relationship between forage legume CT concentration and apparent establishment of abomasal parasites and FLC, and a significant positive relationship between forage legume CT concentration and liveweight gain of infected deer.

### **7.2. INTERNAL PARASITISM IN DEER**

The development of a working model for study of internal parasitism in deer represents a significant advancement in deer science. Such models have been in use for sheep and cattle parasitological research for decades and can be used for a multiplicity of

purposes (see 4.5.).

This study has also demonstrated that anthelmintic-treated, pasture-reared deer calves are suitable for use in some types of parasitological studies, particularly in relation to nutrition and parasite control.

### **7.2.1. Quantifying Sub-clinical Effects of Internal Parasitism in Deer**

This is the first report quantifying effects of sub-clinical parasitism on VFI, liveweight, nitrogen retention, carcass weight, haematology and serum biochemistry in farmed deer (Chapters 4 & 5). Compared with control deer, a 22% reduction in VFI and a 9% reduction in liveweight occurred in the high dose group that, when euthanased, had a mean lungworm count of 62 and a mean GI nematode count of 783 (Chapter 4). By accepted standards these worm counts and associated FLC (group mean <110 lpg) and FEC (group mean <150epg) are considered to be relatively low, yet significant reductions in VFI, liveweight and nitrogen retention were recorded in these deer after trickle-infection for nine weeks. Audige *et al.*, (1998) found in a survey of NZ red deer farms over three years that during the autumn period up to 99% of weaner deer had FLC >0 and up to 34% of weaner deer with FEC>0 had FEC>200epg. The reduction in VFI and liveweight reported in Chapter 4., with relatively low FLC and FEC compared with data of Audige *et al.*, (1998), suggests that significant production losses attributable to sub-clinical parasitism may be widespread in farmed weaner deer in NZ. This represents a loss to deer farmers and to the industry and warrants further investigation.

### **7.2.2. The Effects of Internal Parasitism on Growth and Carcass Production**

This research has shown reduced VFI to be the primary effect of concurrent lungworm and GI nematode infections in deer. Reduced liveweight gain, N-retention and carcass weight of parasitised deer have been shown to be a function of reduced VFI,

except when forages containing CT are fed (see 6.3.2.). Experiments involving pairs of animals, one infected and one uninfected, both fed to the level of intake of the infected animal would allow more detailed investigation of the effect of parasitism on liveweight gain, N metabolism and retention, and other factors such as energy metabolism. Experimentation of this type involving separate infections of lungworm and mixed species of abomasal parasites would allow determination of the relative importance of lung and GI nematode infection on reducing VFI and hence deer production. Further work with individual abomasal nematode spp. of deer would yield information on which species are the most pathogenic, but would require considerable preliminary work to develop single-species isolates and culture sufficient larvae for experimental use.

Data in Chapter 3 showed that reduced liveweight gain or liveweight loss caused by internal parasitism in weaner deer grazing PRG/WC pasture in autumn and early winter was only partly recovered by compensatory growth in spring, leading to lower carcass weights and a lower proportion of deer reaching the target of 50kg carcass weight by one year of age. Where this occurs on commercial deer farms it would represent a direct, unrecoverable loss to the farmer. Additionally, a flow-on effect would be reduced liveweight of yearling hinds affecting future reproductive performance throughout their lifetime (Audige, 1995). Further research is required to investigate the effect of internal parasitism in the first year of life on subsequent lifetime performance of both hinds and stags grazing PRG/WC pasture and specialist forage crops for deer.

### **7.2.3. Diagnosis of Sub-Clinical Parasitism in Deer**

Sub-clinical parasitism occurring in grazed weaned deer calves during the autumn period (Chapter 3) rapidly became clinical, with acute effects on VFI and bodyweight of some animals. This was despite low or zero faecal egg and/or larval counts. Thus these diagnostic tools alone were found inadequate for monitoring parasite infections in deer of

this age. As a result, the diagnosis of parasitism in these animals was based on reduced liveweight gain, coughing and response to treatment, rather than FLC or FEC. If systems incorporating minimal anthelmintic input using forage crops for commercial deer production are to be safely adopted, then accurate methods for monitoring of parasite infections are essential. Clearly, to decrease the risk associated with minimising anthelmintic use, the intensity of monitoring needs to increase as the frequency of anthelmintic treatment decreases.

Reduced VFI and liveweight have been indicative of the level of parasite infection in all experiments described in this thesis. However, reduced VFI and liveweight are not specific to internal parasitism and can only be used in conjunction with more specific diagnostic criteria. In the field, frequent measurement of liveweight will assist in early identification of the onset of parasitism in young deer, in the absence of confounding effects reducing liveweight gain.

The FEC and FLC reported in the field and indoor experiments of this thesis correspond well to 'normal' field data (Audige *et al.*, 1998). However, both FEC and FLC in these studies were found to be poor indicators of level of infection, particularly FEC, although this statement applies only to the range of parasite numbers recorded, which were low compared with those reported by Anderson and Wilson (1984).

In Chapter 3, animals with sub-clinical lung and GI parasitism rapidly developed clinical lungworm disease, yet had low or zero FLC, which was probably due to the presence of an immature lungworm population. This situation prompted the question as to whether FEC could be used as a surrogate measure of lungworm infection in the field when lungworm populations are immature. Figure 7.1. indicates a positive relationship between sub-clinical lung and GI nematode burdens presented in Chapters 4 and 6. However, given that no relationship was found between pooled FEC and FLC data from the sub-clinical infections reported in Chapters 3, 4 and 6, and given the poor relationships

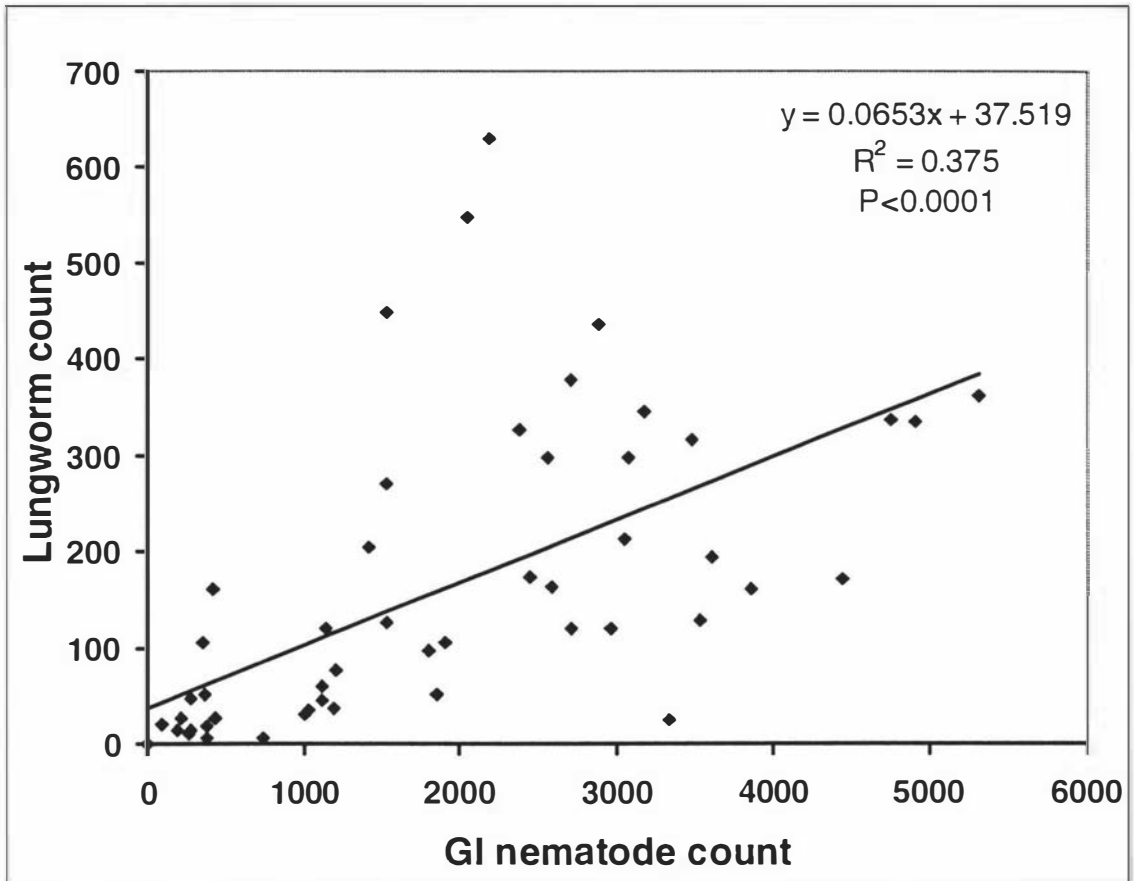


found between FEC and FLC and the GI nematode and lungworm burdens in Chapters 4 and 6, it seems that FEC is not suitable as a surrogate measure of lungworm infection where sub-clinical infections are concerned. Still, the use of FEC as a surrogate measure of immature lungworm infection should be evaluated for use in the differential diagnosis of clinical infections. Investigation of other procedures such as faecal antigen ELISA testing (Qureshi, pers. comm.) may assist in diagnosis of parasite infections in weaner deer during their first autumn/winter.

Further experimentation involving serial slaughter of deer is required to gain knowledge of the effect of stage of infection (mature vs immature), level of infection (sub-clinical to clinical), age of animal and prior exposure to parasites on FEC and FLC before they can be considered as quantitative measures of parasitism in deer. This research should involve a wider range of worm numbers than used in these studies.

This study has yielded some information about the potential for using serum biochemical and haematological parameters for diagnosing internal parasitism in deer. However, little is known of their relationship to internal parasitism of deer and they are subject to large individual animal variation. Consequently, there is debate as to whether individual animal or group mean values are the most appropriate diagnostic measurement for internal parasitism, particularly in relation to serum pepsinogen (Auldige *et al.*, 1998). Further research on these parameters, particularly herd mean serum pepsinogen concentration, in relation to internal parasite infection and sub-clinical effects is warranted in deer.

**Figure 7.1. Gastrointestinal nematode count at slaughter vs lungworm count at slaughter for Chapters 4 & 6 combined.**



This study has confirmed that large individual animal variation is a common feature of internal parasitism in deer, particularly in relation to nematode counts, FEC, FLC, haematological and serum biochemical parameters. This has implications for experimental design, and it necessitates the use of relatively large numbers of animals in parasitological experimentation and investigation of parasitism on commercial farms, to increase the likelihood of significant effects being detected.

### 7.3. FORAGES FOR CONTROL OF INTERNAL PARASITES IN DEER

Niezen (1996) has shown that forage species can affect the growth of parasitised lambs, nematode burdens of grazing lambs, the recovery of larvae from herbage and development and migration of larvae onto herbage.

Semiadi *et al.*, (1993), Soetrisno *et al.*, (1994), Kusmartono *et al.*, (1996a), and Min *et al.*, (1997) have shown superior deer growth and carcass production of deer grazing specialist forage crops such as red clover and chicory, compared with deer grazing conventional PRG/WC pasture. A summary is presented in Table 7.1, including data from Chapter 3 showing the high autumn and spring feeding value of sulla, particularly for hybrid stags. Data reported in Chapter 3 showed that forage species grazed affected the timing of onset of sub-clinical parasitism, the severity of subsequent clinical signs and deer production. Condensed tannins in sulla reduce apparent establishment of abomasal nematodes in deer and reduce FLC whilst increasing liveweight gain (Chapter 6). The potential exists for forage crops, particularly those containing high concentrations of CT, to be used on deer farms to aid in the control of internal parasites, hence reducing anthelmintic use, whilst simultaneously increasing deer production through direct nutritional effects of the forage *per se*. While these are promising results, field research applying a systems approach is required to further evaluate the use of sulla for growth of weaner deer under systems of minimal anthelmintic input before recommendations can be made to the Deer Industry.

**Table 7.1. Growth (g/d) of red (R) and hybrid (H) stags to one year of age grazing perennial ryegrass/white clover, red clover, chicory and sulla, and feeding value of forages relative to perennial ryegrass/white clover .**

Year**	Pasture		R.Clover	Chicory		Sulla	
	<u>R</u>	<u>H</u>	<u>R</u>	<u>R</u>	<u>H</u>	<u>R</u>	<u>H</u>
<b>AUTUMN</b>							
1991	192	-	263	-	-	-	-
1992	207	-	237	-	-	-	-
1993	178	203	-	246	318	-	-
1995	152	199	-	235	271	-	-
1994 (Chapter 3)	223	213	-	223	196	267	319
<b>Mean Relative Feeding Value</b>							
	<b>100</b>	<b>100</b>	<b>126</b>	<b>131</b>	<b>128</b>	<b>120</b>	<b>150</b>
<b>SPRING</b>							
1991	341	-	354	-	-	-	-
1992	281	-	346	-	-	-	-
1993	260	271	-	255	310	-	-
1995	285	298	-	335	331	-	-
1994 (Chapter 3)	221	304	-	247	421	248	378
<b>Mean Relative Feeding Value</b>							
	<b>100</b>	<b>100</b>	<b>114</b>	<b>109</b>	<b>121</b>	<b>112</b>	<b>124</b>

### **7.3.1. Effect of Plant Morphology and Sward Structure on Internal Parasites**

Herbage species affects development of GI nematode larvae from eggs in faeces to infective (L<sub>3</sub>) stage larvae, larval survival and migration of larvae up through the sward to the upper grazed stratum (Niezen, 1996). These effects are thought to be due to differences in plant morphology and sward structure.

Niezen (1996) investigated effects of herbage species on larval dynamics of GI

nematode species from sheep. Although it is to be expected that there would be similar effects with deer lungworm and GI nematode larvae, the interaction between deer and pasture, or between deer-specific parasites and pasture may differ. Therefore the effects of herbage species on deer nematode species larval dynamics warrants investigation using similar methods to those employed by Niezen (1996). The larval dynamics studies of Niezen (1996) were based on a modified Baermann technique which measured the number of larvae recovered from a standard wet weight of herbage. However, Niezen (1996) found that larval recovery rates differed between herbage species and further studies are needed to determine the reasons for differential recovery rates and the fate of larvae not recovered, before experimentation in this area can proceed.

The differences in parasitism and production observed between deer grazing PRG/WC pasture and chicory in Chapter 3 could have been due to differences in dynamics of deer nematode species larvae present in the sward caused by different plant morphology and sward structure and/or to differences in nutritional status of the host caused by differing forage chemical composition. However, experiments in which parasite-free forages are cut and fed to deer individually housed indoors and artificially infected with parasites has allowed investigation of the effect of chemical composition of forages on parasitism, separate from effects of larval dynamics in the sward. Such experimentation needs to be carried out with deer fed chicory and PRG/WC pasture to investigate the relative importance of the effects of plant chemical composition and plant morphology and sward structure on the effects on parasitism, VFI and liveweight observed in Chapter 3.

### **7.3.2. Plant Chemical Composition**

Data in this thesis has shown that abomasal nematode establishment and FLC are reduced in deer by feeding legume forages containing CT and that the magnitude of these

effects are related to condensed tannin concentration. The hypothesis that the CT were responsible for these observations, rather than some other component not measured, needs to be tested by feeding a standard non CT-containing diet to deer to which graded amounts of CT, extracted from a forage legume, are added, while the deer are infected with internal parasites. This method would be more appropriate than the use of polyethylene glycol (PEG) to selectively bind and inactivate CT (Waghorn *et al.*, 1987), since previous research investigating the effect of CT on establishment of sheep nematodes using PEG, found PEG had an effect on some parasitological aspects, irrespective of forage fed (Niezen, unpublished). Condensed tannins extracted from different forage legumes could also be added to a standard diet at the same concentration, in a similar experiment, to determine whether different CT composition and/or molecular structure, and not just total concentration, affect establishment of internal parasites. A similar experimental design involving deer not infected with internal parasites would allow determination of the threshold CT concentration level for deer at which VFI and liveweight become negatively affected. This may be higher for deer than other ruminants, given that VFI was not affected in deer grazing sulla containing up to 8% CT (Chapter 2) whereas VFI in sheep is reduced at >4%CT (Waghorn *et al.*, 1990). Tannin-binding proteins in deer saliva (Hagerman and Robbins, 1993; Semiadi *et al.*, 1995), may contribute to this apparent species difference.

Assuming CT are responsible for the apparent reduced parasite establishment and FLC and increased liveweight gain seen (Chapter 6.), the exact mechanism of CT in reducing abomasal parasite numbers and FLC remains unclear. It needs to be determined whether CT have any direct inhibitory effect on larval, immature or adult parasites, either *in-vitro* or *in-vivo* or whether effects on apparent parasite establishment (Chapter 6) were due to CT supplying bypass protein to the small intestine and hence increasing the nutritional status of the host. Studies involving addition of extracted CT to different

developmental stages of GI and lung nematodes at differing pH should be carried out *in-vitro*. For abomasal nematodes, which appear to be the most important GI nematodes of deer, short term *in-vivo* studies could also be performed involving abomasal cannulated animals that have extracted CT infused directly into the abomasum. This would determine whether CT have any direct effect on parasites *in-vivo*, separately from the effect of increased host nutrition on parasites due to forage CT providing a source of rumen bypass protein. Thus the effect of CT on the parasites and the host could be partitioned.

This study has shown that feeding forages containing CT can reduce the apparent establishment of deer abomasal parasites as determined by slaughter of deer following five weeks of trickle infection. Further research is now required using trickle infections of longer duration, to assess effects of forage CT on acquisition of host immunity and the ability of the host to cope with the effects of parasitism under long-term infection. Studies of this type would require serial slaughter of animals at set intervals in order to accurately determine nematode population dynamics over time.

The reduction in liveweight gain and carcass weight of deer fed lucerne compared with deer fed sulla was not due to reduced VFI, but rather to increased efficiency of utilisation of digested nutrients for growth in the deer fed the high CT-containing forage (Chapter 6). This is an important finding and suggests that deer fed high CT-containing forages are likely to have greater ability to cope with the effects of parasitism than deer fed non-CT containing forages. However, this effect may have been partly a function of the shorter infection period used in this experiment and further experimentation is required to confirm this.

Differences in establishment of GI nematodes were observed between forage legumes that can be explained by differences in forage chemical composition (Chapter 6). Grazing studies are now needed to determine the effect of CT-containing legumes on production of parasitised deer grazing outdoors and on larval dynamics in the grazed

sward. However, in order for forage crops such as sulla and birdsfoot trefoil to be viable for commercial farm use, their establishment and persistence under grazing needs to be improved. Selection of sulla and *Lotus* spp. for stoloniferous growth habit and hence greater persistence under grazing management is underway in NZ (Rumball, pers. comm.), but it will be 3-5 years before these cultivars become available for commercial use. Priority should be given to their study under deer grazing and systems of minimal anthelmintic input.

#### **7.4. CONCLUSIONS**

Feeding forages containing CT reduces apparent establishment of abomasal nematodes and reduces faecal lungworm larval count in weaner deer trickle-infected with lungworm and GI nematode larvae. Parasitised deer fed CT-containing forages exhibit greater liveweight gain than deer fed lucerne. This may be due to a direct inhibitory effect of CT on the nematodes *in-situ*, the effect of CT increasing the protein nutritional status of the host, or to a combination of both effects.

This thesis has highlighted the potential use of sulla as a forage crop particularly suited to deer production under a regime of minimal anthelmintic input. This is allied with the NZ Deer Industry's aim of producing clean, green, natural products using a minimum of chemical inputs and contributes to the potential development of sustainable deer farming systems. Further field trials evaluating the use of pure sulla swards for deer production whilst minimising anthelmintic inputs are required. Also research and development is needed to enhance the persistence of sulla under grazing management. Forages such as chicory also offer potential for deer production under minimal anthelmintic regimes, but further field studies are required to determine how differing plant morphology and sward structure affects parasite larval dynamics in these forages.

The model for study of internal parasitism of deer described in this thesis is an



important tool that can now be used to investigate many aspects of internal parasitism in deer. This model has been used to help quantify sub-clinical parasitism in terms of VFI, liveweight gain and N-retention in young deer. Data of Audige *et al.*, (1998) suggests that in any one season sub-clinical parasitism affects possibly more than 50% of farmed weaner deer in NZ, resulting in a serious loss of production across the Deer Industry. In this context the studies in this thesis and the proposed uses for the parasite study model developed should be viewed as highly relevant and timely.

## LIST OF REFERENCES

1. Abbott, E. M.; Parkins, J. J. & Holmes, P. H. 1985a. Influence of dietary protein on establishment and pathogenesis in Finn Dorset and Scottish Blackface lambs given a single moderate infection of *Haemonchus contortus*. *Research in Veterinary Science* 38: 6-13.
2. Abbott, E. M.; Parkins, J. J. & Holmes, P. H. 1985b. Influence of dietary protein on the pathophysiology of ovine haemonchosis in Finn Dorset and Scottish Blackface lambs given a single moderate infection. *Research in Veterinary Science* 38: 54-60.
3. Abbott, E. M.; Parkins, J. J. & Holmes, P. H. 1986a. The effect of dietary protein on the pathogenesis of acute ovine haemonchosis. *Veterinary Parasitology* 20: 275-289.
4. Abbott, E. M.; Parkins, J. J. & Holmes, P. H. 1986b. The effect of dietary protein on the pathophysiology of acute ovine haemonchosis. *Veterinary Parasitology* 20: 291-306.
5. Abbott, E. M.; Parkins, J. J. & Holmes, P. H. 1988. Influence of dietary protein on the pathophysiology of haemonchosis in lambs given continuous infections. *Research in Veterinary Science* 45: 41-49.
6. Adam, C. L. 1991. Nutrition and the implications of modifying the seasonality of farmed red deer. Recent advances in animal nutrition. W. Haresign, D.J.A. Cole (Eds). Butterworth-Heinmann Ltd, Oxford, United Kingdom: 211-223.
7. Adam, C. L. & Moir, C. E. 1985. Effect of winter nutrition of young farmed red deer on their subsequent growth at pasture. *Animal Production* 40(1): 135-141.
8. Adu, E. K.; Barry, T. N.; Kemp, P. D. & Wilson, P. R. 1996. Evaluation of *Lotus corniculatus* for pre-weaning growth of deer. Proceedings of the Massey University Deer Farmers Conference: 73-75.
10. Anderson, M.V. & Wilson, P.R. 1984. Studies of some aspects of gastrointestinal nematodes and *Dictyocaulus viviparus* of farmed red deer. Proceedings of a Deer Course for Veterinarians. Deer Branch of the New Zealand Veterinary Association 1: 78-88.
11. Anderson, N.; Armour, J.; Jarrett, W.F.H.; Jennings, F.W.; Ritchie, J.S.D. & Urquhart, G.M. 1965. A field study of parasitic gastritis in cattle. *Veterinary Record* 77: 196-204.
12. Anderson, N.; Hansky, J. & Titchen, D.A. 1976a. Hypergastrinaemia during

- parasitic gastritis in sheep. *Journal of Physiology* 256: 51-52.
13. Anderson, N.; Blake, R. & Titchen, D.A. 1976b. Effects of a series of infections of *Ostertagia circumcincta* on gastric secretion of the sheep. *Parasitology* 72: 1-12.
  14. Anderson, N.; Hansky, J. & Titchen, D. A. 1981. Effects of *Ostertagia circumcincta* infections on plasma gastrin in sheep. *Parasitology* 82: 401-410.
  15. Anderson, N.; Hansky, J. & Titchen, D.A. 1985. Effects on plasma pepsinogen, gastrin and pancreatic polypeptide of *Ostertagia* spp. transferred directly into the abomasum of sheep. *International Journal for Parasitology* 2: 159-165.
  16. Anderson, N.; Reynolds, G.W. & Titchen, D.A. 1988. Changes in gastrointestinal mucosal mass and mucosal and serum gastrin in sheep experimentally infected with *Ostertagia circumcincta*. *International Journal for Parasitology* 18: 325-331.
  17. Andrews, J. R. H. 1963. New trichostrongylids from the red deer (*Cervus elaphus* L.) in New Zealand. *Transactions of the Royal Society of New Zealand Zoology* 3:239-246.
  18. Andrews, J. R. H. 1964. The arthropod and helminth parasites of red deer (*Cervus elaphus* L.) in New Zealand. *Transactions of the Royal Society of New Zealand Zoology* 5: 97-121.
  19. Andrews, J. R. H. 1973. A host-parasite checklist of helminths of wild ruminants in New Zealand. *New Zealand Veterinary Journal* 21: 43-47.
  20. Andrews, S. J.; Ferrari, M. M.; Pow, J. D. E. & Lancaster, M. B. 1993. Nematode egg output and plasma concentrations of ivermectin after its administration to red deer (*Cervus elaphus elaphus*). *The Veterinary Record* 132: 161-163.
  21. Asher, G. W.; Barrell, G. K.; Adam, J. L. & Staples, L. D. 1988. Effects of subcutaneous melatonin implants on reproductive seasonality of farmed fallow deer (*Dama dama*). *Journal of Reproduction and Fertility* 84: 679-691.
  22. Asquith, T. N. & Butler, L. G. 1986. Interactions of condensed tannins with selected proteins. *Phytochemistry* 25(7): 1591-1593.
  23. Ataja, A. M.; Wilson, P. R.; Barry, T. N.; Hodgson, J.; Hoskinson, R. M.; Parker, W. J. & Purchas, R. W. 1992a. Early venison production from red deer (*Cervus elaphus*) as affected by grazing perennial or annual ryegrass pastures, pasture surface height and immunization against melatonin. *Journal of Agricultural Science, Cambridge* 118: 353-369.
  24. Ataja, A. M.; Wilson, P. R.; Hodgson, J.; Hoskinson, R. M.; Purchas, R. W.; Varela-Alvarez, H. & Barry, T. N. 1992b. Venison production from grazing young red deer stags: effects of pasture type and immunisation against melatonin. In: *The*

- Biology of Deer. Proceedings of an International Symposium on the Biology of Deer. R.D. Brown (Ed). Springer-Verlag, New York. USA: 203-210.
25. Audige, L. J. M. 1995. Deer herd health and production profiling. PhD Thesis, Massey University, Palmerston North, New Zealand.
  26. Audige, L.J.M.; Wilson, P.R. & Morris, R.S. 1998. A survey of internal parasites and parasite control on New Zealand deer farms. *New Zealand Veterinary Journal* (in press).
  27. Austin, P. J.; Suchar, L. A.; Robbins, C. T. & Hagerman, A. E. 1989. Tannin-binding proteins in saliva of deer and their absence in saliva of sheep and cattle. *Journal of Chemical Ecology* 15: 1335-1347.
  28. Bailey, R. W. 1967. Quantitative studies of ruminant digestion. II. Loss of ingested plant carbohydrates from the reticulo-rumen. *New Zealand Journal of Agricultural Research* 10: 15-32.
  29. Baker, M. R. & Anderson, R. C. 1975. Seasonal changes in abomasal worms (*Ostertagia* spp.) in white-tailed deer (*Odocoileus virginianus*) at Long Point, Ontario. *Canadian Journal of Zoology* 53: 87-96.
  30. Barry, T. N. 1985. The role of condensed tannins in the nutritive value of *Lotus pedunculatus* for sheep 3. Rates of body and wool growth. *British Journal of Nutrition* 54: 211-217.
  31. Barry, T. N. 1989. Condensed tannins: their role in ruminant protein and carbohydrate digestion and possible effects upon the rumen ecosystem. The roles of protozoa and fungi in ruminant digestion. Eds. Nolan, J. V., Leng, R. A. & Demeyer, D. I. Armidale NSW, Australia: Penambul Books: 153-169.
  32. Barry, T. N. & Duncan, S. J. 1984. The role of condensed tannins in the nutritive value of *Lotus pedunculatus* for sheep. 1. Voluntary intake. *British Journal of Nutrition* 51: 484-491.
  33. Barry, T. N. & Forss, D. A. 1983. The condensed tannin content of vegetative *Lotus pedunculatus*, its regulation by fertiliser application, and effect upon protein solubility. *Journal of the Science of Food and Agriculture* 34: 1047-1056.
  34. Barry, T. N. & Manley, T. R. 1984. The role of condensed tannins in the nutritive value of *Lotus pedunculatus* for sheep. 2. Quantitative digestion of carbohydrates and proteins. *British Journal of Nutrition* 51: 493-504.
  35. Barry, T. N. & Manley, T. R. 1986. Interrelationships between the concentrations of total condensed tannin, free condensed tannin and lignin in *Lotus* sp. and their

- possible consequences in ruminant nutrition. *Journal of the Science of Food and Agriculture* 37: 248-254.
36. Barry, T. N. & Wilson, P. R. 1994. Review: Venison production from farmed deer. *Journal of Agricultural Science, Cambridge* 123: 159-165.
  37. Barry, T.N.; Faichney, G.J. & Redekopp, Carolyn. 1985. Gastro-intestinal tract function in sheep infused with somatostatin. *Australian Journal of Biological Science* 38: 393-403.
  38. Barry, T. N.; Manley, T. R. & Duncan, S. J. 1986. The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 4. Sites of carbohydrate and protein digestion as influenced by dietary reactive tannin concentration. *British Journal of Nutrition* 55: 123-137.
  39. Barry, T. N.; Suttie, J. M.; Milne, J. A. & Kay, R. N. B. 1991. Control of food intake in domesticated deer. *Physiological Aspects of Digestion and Metabolism in Ruminants: Proceedings of the Seventh International Symposium on Ruminant Physiology*: 385-401.
  40. Batty, A. F.; Chapman, D. I. & Chapman, N. 1987. Prevalence of nematode parasites in wild fallow deer (*Dama dama*). *Veterinary Record, Short Communication*: 598-599.
  41. Beaudoin, R. L.; Samuel, W. M. & Strome, C. P. A. 1970. A comparative study of the parasites in two populations of white-tailed deer. *Journal of Wildlife Diseases* 6: 56-63.
  42. Beever, D.E.; Kellaway, R.C.; Thomson, D.J.; MacRae, J.C.; Evans, C.C. & Wallace, Annie. S. 1978. A comparison of two non-radioactive digesta marker systems for the measurement of nutrient flow at the proximal duodenum of calves. *Journal of Agricultural Science, Cambridge* 90: 157-163.
  43. Begovic, S.; Dusic, E.; Sacirbegovic, A. & Tafro, A. 1978. Examination of variations of tannase activity in ruminal content and mucosa of goats on oak leaf diet and during intraruminal administration of 3 to 10% tannic acid. *Veterinaria (Sarajevo)* 27: 445-457.
  44. Berg, R. T. & Butterfield, R. M. 1976. *New Concepts of Cattle Growth*. Halsted Press, John Wiley and Sons, New York. USA.
  45. Bines, J. A. & Davey, A. W. F. 1970. Voluntary intake, digestion, rate of passage, amount of material in the alimentary tract and behaviour in cows receiving complete diets containing straw in different proportions. *British Journal of Nutrition* 24: 1013-1027.

46. Bjorn, H.; Roepstorff, A. & Nansen, P. A 1996. Possible influence of diet composition on the establishment of nematodes in the pig. *Veterinary Parasitology* 63(1-2): 167-171.
47. Black, J. L. 1990. Nutrition of the grazing ruminant. *Proceedings of the New Zealand Society of Animal Production* 50: 7-27.
48. Black, J. L.; Kenney, P. A. & Colebrook, W. F. 1987. Diet selection by sheep. In: *Temperate Pastures; Their Production, Use and Management*, J.L. Wheeler, C.J. Pearson and G.E. Robards (Eds). 1987; Australian Wool Corporation / CSIRO, Melbourne: 331-334.
49. Blackburn, H. D.; Rocha, J. L.; Igueiredo, E. P.; Berne, M. E.; Vieira, L. S.; Cavalcante, A. R. & Rosa, J. S. 1991. Interaction of parasitism and nutrition and their effects on production and clinical parameters in goats. *Veterinary Parasitology* 40: 99-112.
50. Blair, E.L.; Greenwell, J.R.; Grund, E.R.; Reed, J.D. & Sanders, D.J. 1975. Gastrin response to meals of different composition in normal subjects. *Gut* 16: 766-773.
51. Blaxter, K. L. 1989. *Energy Metabolism in Animals and Man*. Cambridge University Press, Cambridge, United Kingdom.
52. Bootsma, A.; Ataja, A. M. & Hodgson, J. 1990. Diet selection by young deer grazing mixed ryegrass/white clover pastures. *Proceedings of the New Zealand Grassland Association* 51: 187-190.
53. Bowie, J.; Mackintosh, C. G. & Mason, P. C. 1987. Effect of ivermectin and oxfendazole on shedding of larvae of the lungworm (*Dictyocaulus viviparus*) by red deer (*Cervus elaphus*). *New Zealand Veterinary Journal* 35: 8-10.
54. Bown, M. D.; Poppi, D. P. & Sykes, A. R. 1991a. The effect of post-ruminal infusion of protein or energy on the pathophysiology of *Trichostrongylus colubriformis* infection and body composition in lambs. *Australian Journal of Agricultural Research* 42: 253-267.
55. Bown, M. D.; Poppi, D. P. & Sykes, A. R. 1991b. Nitrogen transactions along the digestive tract of lambs concurrently infected with *Trichostrongylus colubriformis* and *Ostertagia circumcincta*. *British Journal of Nutrition* 66: 237-249.
56. Broadhurst, R. B. & Jones, W. T. 1978. Analysis of condensed tannins using acidified vanillin. *Journal of the Science of Food and Agriculture* 29: 788-794.
57. Brunsdon, R. V. 1980. Principles of helminth control. *Veterinary Parasitology* 6: 185-215.

58. Buddle, B. M.; Jowett, G.; Green, R. S.; Douch, P. G. C. & Risdon, P. L. 1992. Association of blood eosinophilia with the expression of resistance in Romney lambs to nematodes. *International Journal for Parasitology* 22: 955-960.
59. Buntinx, S. E.; Pond, K. R.; Fisher, D. S. & Burns, J. C. 1992. Evaluation of the Captec chromium controlled-release device for the estimation of faecal output by grazing sheep. *Journal of Animal Science* 70: 2243-2249.
60. Butterworth, A.E. 1984. Cell-mediated damage to helminths. *Advances in Parasitology* 23: 143-211.
61. Bye, K. & Halvorsen, O. 1983. Abomasal nematodes of the Svalbard reindeer (*Rangifer tarandus platyrhynchus* Vrolik). *Journal of Wildlife Diseases* 19(2): 101-105.
62. Callinan, A. P. L. & Westcott, J. M. 1986. Vertical distribution of trichostrongylid larvae on herbage and in soil. *International Journal for Parasitology* 16(3): 241-244.
63. Cameron, T. W. M. 1931. On two species of nematodes from the Scottish red deer. *Journal of Helminthology* 9: 213-216.
64. Catchpole, J. & Harris, T. J. 1989. Interaction between coccidia and *Nematodirus battus* in lambs on pasture. *Veterinary Record* 124: 603-605.
65. Challies, C. N. 1985. Establishment, control and commercial exploitation of wild deer in New Zealand. In: *Biology of Deer Production*, P.F. Fennessy, K.R. Drew (Eds). The Royal Society of New Zealand, Bulletin 22: 23-26.
66. Charleston, W. A. G. 1980. Lungworm and lice of the red deer (*Cervus elaphus*) and the fallow deer (*Dama dama*) - a review. *New Zealand Veterinary Journal* 28(8): 150-152.
67. Chiquette, J.; Cheng, K. J.; Costerton, J. W. & Millian, L. P. 1988. Effects of tannins on the digestibility of two isosynthetic strains of birdsfoot trefoil (*Lotus corniculatus* L.) using *in-vitro* and *in-sacco* techniques. *Canadian Journal of Animal Science* 68: 751-760.
68. Christensen, C.M.; Barnes, E.H. & Nansen, P. 1997. Experimental *Oesophagostomum dentatum* infections in the pig: worm populations at regular intervals during trickle infections with three dose levels of larvae. *Parasitology* 115: 545-552.
69. Connan, R. M. 1996. Observations on the epidemiology of gastrointestinal nematodes of farmed red deer in central southern England. *Veterinary Record* 139: 228-232.

70. Connan, R. M. 1991. Type II ostertagiosis in farmed red deer. *Veterinary Record* 128: 233-235.
71. Connan, R.M. 1997. Hypobiosis in the ostertagids of red deer and the efficacy of ivermectin and fenbendazole against them. *Veterinary Record* 140:203-205.
72. Conti, J. A. & Howerth, E. W. 1987. Ostertagiosis in a white-tailed deer due to *Ostertagia ostertagi*. *Journal of Wildlife Diseases* 23(1): 159-162.
73. Coop, R.L. & Holmes, P.H. 1996. Nutrition and parasite interaction. *International Journal for Parasitology* 26 (8/9): 951-962.
74. Coop, R. L.; Sykes, A. R. & Angus, K. W. 1977. The effect of a daily intake of *Ostertagia circumcincta* larvae on body weight, food intake and concentration of serum constituents in sheep. *Research in Veterinary Science* 23: 76-83.
75. Coop, R. L.; Sykes, A. R. & Angus, K. W. 1982. The effect of three levels of intake of *Ostertagia circumcincta* larvae on growth rate, food intake and body composition in lambs. *Journal of Agricultural Science, Cambridge* 98: 274-255.
76. Coop, R. L.; Huntley, J. F. & Smith, W. D. 1995. Effect of dietary protein supplementation on the development of immunity to *Ostertagia circumcincta* in growing lambs. *Research in Veterinary Science* 59: 24-29.
77. Cope, W. A.; Bell, T. A. & Smart, W. W. G. 1971. Seasonal changes in an enzyme inhibitor and tannin content in *Sericea lespedeza*. *Crop Science* 11: 893-895.
78. Corrigan, W. 1985. *Dictyocaulus viviparus* infection in red deer. In: *Biology of Deer Production*. P.F. Fennessy, K.R. Drew (Eds). The Royal Society of New Zealand, Bulletin 22: 123-126.
79. Corrigan, W.; Easton, J. F. & Hamilton, W. J. 1980. *Dictyocaulus* infection in farmed red deer (*Cervus elaphus*). *Veterinary Record* 106: 335-339.
80. Corrigan, W.; Coutts, A. G. P.; Watt, C. F.; Hunter, A. R. & Munro, R. 1982. Experimental infections with *Dictyocaulus viviparus* in vaccinated and unvaccinated red deer. *Veterinary Record* 111: 179-184.
81. Corrigan, W.; Coutts, A. G. P. & Watt, C. F. 1986. Naturally occurring parasitic bronchitis in red deer: effect of vaccination using live bovine lungworm vaccine. *Veterinary Record* 118: 578-580.
82. Corrigan, W.; Coutts, A. G. P.; Watt, C. F.; Hunter, A. R. & Munro, R. 1988. Comparison by experimental infections in cattle of a *Dictyocaulus* species occurring naturally in red deer and a *Dictyocaulus* of bovine origin. *Veterinary Record* 122: 302-304.



83. Costigan, P. & Ellis, K. J. 1987. Analysis of faecal chromium from controlled release marker devices. *New Zealand Journal of Technology* 3: 89-92.
84. Cruickshank, G. J.; Poppi, D. P. & Sykes, A. R. 1985. Intake and duodenal proetin flow in early weaned lambs grazing white clover, lucerne, ryegrass and prairie grass. *Proceedings of the New Zealand Society of Animal Production* 45: 113-116.
85. Cruickshank, G. J.; Poppi, D. P. & Sykes, A. R. 1992. The intake, digestion and protein degradation of grazed herbage by early weaned lambs. *British Journal of Nutrition* 68: 349-364.
86. Crush, J. R. & Evans, J. P. M. 1990. Shoot growth and herbage element concentrations of Grasslands Puna chicory (*Cichorium intybus* L.) under varying soil pH. *Proceedings of the New Zealand Grassland Association* 51: 163-166.
87. Davidson, W. R.; McGhee, M. B.; Nettles, V. F. & Chappell, L. C. 1980. Haemonchosis in white-tailed deer in the southeastern United States. *Journal of Wildlife Diseases* 16(4): 499-508.
88. Davidson, W. R.; Crum, J. M.; Blue, J. L.; Sharp, D. W. & Phillips, J. H. 1985. Parasites, diseases and health status of sympatric populations of fallow deer and white-tailed deer in Kentucky. *Journal of Wildlife Diseases* 21(2): 153-159.
89. Davis, L. R.; Herlich, H. & Bowman, G. W. 1959. Studies on experimental concurrent infections of dairy calves with coccidia and nematodes. I *Eimeria spp* and the small intestine worm, *Cooperia punctata*. *American Journal of Veterinary Research* 20: 281-286.
90. Degen, A. A.; Becker, K.; Makkar, H. P. S. & Borowy, N. 1995. *Acacia saligna* as a fodder tree for desert livestock and the interaction of its tannins with fibre fractions. *Journal of Science of Food and Agriculture* 68: 65-71.
91. Deinum, B. 1966. Climate nitrogen and grass. Research into the influence of light intensity, temperature, water supply and nitrogen on the production and chemical composition of grass. *Mededelingen Landbouwhogeschool, Wageningen* 66: 91.
92. DelValle, J. & Yamada, T. 1990. Amino acids and amines stimulate gastrin release from canine antral G-cells via different pathways. *The Journal of Clinical Investigation* 85: 139-143.
93. Demarais, S.; Jacobson, H. A. & Guynn, D. C. 1983. Abomasal parasites as a health index for white-tailed deer in Mississippi. *Journal of Wildlife Management* 47(1): 247-252.
94. Distel, R. A. & Provenza, F. D. 1991. Experience early in life affects voluntary intake of blackbrush by goats. *Journal of Chemical Ecology* 17: 431-450.

95. Dobson, C. & Bawden, R. J. 1974. Studies on the immunity of sheep to *Oesophagostomum columbianum*: Effects of low-protein diet on resistance to infection and cellular reactions in the gut. *Parasitology* 69: 239-255.
96. Dobson, R.J.; Waller, P.J. & Donald, A.D. 1990a. Population dynamics of *Trichostrongylus colubriformis* in sheep: the effect of infection rate on loss of adult parasites. *International Journal for Parasitology* 20(3): 359-363.
97. Domingue, B. M. F.; Dellow, D. W.; Wilson, P. R. & Barry, T. N. 1991a. Comparative digestion in deer, goats, and sheep. *New Zealand Journal of Agricultural Research* 34(1): 45-53.
98. Domingue, B. M. F.; Dellow, D. W.; Wilson, P. R. & Barry, T. N. 1991b. Nitrogen metabolism, rumen fermentation, and water absorption in red deer, goats and sheep. *New Zealand Journal of Agricultural Research* 34: 391-400.
99. Donaldson, J.; van Houtert, M.F.J.; Sykes, A.R. 1997. The effect of protein supply on the periparturient parasite status of the mature ewe. *Proceedings of the New Zealand Society of Animal Production* 57, 186-189.
100. Doster, G. L. & Friend, M. 1971. Spiculopteragia (Nematoda) from deer in North America. *Journal of Parasitology* 57(3): 468.
101. Douglas, G. B.; Donkers, P.; Foote, A. G. & Barry, T. N. 1993. Determination of extractable and bound condensed tannins in forage species. *Proceedings of the XVII International Grassland Congress, XVII*: 204-206.
102. Douglas, G.B.; Wang, Y.; Waghorn, G.C.; Barry, T.N.; Purchas, R.W.; Foote, A.G. & Wilson, G.F. 1995. Liveweight gain and wool production of sheep grazing *Lotus corniculatus* and lucerne (*Medicago sativa*). *New Zealand Journal of Agricultural Research* 38: 95-104.
103. Dove, H. 1993. Advances in the estimation of intake and diet selection in the grazing animal. In: *Recent Advances in Animal Nutrition in Australia*, D.J. Farrell (Ed). University of New England, Armidale, Australia: 34-44.
104. Dove, H.; Mayes, R. W.; Lamb, C. S. & Ellis, K. J. 1991. Evaluation of an intraruminal controlled release device for estimating herbage intake using synthetic and plant cuticular wax alkanes. *Proceedings of the 3rd International Symposium on the Nutrition of Herbivores*: pp?-?
105. Drew, K. R. 1985. Meat production from farmed deer. In: *Biology of Deer Production*. P.F. Fennessy, K.R. Drew (Eds). The Royal Society of New Zealand, Bulletin 22: 285-290.

106. Drew, K. R. 1992. Venison and other deer products. In: The Biology of Deer. Proceedings of an International Symposium on the Biology of Deer. R.D. Brown (Ed). Springer-Verlag, New York. USA: 225-232.
107. Drew, K. R. & Hogg, B. W. 1990. Comparative carcass production from red, wapiti and fallow deer. Proceedings of the Australian Association of Animal Breeding and Genetics 8: 491-496.
108. Drew, K. R. & Suttie, J. M. 1982. Quality meat from farmed deer. Proceedings of the New Zealand Deer Farmers Association Annual Conference No. 7.: 39-47.
109. Drozd, J. 1966. Studies on helminths and helminthiasis in cervidae II. The helminth fauna in Cervidae in Poland. Acta Parasitologica Polonica 14: 1-13.
110. Dunn, A. M. 1965. The gastrointestinal helminths of wild ruminants in Britain. Parasitology 55: 739-745.
111. English, A. W. 1991. Gastrointestinal helminthosis in farmed fallow deer. Proceedings of a Deer Course for Veterinarians. Deer Branch of the New Zealand Veterinary Association 8: 237-242.
112. Epe, C.; Vonsamsonhimmelstjerna, G.; Stoye, M. & Schnieder, T. 1996. Comparative molecular characterization of *Dictyocaulus viviparus* and *Dictyocaulus eckerti*. Berliner und Münchener Tierärztliche Wochenschrift 109(6-7): 227-231.
113. Eve, J. H. & Kellogg, F. E. 1977. Management implications of abomasal parasites in southeastern white-tailed deer. Journal of Wildlife Management 41(2): 169-177.
114. Eysselein, V.E.; Kovacs, T.O.G.; Kleibeuker, J.H.; Maxwell, V.; Reedy, T. & Walsh, J.H. 1992. Regulation of gastric acid secretion by gastrin in duodenal ulcer patients and healthy subjects. Gastroenterology 102: 1142-1148.
115. Faichney, G.J. 1972. An assessment of chromic oxide as an indigestible marker for digestion studies in sheep. Journal of Agricultural Science, Cambridge 79: 493-499.
116. Faichney, G.J. 1975. The use of markers to partition digestion within the gastrointestinal tract of ruminants. In: Digestion and Metabolism of the Ruminant, Eds. I.W. McDonald & A.C.I. Warner. Proceedings of the IV International Symposium on Ruminant Physiology, The University of New England Publishing Unit.
117. Feeny, P. 1976. Plant apparency and chemical defense. In: Recent Advances in Phytochemistry. Biochemical Interaction Between Plants and Insects, J.W. Wallace, R.L. Mansell (Eds). Plenum Press, New York, USA: 10 (pp1-40).

118. Fennessy, P. F. & Mackintosh, C. G. 1992. Hybridisation of red deer and Pere Davids deer. Proceedings of a Deer Course for Veterinarians. Deer Branch of the New Zealand Veterinary Association 9: 181-186.
119. Fennessy, P. F. & Milligan, K. E. 1987. Grazing management of red deer. In: Livestock Feeding on Pasture. A.M. Nicol (Ed). New Zealand Society of Animal Production Occasional Publication 10: 111-118.
120. Fennessy, P. F. & Pearse, A. J. 1990. The relative performance of Canadian wapiti and their hybrids. Proceedings of the Australian Association of Animal Breeding and Genetics 8: 497-501.
121. Fennessy, P. F.; Moore, G. H. & Corson, I. D. 1981. Energy requirements of red deer. Proceedings of the New Zealand Society of Animal Production 41: 167-173.
122. Fletcher, T.J. 1982. Management problems and disease in farmed deer. Veterinary Record 111(11).
123. Fennessy, P. F.; Greer, G. J. & Bass, J. J. 1982. Progeny test of selected lean and fat rams. Proceedings of the New Zealand Society of Animal Production 42: 137-140.
124. Foreyt, W. & Trainer, D. O. 1970. Experimental haemonchosis in white-tailed deer. Journal of Wildlife Diseases 6: 35-42.
125. Forrester, D. J.; Taylor, W. J. & Nair, K. P. C. 1974. Strongyloidosis in captive white-tailed deer. Journal of Wildlife Diseases 10: 11-17.
126. Fox, M.T. 1993. Pathophysiology of infection with *Ostertagia ostertagi* in cattle. Veterinary Parasitology 46: 143-158.
127. Fox, M. T.; Gerrelli, D.; Pitt, S. R.; Jacobs, D. E.; Hart, I. C. & Simmonds, A. D. 1987. Endocrine effects of a single infection with *Ostertagia ostertagi* in the calf. International Journal for Parasitology 17: 1181-1185.
128. Fox, M. T.; Gerrelli, D.; Pitt, S. R.; Jacobs, D. E.; Gill, M. & Gale, D. L. 1989a. *Ostertagia ostertagi* infection in the calf: effects of a trickle challenge on appetite, digestibility, rate of passage of digesta and liveweight gain. Research in Veterinary Science 47: 294-298.
129. Fox, M.T.; Gerrelli, D.; Shivalkar, P. & Jacobs, D.E. 1989b. Effect of omeprazole treatment on feed intake and blood gastrin and pepsinogen levels in the calf. Research in Veterinary Science 47: 280-282.
130. Freudenberger, D. O.; Toyokawa, K.; Ball, A. J.; Suttie, J. M. & Barry, T. N. 1994a. Seasonality in digestion and rumen metabolism in red deer (*Cervus elaphus*) fed a forage diet. British Journal of Nutrition 71: 489-499.

131. Freudenberger, D. O.; Burns, C. J.; Toyokawa, K. & Barry, T. N. 1994b. Digestion and rumen metabolism of red clover and perennial ryegrass/white clover forages by red deer. *Journal of Agricultural Science, Cambridge*, in press.
132. GIB. 1996. Deer Quality Assurance On-Farm Programme Operating Standards. New Zealand Game Industry Board.
133. GIB. 1997. New Zealand Game Industry Board Annual Report.
134. Gladden, N. 1981. Deer population and health survey. New Zealand Ministry of Agriculture and Fisheries AgLink, FPP 259.
135. Guild, J. 1993. An introduction to New Zealand deer farming. In: A Salute to World Deer Farming, Proceedings of the First World Deer Congress. I. Woodhouse (Ed). New Zealand Deer Farmers Association. The Raven Press, Wellington, New Zealand: 33-35.
136. Hagerman, A. E. & Robbins, C. T. 1993. Specificity of tannin-binding salivary proteins relative to diet selection by mammals. *Canadian Journal of Zoology* 71: 628-633.
137. Haigh, J. C. & Hudson, R. J. 1993. Parasitic diseases. In: Farming Wapiti and Red Deer. J.C. Haigh, R.J. Hudson (Eds). Mosby-Year Book Inc, St Louis, Missouri: 255-297.
138. Hamilton, W. J. & Blaxter, K. L. 1981. Growth of red deer stags under farm conditions. *Journal of Agricultural Science, Cambridge* 97: 329-334.
139. Hamilton, W. J.; Sibbald, M. A. & Feist, D. 1995. The effect of sward height on the liveweight gain of farmed yearling red deer (*Cervus elaphus*) stags. *Grass and Forage Science* 50(4): 399-404.
140. Hanley, T. A.; Robbins, C. T.; Hagerman, A. E. & McArthur, C. 1992. Predicting digestible protein and digestible dry matter in tannin-containing forages consumed by ruminants. *Ecology* 73: 537-541.
141. Hansky, J. & Cain, M. D. 1969. Radioimmunoassay of gastrin in human serum. *Lancet* 2: 1388-1390.
142. Hart, S. P. & Sahlu, T. 1993. Mohair production and body-weight gains of yearling Angora goats grazing forages with different tannin levels. Proceedings of the XVII International Grassland Congress: 575-576.
143. Heath, M. F. & Connan, R. M. 1991. Interaction of *Ostertagia* and *Nematodirus* species in sheep and the potential of serum fructosamine determination in monitoring gastrointestinal parasitism. *Research in Veterinary Science* 51: 322-326.

144. Hendrickson, R. E.; Poppi, D. P. & Minson, D. J. 1981. The voluntary intake, digestibility and retention time by cattle and sheep of stem and leaf fractions of a tropical legume (*Lablab purpureus*). Australian Journal of Agricultural Research 32: 389-398.
145. Hendriksen, S. A. 1965. En forbedret teknik ved undersogelse for lungeormelarver i faeces. Nordisk Veterinaemedivin 17: 446-454.
146. Heydon, M. J.; Sibbald, A. M.; Milne, J. A.; Brinklow, B. R. & Loudon, A. S. 1993. I. The interaction of food availability and endogenous physiological cycles on the grazing ecology of red deer hinds (*Cervus elaphus*). Functional Ecology 7: 216-222.
147. Hoberg, E. P.; Lichtenfels, J. R. & Pilitt, P. A. 1993. Comparative morphology of *Ostertagia mossi* and *Ostertagia dikmansi* (Trichostrongylidae) from *Odocoileus virginianus* and comments on other *Ostertagia* spp. from the Cervidae. Systematic Parasitology 24: 111-127.
148. Hodgson, J. 1977. Factors limiting herbage intake by the grazing animal. Proceedings of the International Meeting on Animal Production from Temperate Grassland, Dublin: 70-78.
149. Hodgson, J. 1981. Variations in the surface characteristics of the sward and the short-term rate of herbage intake by calves and lambs. Grass and Forage Science 36: 49-57.
150. Hodgson, J. 1982. Ingestive Behaviour. In: Herbage Intake Handbook, J.D. Leaver (Ed). The British Grassland Society, Hurley, Maidenhead, Berkshire, United Kingdom: 113-138.
151. Hodgson, J. 1985. The control of herbage intake in the grazing ruminant. Proceedings of the Nutrition Society 44: 339-346.
152. Hodgson, J. 1990. Grazing Management, Science into Practice. Longman Books in Agriculture, New Zealand.
153. Hogan, J.P.; Weston, R.H. & Lindsay, J.R. 1970. The effects of ruminal digestion of forages on the quantities of amino-acids supplied to the sheep. Proceedings of the XI International Grassland Congress, Ed. M.J.T. Norman, 706-709.
154. Holmes, P. H. 1985. Pathogenesis of trichostrongylosis. Veterinary Parasitology 18: 89-101.
155. Holmes, P. H. 1993. Interactions between parasites and animal nutrition: the veterinary consequences. Proceedings of the Nutrition Society 52: 113-120.

156. Holmes, P.H. & McLean, J.M. 1971. The pathophysiology of ovine ostertagiasis: a study of the changes in plasma protein metabolism following single infections. *Research in Veterinary Science* 12: 265-271.
157. Hoskin, S. O.; Stafford, K. J. & Barry, T. N. 1995. Digestion, rumen fermentation and chewing behaviour of red deer fed chicory and perennial ryegrass. *Journal of Agricultural Science, Cambridge* 124: 289-295.
158. Hunt, W. F. & Hay, J. M. 1990. A photographic technique for assessing the pasture species performance of grazing animals. *Proceedings of the New Zealand Grassland Association* 51: 191-196.
159. Inderbitzen, F. 1976. Recovery of lungworm from bovine lung by perfusion. University of Zurich: PhD Thesis.
160. Jagusch, K. T.; Rattray, P. V.; Petch, M. & Vlassoff, A. 1980. Effects of level of nutrition and drenching strategy on pasture utilisation during autumn by set-stocked hoggets infected with gastrointestinal nematodes. *Proceedings of the New Zealand Society of Animal Production* 40: 12-17.
161. Jansen, J. & Borgesteede, F. H. M. 1990. De *Dictyocaulus*-soorten longwormen van rund en hertachtigen in Nederland. *Tijdschrift Voor Diergeneeskunde* 115(4): 155-158.
162. Jarrett, W. F. H. & Sharp, N. C. C. 1963. Vaccination against parasitic disease: reactions in vaccinated and immune hosts in *Dictyocaulus viviparus* infection. *Journal of Parasitology* 49(2): 177-189.
163. Jennings, F.W.; Armour, J.; Lawson, D.D. & Roberts, R. 1966. Experimental *Ostertagia ostertagi* infections in calves: studies with abomasal cannulas. *American Journal of Veterinary Research* 27: 1249.
164. Johnston, J. T.; Familton, A. S.; McAnulty, R. & Sykes, A. R. 1984. Pathogenicity of *O.circumcincta*, *O.ostertagia* and *H.contortus* in weanling stag fawns (*Cervus elaphus*). *New Zealand Veterinary Journal* 32: 177-179.
166. Jones, W. T. & Mangan, J. L. 1977. Complexes of the condensed tannins of sainfoin (*Onobrychis viciifolia* Scop.) with fraction 1 leaf protein and with submaxillary mucoprotein, and their reversal with polyethylene glycol and pH. *Journal of the Science of Food and Agriculture* 28: 126-136.
167. Jones, W. T.; Anderson, L. B. & Ross, M. D. 1973. Bloat in cattle. XXXIX. Detection of protein precipitants (flavolans) in legumes. *New Zealand Journal of Agricultural Research* 16: 441-446.

168. Kambara, T.; McFarlane, R. G.; Abell, T. T.; McAnulty, R. W. & Sykes, A. R. 1993. The effect of age and dietary protein on the immunity and resistance in lambs vaccinated with *Trichostrongylus colubriformis*. *International Journal for Parasitology* 23: 471-476.
169. Kay, R. N. B. 1979. Seasonal changes of appetite in deer and sheep. *Animal Research Council Review* 5: 13-15.
170. Kay, R. N. B. & Staines, B. W. 1981. The nutrition of the red deer (*Cervus elaphus*). *Nutrition Abstracts & Reviews B* 51(9): 601-622.
171. Kay, R. N. B.; Engelhardt, W. V. & White, R. G. 1980. The digestive physiology of wild ruminants. In: *Digestive Physiology and Metabolism in Ruminants*. Y.Ruckebusch & P.Thivend (Eds). MTP Press: Lancaster, United Kingdom: 743-761.
172. Kemp, P. D. 1996. Using research results to achieve targets on a deer farm. *Proceedings of the Massey University Deer Farmers Conference* 31-38.
173. Kennedy, P. M. & Murphy, M. R. 1988. The nutritional implications of differential passage of particles through the ruminant alimentary tract. *Nutrition Research Reviews* 1: 189-208.
174. Kenney, P. A.; Black, J. L. & Colebrook, W. F. 1984. Factors affecting diet selection by sheep. III. Dry matter content and particle length of forage. *Australian Journal of Agricultural Research* 35: 831-838.
175. Ketelaars, J. J. M. H. & Tolkamp, B. J. 1992. Toward a new theory of feed intake regulation in ruminants 1. causes of differences in voluntary feed intake: critique of current reviews. *Livestock Production Science* 30: 269-296.
176. Kimambo, A.E.; MacRae, J.C.; Walker, A.; Watt, C.F. & Coop, R.L. 1988. Effect of prolonged subclinical infection with *Trichostrongylus colubriformis* on the performance and nitrogen metabolism of growing lambs. *Veterinary Parasitology* 28: 191-203.
177. Knapp, S. E. 1964. Relationship of different species of forage to the survival and infectivity of *Haemonchus contortus* in lambs. *Journal of Parasitology* 50: 144-148.
178. Knox, M. & Steel, J. 1996. Nutritional enhancement of parasite control in small ruminant production systems in developing countries of South-East Asia and the Pacific. *International Journal for Parasitology* 26 (8/9): 963-970.
179. Krishna, H.; Kemp, P. D. & Newton, S. D. 1990. Necton sulla - a preliminary agronomic evaluation. *Proceedings of the New Zealand Grassland Association* 51: 157-159.



180. Kroonen, J. E. G. M.; Verstegen, M. W. A.; Boon, J. H. & Van der hel, W. 1986. Effect of infection with lungworms (*Dictyocaulus viviparus*) on energy and nitrogen metabolism in growing calves. *British Journal of Nutrition* 55: 351-360.
181. Kusmartono. 1996. Nutritive value of chicory (*Cichorium intybus*) as a special purpose forage for deer production. PhD Thesis, Massey University, Palmerston North, New Zealand.
182. Kusmartono; Barry, T.N.; Wilson, P.R.; Kemp, P.D. & Stafford, K.J. 1996a. Effects of grazing chicory (*Cichorium intybus*) and perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture upon the growth and voluntary feed intake of red and hybrid deer during lactational and post-weaning growth. *Journal of Agricultural Science, Cambridge* 127:387-401.
183. Kusmartono; Shimada, A.; Stafford, K.J. & Barry, T.N. 1996b. Intra-ruminal particle size reduction in deer fed fresh perennial ryegrass (*Lolium perenne*) or chicory (*Cichorium intybus*). *Journal of Agricultural Science, Cambridge* 127:525-531.
184. Kyriazakis, I.; Anderson, D. H.; Coop, R. L. & Jackson, F. 1996. The pathophysiology and development of immunity during long-term subclinical infection with *Trichostrongylus colubriformis* of sheep receiving different nutritional treatments. *Veterinary Parasitology* 65: 41-54.
185. Laca, E. A.; Ungar, E. D.; Seligman, N. & Demment, M. W. 1992. Effects of sward height and bulk density on bite dimensions of cattle grazing homogeneous swards. *Grass and Forage Science* 47: 91-102.
186. Lancaster, M. B. & Andrews, S. J. 1991. Observations on the output of nematode eggs in faeces and on the subsequent pasture infestation with third stage larvae produced by a herd of farmed red deer (*Cervus elaphus*). *Veterinary Record* 129(25/26): 549-551.
187. Langer, R. H. M. 1990. Pasture plants. In: Pastures. Their ecology and management. R.H.M. Langer (Ed). Oxford University Press, Oxford, United Kingdom: 39-74.
188. Lawton, D.E.B. 1995. Abomasal secretion in parasitised sheep. PhD Thesis, Massey University, Palmerston North, New Zealand.
189. Lawton, D.E.B.; Reynolds, G.W.; Hodgkinson, S.M.; Pomroy, W.E. & Simpson, H.V. 1996. Infection of sheep with adult and larval *Ostertagia circumcincta*: effects on abomasal pH and serum gastrin and pepsinogen. *International Journal for Parasitology* 10: 1063-1074.

190. Leaver, J. D. 1985. Effects of supplements on herbage intake and performance. J. Frame (Ed). Proceedings of the British Grassland Society Occasional Publication 19: 79-88.
191. Lichtenfels, J. R. & Hoberg, E. P. 1993. The systematics of nematodes that cause ostertagiasis in domestic and wild ruminants in North America: an update and a key to species. *Veterinary Parasitology* 46: 33-53.
192. Lowther, W. L.; Manley, T. R. & Barry, T. N. 1987. Condensed tannin concentrations in *Lotus corniculatus* and *L. pedunculatus* cultivar grown under low soil fertility conditions. *New Zealand Journal of Agricultural Research* 30: 23-25.
193. McAllum, H. J. F. 1976. Animal health and deer farming. Proceedings of the New Zealand Society of Animal Production; Occasional publication No5: 15-19.
194. McGhee, M. B. 1981. Failure of white-tailed deer (*Odocoileus virginianus*) to serve as experimental hosts for *Ostertagia ostertagi* (Stiles, 1892). *Journal of Parasitology* 67(6): 969-970.
195. McCall, D. G.; Townsley, R. J.; Bircham, J. S. & Sheath, G. W. 1986. The interdependence of animal intake, pre-and post-grazing pasture mass and stocking density. Proceedings of the New Zealand Grassland Association 47: 255-261.
196. McDiarmid, A. 1975. Some disorders of wild deer in the United Kingdom. *Veterinary Record* 97: 6-9.
197. McKellar, Q. A. & Benchaoui, H. A. 1996. Avermectins and milbemycins - a review. *Journal of Veterinary Pharmacology and Therapeutics* 19(5): 331-351.
198. McKellar, Q.A.; Duncan, J.L.; Armour, J.; Lindsay, F.E.F. & McWilliam. 1985. Further studies on the response to transplanted adult *Ostertagia ostertagi* in calves. *Research in Veterinary Science* 42: 29-34.
199. McKenna, P. B.; Charleston, W. A. G. & Hughes, P. L. 1981. *Cooperia pectinata* (Nematoda: Trichostrongylidae) in New Zealand. *New Zealand Veterinary Journal* 29: 26-27.
200. Mackintosh, C. G. 1992. Observations on the relative susceptibility to disease of different species of deer farmed in New Zealand. In: *The Biology of Deer. Proceedings of an International Symposium on the Biology of Deer.* R.D. Brown (Ed). Springer-Verlag, New York. USA: 113-119.
201. Mackintosh, C. G. & Mason, P. C. 1985. Anthelmintics and lungworm in red deer. In: *Biology of deer production.* P.F. Fennessy, K.R. Drew (Eds). The Royal Society of New Zealand, Bulletin 22: 131-133.

202. Mackintosh, C. G.; Mason, P. C.; Bowie, J. Y. & Beatson, N. S. 1984. Anthelmintics against lungworm (*Dictyocaulus viviparus*) in red deer (*Cervus elaphus*). Proceedings of a Deer Course for Veterinarians. Deer Branch of the New Zealand Veterinary Association 1: 69-77.
203. Mackintosh, C. G.; Mason, P. C.; Manley, T.; Baker, K. & Littlejohn, R. 1990a. Efficacy and pharmacokinetics of febantel and ivermectin in red deer (*Cervus elaphus*). New Zealand Veterinary Journal 38(3): 112-113.
204. Mackintosh, C. G.; Mason, P. C. & Taylor, M. 1990b. A pilot study of the efficacy of topical ivermectin against lungworm in young red deer (*Cervus elaphus*). New Zealand Veterinary Journal 38(3): 112-113.
205. Mackintosh, C. G.; Waldrup, K.; Labes, R. & Taylor, T. 1993. Efficacy of Ivermectin injection and moxidectin pour-on formulations in young red deer (*Cervus elaphus*). Proceedings of a Deer Course for Veterinarians. Deer Branch of the New Zealand Veterinary Association 10: 143-150.
206. McLeod, M. N. 1974. Plant tannins - Their role in forage quality. Nutrition Abstracts and Reviews 44: 905-913.
207. McNabb, W. C.; Waghorn, G. C.; Barry, T. N. & Shelton, I. D. 1993. The effect of condensed tannins in *Lotus pedunculatus* upon the digestion and metabolism of methionine, cystine and inorganic sulphur in sheep. British Journal of Nutrition 70: 647-661.
208. MacRae, J. C. 1993. Metabolic consequences of intestinal parasitism. Proceedings of the Nutrition Society 52: 121-130.
209. MacRae, J. C.; Smith, J. S.; Sharman, G. A. M.; Corrigan, W. & Coop, R. L. 1982. Energy metabolism of lambs infected with *Trichostrongylus colubriformis*. European Association of Animal Production 29: 112-115.
210. Mangan, J. L.; Vetter, R. L.; Jordan, D. J. & Wright, P. C. 1976. The effect of condensed tannins of sainfoin (*Onobrychis viciaefolia*) on the release of soluble leaf protein into the food bolus of cattle. Proceedings of the Nutrition Society 35: 95A-97A.
211. Mason, P. C. 1977. Gastrointestinal parasitism in red deer. New Zealand Journal of Agricultural Science 11(4): 182-183.
212. Mason, P. C. 1979. Lungworm in red deer, biology, symptoms and control. New Zealand Ministry of Agriculture and Fisheries; AgLink, FPP 248.

213. Mason, P. C. 1981. Parasites of deer in New Zealand. Proceedings of a Deer Course for Veterinarians. Deer Advisory Panel of the New Zealand Veterinary Association:63-70.
214. Mason, P. C. 1984. Gastro-intestinal nematodes in farmed deer. Surveillance 11(1): 24-25.
215. Mason, P. C. 1985. Biology and control of the lungworm *Dictyocaulus viviparus* in farmed red deer in New Zealand. In: Biology of Deer Production. P.F. Fennessy, K.R. Drew (Eds). The Royal Society of New Zealand, Bulletin 22: 119-121.
216. Mason, P. 1994. Parasites of deer in New Zealand. New Zealand Journal of Zoology 21: 39-47.
217. Mason, P. C. & Beatson, N. S. 1985. Anthelmintic activity against *Dictyocaulus viviparus* in farmed red deer. In: Biology of Deer Production. P.F. Fennessy, K.R. Drew, (Eds). The Royal Society of New Zealand; Bulletin 22: 127-129.
218. Mason, P. C. & Gladden, N. R. 1983. Survey of internal parasitism and anthelmintic use in farmed deer. New Zealand Veterinary Journal 31(12): 217-220.
219. Mason, P. C. & McAllum, H. J. F. 1976. *Dictyocaulus viviparus* and *Elaphostrongylus cervi* in wapiti. New Zealand Veterinary Journal 24: 23.
220. Mason, P. C. & Moore, G. H. 1983. *Monezia expansa* in farmed red deer (*Cervus elaphus*). New Zealand Veterinary Journal 31: 226.
221. Mason, P. C.; Mackintosh, C. G.; Alexander, A.; Taylor, M. J. & Booth, D. 1990. Activity of Ivomec pour-on against lungworm in deer. Bulletin de la Societe Francaise de Parasitologie 8(2): 1101.
222. Mayes, R.W.; Lamb, C.S. & Colgrove, Patricia, M. 1986. The use of dosed and herbage n-alkanes as markers for the determination of herbage intake. Journal of Agricultural Science, Cambridge 107: 161-170.
223. Meissner, H. H.; Smuts, M.; van Niekerk, W. A. & Acheampong-Boateng, O. 1993. Rumen ammonia concentrations, and non-ammonia nitrogen passage to and apparent absorption from the small intestine of sheep ingesting subtropical, temperate and tannin-containing forages. South African Journal of Animal Science 23(3): 92-97.
224. Mertens, D. R. & Ely, L. O. 1982. Relationship of rate and extent of digestion to forage utilization - A dynamic model. Journal of Animal Science 54: 895-905.
225. Middelberg, A. 1994. Efficacy of moxidectin pour on in young red deer. Proceedings of a Deer Course for Veterinarians. Deer Branch of the New Zealand Veterinary Association 11: 203-205.

226. Milne, J. A. 1980. Comparative digestive physiology and metabolism of the red deer and the sheep. *Proceedings of the New Zealand Society of Animal Production* 40: 151-157.
227. Milne, J. A.; MacRae, J. C.; Spence, A. M. & Wilson, S. 1978. A comparison of the voluntary intake and digestion of a range of forages at different times of the year by the sheep and the red deer (*Cervus elaphus*). *British Journal of Nutrition* 40(2): 347-357.
229. Min, B.R.; Barry, T.N.; Wilson, P.R. & Kemp, P.D. 1997. The effects of grazing chicory (*Cichorium intybus*) and birdsfoot trefoil (*Lotus corniculatus*) on venison and velvet by weaner red and hybrid deer. *New Zealand Journal of Agricultural Research* 40: 335-347.
230. Minson, D. J. 1981. Nutritional differences between tropical and temperate pastures. In: *World Animal Science, B1, Grazing Animals*. F.H.W. Morley (Ed). Elsevier Publishing Company, Amsterdam, Holland: 143-157.
231. Minson, D. J. 1982. Effects of chemical and physical composition of herbage eaten upon intake. In: *Nutritional Limits to Animal Production from Pastures*, J.B. Hacker (Ed). Commonwealth Agricultural Bureaux, Farnham Royal, United Kingdom: 167-182.
232. Mitchell, R. J.; Hodgson, J. & Clark, D. A. 1991. The effect of varying leafy sward height and bulk density on the ingestive behaviour of young deer and sheep. *Proceedings of the New Zealand Society of Animal Production* 51: 159-165.
233. Moseley, G. 1981. Herbage quality and physical breakdown in the foregut of sheep. *Proceedings of the New Zealand Society of Animal Production*; 41: 142-151.
234. Moss, R. A. & Vlassoff, A. 1993. Effect of herbage species on gastrointestinal roundworm populations and their distribution. *New Zealand Journal of Agricultural Research* 36: 371-375.
235. Muir, P.D. & Sykes, A. R. 1988. Effect of winter nutrition on antler development in red deer (*Cervus elaphus*): a field study. *New Zealand Journal of Agricultural Research* 31(2): 145-150.
236. Mylrea, G. E.; Mulley, R. C. & English, A. W. 1991. Gastrointestinal helminthosis in fallow deer (*Dama dama*) and their response to treatment with anthelmintics. *Australian Veterinary Journal* 68(2): 74-75.
237. Niezen, J.H. 1996. The effect of herbage species on internal parasite dynamics in sheep. PhD Thesis, Massey University, Palmerston North, New Zealand.

238. Niezen, J.; Barry, T. N.; Hodgson, J.; Wilson, P. R.; Ataja, A.; Parker, W. J. & Holmes, C. W. 1993a. Growth responses in red deer calves and hinds grazing red clover, chicory or perennial ryegrass/white clover swards during lactation. *Journal of Agricultural Science, Cambridge* 121: 255-263.
239. Niezen, J. H.; Charleston, W. A. G.; Hodgson, J. & Waghorn, T. S. 1993b. Effect of four grass species on lamb parasitism and growth. *Proceedings of the New Zealand Grassland Association* 55: 203-206.
240. Niezen, J., Barry, T. N., Hodgson, J., Wilson, P. R., Ataja, A., Parker, W. J. & Holmes, C. W. 1993c. Growth responses in red deer calves and hinds grazing red clover, chicory or perennial ryegrass/white clover swards during lactation. *Journal of Agricultural Science, Cambridge* 121: 255-263.
241. Niezen, J., Waghorn, T. S., Waghorn, G. C. & Charleston, W. A. G. 1993d. Internal parasites and lamb production - a role for plants containing condensed tannins? *Proceedings of the New Zealand Society of Animal Production* 53: 235-238.
242. Niezen, J. H.; Waghorn, G. C.; Charleston, W. A. G. & Waghorn, T. S. 1994a. Lamb growth, faecal egg count and worm burdens when fed *Lotus pedunculatus* or ryegrass and challenged with *Ostertagia* and *Trichostrongylus*. *New Zealand Journal of Zoology* 21: 109.
243. Niezen, J.; Waghorn, T. & Rufaut, K. 1994b. Lamb weight gain and faecal egg count when grazing a range of herbage. *New Zealand Journal of Zoology* 21: 108.
244. Niezen, J. J.; Waghorn, T. S.; Raufaut, K.; Robertson, H. A. & McFarlane, R. G. 1994c. Lamb weight gain and faecal egg count when grazing one of seven herbage and dosed with larvae for six weeks. *Proceedings of the New Zealand Society of Animal Production* 54: 15-18.
246. Niezen, J. H.; Waghorn, T. S.; Charleston, W. A. G. & Waghorn, G. C. 1995. Growth and gastrointestinal nematode parasitism in lambs grazing either lucerne (*Medicago sativa*) or sulla (*Hedysarum coronarium*) which contains condensed tannins. *Journal of Agricultural Science, Cambridge* 125: 281-289.
247. Niezen, J. H.; Charleston, W. A. G.; Hodgson, J.; MacKay, A. D. & Leathwick, D. M. 1996. Controlling internal parasites in grazing ruminants without recourse to anthelmintics: approaches, experience and prospects. *International Journal for Parasitology* 26(8/9): 983-992.
248. Orr, M. 1991. A review of respiratory disease in New Zealand deer. *Surveillance* 18(2): 17-18.

249. Orr, M. B.; Mackintosh, C. G. & Walker, G. 1990. Chronic illthrift in elk - a review of 1988 Invermay Animal Health laboratory cases. *Surveillance* 17: 11-12.
250. Otway, W. 1992. Establishment of Mesopotamian fallow deer in New Zealand and their hybridisation with European fallow deer. In: *The Biology of Deer. Proceedings of an International Symposium on the Biology of Deer*. R.D. Brown (Ed). Springer-Verlag, New York. USA: 400.
251. Otway, W. 1992. Hybridisation of Pere Davids and red deer. In: *The Biology of Deer, Proceedings of an International Symposium on the Biology of Deer*. R.D. Brown (Ed). Springer-Verlag, New York. USA: 401.
252. Ozanne, P. G.; Purser, D. B.; Howes, K. M. W. & Southey, I. 1976. Influence of phosphorous content on feed intake and weight gain in sheep. *Australian Journal of Experimental Agriculture and Animal Husbandry* 16: 353-360.
253. Parker, W. J. 1990. Application of intraruminal chromium controlled release capsules to the measurement of herbage intake of sheep at pasture. PhD Thesis, Massey University, Palmerston North, New Zealand.
254. Parker, W. J.; McCutcheon, S. N. & Carr, D. H. 1989. Effect of herbage type and level of intake on the release of chromic oxide from intraruminal controlled release capsules in sheep. *New Zealand Journal of Agricultural Research* 32: 537-546.
255. Parker, W. J.; McCutcheon, S. N. & Wickham, G. A. 1991. Effect of administration and ruminal presence of chromic oxide controlled release capsules on herbage intake of sheep. *New Zealand Journal of Agricultural Research* 34: 193-200.
256. Parkins, J.J. & Holmes, P.H. 1989. Effects of gastrointestinal helminth parasites on ruminant nutrition. *Nutrition Research Reviews* 2: 227-246.
257. Parkins, J. J.; Holmes, P. H. & Bremner, K. C. 1973. The pathophysiology of ovine ostertagiasis. Some nitrogen balance and digestibility studies. *Research in Veterinary Science* 14: 21-28.
258. Pearse, A. J. 1992. Farming of wapiti and wapiti hybrids in New Zealand. In: *The Biology of Deer. Proceedings of an International Symposium on the Biology of Deer*. R.D. Brown (Ed). Springer-Verlag, New York. USA: 173-179.
259. Pollock, A. M. 1975. Seasonal changes in appetite and sexual condition in red deer stags maintained on a six month photoperiod. *Journal of Physiology* 244(1): 95P-96P.
260. Pomroy, W. E. & Charleston, W. A. G. 1989. Failure of young goats to acquire resistance to *Haemonchus contortus*. *New Zealand Veterinary Journal* 37: 23-26.

261. Poppi, D. P.; MacRae, J. C.; Brewer, A. & Coop, R. L. 1986. Nitrogen transactions in the digestive tract of lambs exposed to the intestinal parasite *Trichostrongylus colubriformis*. *British Journal of Nutrition* 55: 593-602.
262. Poppi, D. P.; Hughes, T. P. & L'Huillier, J. H. 1987. Intake of pasture by the grazing animal. In: *Livestock Feeding on Pasture*. A.M. Nicol (Ed). The New Zealand Society of Animal Production Occasional Publication 10: 55-63.
263. Poppi, D. P.; Sykes, A. R. & Dynes, R. A. 1990. The effect of endoparasitism on host nutrition - the implications for nutrient manipulation. *Proceedings of the New Zealand Society of Animal Production* 50: 237-243.
264. Presidente, P. J. A. 1979. Endoparasites of captive and free-ranging deer in Australia. *Proceedings of a Refresher Course for Veterinarians, The University of Sydney Post-Graduate Committee in Veterinary Science* 49A: 235-251.
265. Presidente, P. J. A.; Whorley, D. E. & Catlin, J. E. 1972. Cross-transmission experiments with *Dictyocaulus viviparus* isolates from rocky mountain elk and cattle. *Journal of Wildlife Diseases* 8: 57-62.
266. Presidente, P. J. A.; Knapp, S. E. & Dean, R. E. 1973. Treatment and control of *Dictyocaulus viviparus* in captive Black-tailed deer. *Journal of Wildlife Diseases* 9: 34-40.
267. Prestwood, A. K. & Kellogg, F. E. 1971. Naturally occurring haemonchosis in a white-tailed deer. *Journal of Wildlife Diseases* 7: 133-134.
268. Prestwood, A. K.; Hayes, F. A.; Eve, J. H. & Smith, J. F. 1973. Abomasal helminths of white-tailed deer in Southeastern United States, Texas, and the Virgin Islands. *Journal of the American Veterinary Medical Association* 163(6): 556-561.
269. Pritchard, D. A.; Stocks, D. C.; O'Sullivan, B. M.; Martin, P. R.; Hurwood, I. S. & O'Rourke, P. K. 1988. The effect of polyethylene glycol (PEG) on wool growth and liveweight of sheep consuming a mulga (*Acacia aneura*) diet. *Proceedings of the Australian Society of Animal Production* 17: 290-293.
270. Pritchard, D. A.; Martin, P. R. & O'Rourke, P. K. 1992. The role of condensed tannins in the nutritional value of mulga (*Acacia aneura*) for sheep. *Australian Journal of Agricultural Research* 43: 1739-1746.
271. Pritchard, M. H. & Kruse, G. O. W. 1982. *The collection and preservation of animal parasites*. University of Nebraska Press, USA.
272. Purchas, R. W. & Keogh, R. G. 1984. Fatness of lambs grazed on 'Grasslands Maku' Lotus and 'Grasslands Huia' white clover. *Proceedings of the New Zealand Society of Animal Production* 44: 219-221.



273. Rattray, P. V. & Clark, D. A. 1984. Factors affecting the intake of pasture. *New Zealand Journal of Agricultural Science* 18: 141-146.
274. Rattray, P. V. & Joyce, J. P. 1974. Nutritive value of white clover and perennial ryegrass IV utilisation of dietary energy. *New Zealand Journal of Agricultural Research* 17: 401-406.
275. Reed, J. D.; McDowell, R. E.; van Soest, P. J. & Horvath, P. J. 1982. Condensed tannins: a factor limiting the use of cassava forage. *Journal of the Science of Food and Agriculture* 33: 213-220.
276. Rees, M. C. & Minson, D. J. 1978. Fertiliser sulphur as a factor affecting voluntary intake, digestibility and retention time of pangola grass (*Digitaria decumbens*) by sheep. *British Journal of Nutrition* 39: 5-11.
277. Rees, S.B. & Harborne, J.B. 1985. The role of sesquiterpene lactones and phenolics in the chemical defence of the chicory plant. *Phytochemistry* 24(10): 2225-2231.
278. Rehbein, S.; Haupt, W.; Kunz, G. & Hock, W. 1995. Study on the infestation of a pasture for farmed fallow deer (*Dama dama* L.) with larvae of gastrointestinal nematodes and lungworms. *Zeitschrift für Jagdwissenschaft* 41(2): 93-99.
279. Reid, C. S. W.; Ulyatt, M. J. & Wilson, J. M. 1974. Plant tannins, bloat and nutritive value. *Proceedings of the New Zealand Society of Animal Production* 34: 82-92.
280. Rhodes, A. P. 1993. Efficacy of slow-release albendazole capsules in controlling lungworms and gastro-intestinal nematodes in red deer (*Cervus elaphus*). *New Zealand Veterinary Journal* 41(3): 131-133.
281. Robbins, C. T.; Hanley, T. A.; Hagerman, A. E.; Hjeljord, O.; Baker, D. L.; Schwartz, C. C. & Mautz, W. W. 1987a. Role of tannins in defending plants against ruminants: reduction in protein availability. *Ecology* 68(1): 98-107.
282. Robbins, C. T.; Mole, S.; Hagerman, A. E. & Hanley, T. A. 1987b. Role of tannins in defending plants against ruminants: reduction in dry matter digestion? *Ecology* 68(6): 1606-1615.
283. Robbins, C. T.; Hagerman, A. E.; Austin, P. J.; McArthur, C. & Hanley, T. A. 1991. Variation in mammalian physiological responses to a condensed tannin and its ecological implications. *Journal of Mammalogy* 72: 480-486.
284. Robertson, H. A.; Niezen, J. H.; Waghorn, G. C.; Charleston, W. A. G. & Jinlong, M. 1995. The effect of six herbage on liveweight gain, wool growth and faecal

- egg count of parasitised ewe lambs. Proceedings of the New Zealand Society of Animal Production 55: 199-201.
285. Roughan, P. G. & Holland, R. 1977. Predicting in-vitro digestibilities of herbage by exhaustive enzymatic hydrolysis of cell walls. Journal of the Science of Food and Agriculture 28: 1057-1064.
286. Rys, G. J.; Smith, N. & Slay, M. W. 1988. Alternative forage species for Hawkes Bay. Proceedings of the Agronomy Society of New Zealand 18: 75-80.
287. Scales, G. H.; Knight, T. L. & Saville, D. J. 1995. Effect of herbage species and feeding level on internal parasites and production performance of grazing lambs. New Zealand Journal of Agricultural Research 38: 237-247.
288. Schnieder, T.; Epe, C. & Vonsamsonhimmelstjerna, G. 1996. Species differentiation of lungworms (Dictyocaulidae) by polymerase chain reaction/restriction-fragment-length polymorphism of second internal transcribed spacers of ribosomal DNA. Parasitology Research 82(5): 392-394.
289. Schultz, S. R.; Johnson, M. K.; Barry, R. X. & Forbes, W. A. 1993. White-tailed deer abomasal parasite and fecal egg counts in Louisiana. Wildlife Society Bulletin 21: 256-263.
290. Scott, I. 1989. Pasture preferences of deer. Proceedings of a Deer Course for Veterinarians. Deer Branch of the New Zealand Veterinary Association 6: 176-180.
291. Scales, G.H., Knight, T.L. & Saville, D.J. 1995. Effect of herbage species and feeding level on internal parasites and production performance of grazing lambs. New Zealand Journal of Agricultural Research 38: 237-247.
292. Seman, D. L. & McKenzie-Parnell, J. M. 1989. The nutritive value of meat as a food. In: Meat Production and Processing, R.W. Purchas, B.W. Butler-Hogg, A.S. Davies (Eds). The New Zealand Society of Animal Production. Occasional Publication 11: 13-28.
293. Semiadi, G. 1995. PhD Thesis, Massey University, Palmerston North, New Zealand.
294. Semiadi, G.; Barry, T. N.; Wilson, P. R.; Hodgson, J. & Purchas, R. W. 1993. Growth and venison production from red deer (*Cervus elaphus*) grazing red clover (*Trifolium pratense*) or perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture. Journal of Agricultural Science, Cambridge 121: 265-271.

295. Semiadi, G.; Barry, T. N.; Muir, P. D. & Hodgson, J. 1995. Dietary preferences of sambar (*Cervus unicolor*) and red deer (*Cervus elaphus*) offered browse, forage legume and grass species. *Journal of Agricultural Science, Cambridge* 125: 99-107.
296. Sheath, G. W. 1983. Pasture utilisation in hill country. 1. Influence of grazing duration and land contour. *New Zealand Journal of Experimental Agriculture* 11: 309-319.
297. Sibbald, A. M. & Milne, J. A. 1993. Physical characteristics of the alimentary tract in relation to seasonal changes in voluntary food intake by the red deer (*Cervus elaphus*). *Journal of Agricultural Science, Cambridge* 120: 99-102.
298. Simpson, H. V.; Reynolds, G. W. & Carr, D. H. 1993. Low tissue gastrin content in the ovine distal duodenum is associated with increased percentage of G34. *Comparative Biochemistry and Physiology* 104A: 461-468.
299. Smith, L. W.; Goering, H. K. & Gordon, C. H. 1972. Relationships of forage composition with rates of cell wall digestion and indigestibility of cell walls. *Journal of Dairy Science* 55: 1140-1147.
300. Smith, R. M.; Hocking-Edwards, J. E. & Dynes, R. A. 1996. Protected protein reduces the faecal egg count of weaners exposed to gastrointestinal parasites. *Proceedings of the Australian Society of Animal Production* 21: 386.
301. Smits, J. E. G. 1991. A brief review of infectious and parasitic diseases of wapiti, with emphasis on Western Canada and the Northwestern United-States. *Canadian Veterinary Journal - Revue Veterinaire Canadienne* 32(8): 471-479.
302. Soetrisno, E.; Barry, T. N.; Wilson, P. R.; Hodgson, J. & Purchas, R. W. 1994. Effects of grazing red clover (*Trifolium pratense*) or perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pastures upon growth and venison production from weaner red deer (*Cervus elaphus*). *New Zealand Journal of Agricultural Research* 37: 19-27.
303. Spalinger, D.E.; Hanley, T.A. & Robbins, C.T. 1988. Analysis of the functional response in foraging in the sitka black-tailed deer. *Ecology* 69(4): 1166-1175.
304. Spiers, I. B. 1987. The development of the deer industry. *Proceedings of a Deer Course for Veterinarians. Deer Branch of the New Zealand Veterinary Association* 4: 1-8.
305. Stafford, K.J.; West, D.M. & Pomroy, W.E. 1994. Nematode worm egg output by ewes. *New Zealand Veterinary Journal* 42: 30-32.

306. Stevenson, J. M.; Seman, D. L. & Littlejohn, R. P. 1992. Seasonal variation in venison quality of mature, farmed red deer stags in New Zealand. *Journal of Animal Science* 70(5): 1389-1396.
307. Steel, J. W. 1974. Pathophysiology of gastrointestinal nematode infections in the ruminant. *Proceedings of the Australian Society of Animal Production* 10: 139-147.
308. Stienezen, M.; Waghorn, G.C. & Douglas, G.B. 1996. Digestibility and effects of condensed tannins on digestion of sulla (*Hedysarum coronarium*) when fed to sheep. *New Zealand Journal of Agricultural Research* 39: 215-221.
309. Storm, E. & Orskov, E. R. 1984. The nutritive value of rumen microorganisms in ruminants. 4. The limiting amino acids of microbial protein in growing sheep determined by a new approach. *British Journal of Nutrition* 52: 613-620.
310. Strunz, U.T.; Walsh, J.H. & Grossman, M.I. 1978. Stimulation of gastrin release in dogs by individual amino acids. *Proceedings of the Society for Experimental Biology and Medicine* 157: 440-441.
311. Suarez, V. H.; Buseti, M. R.; Fort, M. C. & Bedotti, D. O. 1991. *Spiculopteria spiculoptera*, *S. asymmetrica* and *Ostertagia leptospicularis* from *Cervus elaphus* in La-Pampa, Argentina. *Veterinary Parasitology* 40(1-2): 165-168.
312. Suttie, J. M. & Corson, I. D. 1991. Deer growth and production: a review. *Proceedings of a Deer Course for Veterinarians. Deer Branch of the New Zealand Veterinary Association* 8: 53-67.
313. Suttie, J. M.; Goodall, E. D.; Pennie, K. & Kay, R. N. B. 1983. Winter food restriction and summer compensation in red deer stags (*Cervus elaphus*). *British Journal of Nutrition* 50(3): 737-747.
314. Suttie, J. M.; Fennessy, P. F.; Veenvliet, B. A.; Littlejohn, R. P.; Fisher, M. W.; Corson, I. D. & Labes, R. E. 1987. Energy nutrition of young red deer (*Cervus elaphus*) hinds and a comparison with young stags. *Proceedings of the New Zealand Society of Animal Production* 47: 111-113.
315. Suttie, J. M.; Corson, I. D.; Webster, J. R. & Woodford, K. B. 1992. Photoperiodism and growth. *Proceedings of a Deer Course for Veterinarians. Deer Branch of the New Zealand Veterinary Association* 9: 136-142.
316. Swain, T. 1979. Tannins and lignins. In: *Herbivores: Their Interaction with Secondary Plant Metabolites*. G.A.Rosenthal & D.H.Janzen (Eds). Academic Press. New York, USA: 657-682.

317. Sykes, A. R. 1978. The effect of subclinical parasitism in sheep. *Veterinary Record* 102: 32-34.
318. Sykes, A. R. 1994. Parasitism and production in farm animals. *Animal Production* 59: 155-172.
319. Sykes, A.R. & Coop, R.L. 1976. Intake and utilisation of food by growing lambs with parasitic damage to the small intestine caused by daily dosing with *Trichostrongylus colubriformis* larvae. *Journal of Agricultural Science, Cambridge* 86: 507-515.
320. Sykes, A. R. & Coop, R. L. 1977. Intake and utilisation of food by growing sheep with abomasal damage caused by daily dosing with *Ostertagia circumcincta* larvae. *Journal of Agricultural Science, Cambridge* 88: 671-677.
321. Syme, J. 1993. New era for New Zealand's farmed deer industry. *The Food Technologist*: 12-14.
322. Symons, L.E.A. 1985. Anorexia: occurrence, pathophysiology and possible causes in parasitic infections. *Advances in Parasitology* 24: 103-133.
323. Taylor, I.N.; Bryne, W.J.; Christie, D.L.; Ament, M.E. & Walsh, J.H. 1982. Effect of individual L-amino acids on gastric acid and serum gastrin and pancreatic polypeptide release in humans. *Gastroenterology* 83: 273-278.
324. Terrill, T. H.; Rowan, A. M.; Douglas, G. B. & Barry, T. N. 1992a. Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. *Journal of Science of Food and Agriculture* 58: 321-329.
325. Terrill, T. H.; Douglas, G. B.; Foote, A. G.; Purchas, R. W.; Wilson, G. F. & Barry, T. N. 1992b. The effect of condensed tannins upon body growth, wool growth and rumen metabolism in sheep grazing sulla (*Hedysarium coronarium*) and perennial pasture. *Journal of Agricultural Science, Cambridge* 119: 265-273.
326. Terrill, T.H.; Waghorn, G.C.; Woolley, D.J.; McNabb, W.C. & Barry, T.N. 1994. Assay and digestion of <sup>14</sup>C-labelled condensed tannin in the gastrointestinal tract of sheep. *British Journal of Nutrition* 72: 467-477.
327. Thienpont, D.; Rochette, F. & Vanparijs, O. F. J. 1979. Diagnosing helminthiasis through coprological examination. Janssen Research Foundation, Beerse, Belgium.
328. Thouless, C. R. 1990. Feeding competition between grazing red deer hinds. *Animal Behaviour* 40: 105-111.
329. Tinworth, T.R.H. & Barry, T.N. 1998. The effect of wilting chicory on the voluntary feed intake and digestibility by red deer. B.App.Sc. (Hons) Dissertation,

Massey University, Palmerston North, New Zealand.

330. Titchen, D.A. & Anderson, N. 1977. Aspects of the physio-pathology of parasitic gastritis in sheep. *Australian Veterinary Journal* 53: 369-373.
331. Tustin, K. G. 1974. Status of moose in New Zealand. *Journal of Mammalogy* 55: 199-200.
332. Ulyatt, M. J. 1970. Factors contributing to differences in the quality of short-rotation ryegrass, perennial ryegrass and white clover. *Proceedings of the XI International Grassland Congress*. Queensland, Australia: 709-714.
333. Ulyatt, M. J. 1973. The feeding value of herbage. In: *Chemistry and Biochemistry of Herbage*. Butler, G. W.; Bailey, R. W. (Eds). Academic Press. London, United Kingdom: 131-178.
334. Ulyatt, M. J. 1981. The feeding value of temperate pastures. In: *World Animal Science, B1. Grazing Animals*, F.H.W. Morley (Ed): Elsevier Publishing Company, Amsterdam, Holland.
335. Ulyatt, M. J. 1984. Determinants of voluntary feed consumption. In: *Plant Breeding and Feeding Value of Pastures and Forages*, M.J. Ulyatt, J.L. Black (Eds). DSIR, Palmerston North, New Zealand.
336. Ulyatt, M. J. & Egan, A. R. 1979. Quantitative digestion of fresh herbage by sheep V. the digestion of four herbage and prediction of sites of digestion. *Journal of Agricultural Science, Cambridge* 92: 605-616.
337. Ulyatt, M. J. & MacRae, J. C. 1974. Quantitative digestion of fresh herbage by sheep I. The sites of digestion of organic matter, energy, readily fermentable carbohydrate, structural carbohydrate, and lipid. *Journal of Agricultural Science, Cambridge* 82: 295-307.
338. Ulyatt, M. J.; Barclay, P. C.; Lancashire, J. A. & Armstrong, C. S. M. 1974. The feeding value to sheep of 'Grassland Tama' Westerwolds ryegrass, 'Grassland Paroa' Italian ryegrass and 'Grassland Ruanui' perennial ryegrass under rotational grazing management. *New Zealand Journal of Experimental Agriculture* 2: 231-236.
339. Ulyatt, M. J.; MacRae, J. C.; Clarke, R. T. J. & Pearce, P. D. 1975. Quantitative digestion of fresh herbage by sheep. IV. Protein synthesis in the stomach. *Journal of Agricultural Science, Cambridge* 84: 453-458.
340. Ulyatt, M. J.; Lancashire, J. A. & Jones, W. T. 1976. The nutritive value of legumes. *Proceedings of the New Zealand Grassland Association* 38: 107-118.

341. Van Bruchem, J. 1977. Abomasal secretion and motility in sheep. Verslag Landbouwkunde Onderstepoort 868: 1-140.
342. van Houtert, M.; Barger, I. & Steel, J. 1992. Effects of bypass protein intake on responses of Merino lambs to infection with *Trichostrongylus colubriformis*. Joint Conference of the New Zealand and Australian Societies for Parasitology, Auckland, New Zealand.
343. van Houtert, M. J. F.; Barger, I. A. & Steel, J. W. 1995a. Dietary protein for young grazing sheep: interactions with gastrointestinal parasitism. *Veterinary Parasitology* 60: 283-295.
344. van Houtert, M. F. J.; Barger, I. A.; Steel, J. W.; Windon, R. G. & Emery, D. L. 1995b. Effects of dietary protein on responses of young sheep to infection with *Trichostrongylus colubriformis*. *Veterinary Parasitology* 59: 163-180.
345. van Reenen, G. 1982. Diseases. The Farming of Deer, World Trends and Modern Techniques. D. Yerex (Ed). Edition 1. Agricultural Promotion Associates Ltd. Wellington, New Zealand: 129-133.
346. van Soest, P. J. 1994. Nutritional Ecology of the Ruminant. Ithaca & London, Cornell University Press.
347. Waghorn, G. C. & Barry, T. N. 1987. Pasture as a nutrient source. In: Livestock Feeding on Pasture. A.M. Nicol (Ed). New Zealand Society of Animal Production Occasional Publication No 10: 21-37.
348. Waghorn, G. C. & Jones, W. T. 1989. Bloat in cattle: 46. Potential of dock (*Rumex obtusifolius*) as an antibloat agent for cattle. *New Zealand Journal of Agricultural Research* 32: 227-235.
349. Waghorn, T. & Niezen, J. 1994. Lamb growth and gastrointestinal nematode tolerance when grazing two fodder crops. *New Zealand Journal of Zoology* 21: 101.
350. Waghorn, G. C.; John, A.; Jones, W. T. & Shelton, I. D. 1987a. Nutritive value of *Lotus corniculatus* L. containing low and medium concentrations of condensed tannins for sheep. *Proceedings of the New Zealand Society of Animal Production* 47: 25-30.
351. Waghorn, G. C.; Ulyatt, M. J.; John, A. & Fisher, M. T. 1987b. The effect of condensed tannins on the site of digestion of amino acids and other nutrients in sheep fed on *Lotus corniculatus*. *British Journal of Nutrition* 57: 115-126.

352. Waghorn, G. C.; Jones, W. T.; Shelton, I. D. & McNabb, W. C. 1990. Condensed tannins and the nutritive value of herbage. *Proceedings of the New Zealand Grassland Association* 51: 171-176.
353. Waghorn, G. C.; Shelton, I. D.; McNabb, W. C. & McCutcheon, S. N. 1994. Effects of condensed tannins in *Lotus pedunculatus* on its nutritive value for sheep 2. Nitrogenous aspects. *Journal of Agricultural Science, Cambridge* 123: 109-119.
354. Wagner, J. & Mackintosh, C. G. 1993. Ivermectin and copper response trial in mixed age red deer hinds. *Proceedings of a Deer Course for Veterinarians. Deer Branch of the New Zealand Veterinary Association* 10: 134-142.
355. Waldrup, K. & Mackintosh, C. G. 1992. Fading elk syndrome research. *Proceedings of a Deer Course for Veterinarians. Deer Branch of the New Zealand Veterinary Association* 9: 170-174.
356. Waldrup, K. A. & Mackintosh, C. G. 1993. A difference in abomasal pH between Canadian wapiti affected with chronic illthrift and unaffected deer. *New Zealand Veterinary Journal* 41(3): 142-143.
357. Waldrup, K.; Mackintosh, C. G.; Labes, R. E. & Rhodes, A. P. 1993. The use of Profril boluses in weaner red deer hinds (*Cervus elaphus*). *Proceedings of a Deer Course for Veterinarians. Deer Branch of the New Zealand Veterinary Association* 10: 155-161.
358. Waldrup, K. A.; Mackintosh, C. G.; Johnstone, P. D. & Labes, R. E. 1994a. The effects of parasitism on weaner deer: parallel studies with red deer hinds and wapiti hybrid hinds. *Proceedings of a Deer Course for Veterinarians. Deer Branch of the New Zealand Veterinary Association* 11: 194-202.
359. Waldrup, K. A.; Mackintosh, C. G.; Rhodes, A. P. & Labes, R. E. 1994b. The use of Profril boluses in weaner red deer hinds (*Cervus elaphus*): a seven month study. *Proceedings of a Deer Course for Veterinarians*, Ed. P.R. Wilson. Deer Branch of the New Zealand Veterinary Association 11: 213-223.
360. Wallace, D. S.; Bairden, K.; Duncan, J. L.; Fishwick, G.; Gill, M.; Holmes, P. H.; McKellar, Q. A.; Murray, M.; Parkins, J. J. & Stear, M. 1996. Influence of soyabean meal supplementation on the resistance of Scottish blackface lambs to haemonchosis. *Research in Veterinary Science* 60: 138-143.
361. Walters, J. K. & Evans, E. M. 1979. Evaluation of a sward sampling technique for estimating herbage intake by grazing sheep. *Grass and Forage Science* 34: 37-44.
362. Wang, Y.; Waghorn, G. C.; Douglas, G. B.; Barry, T. N. & Wilson, G. F. 1994a. The effects of condensed tannin in *Lotus corniculatus* upon nutrient metabolism



- and upon body and wool growth in sheep. Proceedings of the New Zealand Society of Animal Production 54: 219-222.
363. Wang, Y.; Waghorn, G. C.; Barry, T. N. & Shelton, I. D. 1994b. Effect of condensed tannins in *Lotus corniculatus* upon plasma metabolism of methionine, cysteine and inorganic sulphate by sheep. British Journal of Nutrition 72: 923-935.
364. Wang, Y. 1995. The effects of condensed tannins upon nutrient digestion and metabolism and upon animal production in sheep fed *Lotus corniculatus*. PhD Thesis, Massey University, Palmerston North, New Zealand.
365. Wang, Y.; Douglas, G. B.; Waghorn, G. C.; Barry, T. N.; Foote, A. G. & Purchas, R. W. 1996a. Effect of condensed tannins upon the performance of lambs grazing *Lotus corniculatus* and lucerne (*Medicago sativa*). Journal of Agricultural Science, Cambridge 126: 87-98.
366. Wang, Y.; Douglas, G. B.; Waghorn, G. C.; Barry, T. N. & Foote, A. G. 1996b. Effect of condensed tannins in *Lotus corniculatus* upon lactation performance in ewes. Journal of Agricultural Science, Cambridge 126: 353-362.
367. Warner, A. C. I. 1981. Rate of passage of digesta through the gut of mammals and birds. Nutrition Abstracts and Reviews 51(B): 789-820.
368. Watkins, W. G. & Hudson, R. J. 1984. In: 63rd Annual Feeders Day Report. University of Alberta, Edmonton, Alberta, Canada: 60-61.
369. Watson, M. J. 1982. *Hedysarum coronarium* - a legume with potential for soil conservation and forage. New Zealand Journal of Agricultural Science 16: 189-194.
370. Watson, T. G. 1986. Immunity to gastrointestinal nematode parasites in domestic stock with particular reference to sheep: a review. Proceedings of the New Zealand Society of Animal Production 46: 15-22.
371. Watson, T. G. & Charleston, W. A. G. 1985. The significance of parasites in farmed deer. In: Biology of Deer Production. P.F. Fennessy, K.R. Drew (Eds). The Royal Society of New Zealand Bulletin 22: 105-117.
372. Weston, R. H. 1984. Principles of feed intake in ruminants. In: The Utilization of Fibrous Agricultural Residues as Animal Feed, P.T. Doyle (Ed). Proceedings of the Third Annual Workshop of the Australian-Asian Fibrous Agricultural Residue Network. School of Agriculture and Forestry, University of Melbourne, Parkville, Victoria, Australia: 14-27.

373. Wickstrom, M. L.; Robbins, C. T.; Hanley, T. A.; Spalinger, D. E. & Parish, S. M. 1984. Food intake and foraging energetics of elk and mule deer. *Journal of Wildlife Management* 48: 1285-1301.
374. Wilson, A. D. 1977. The digestibility and voluntary intake of the leaves of trees and shrubs by sheep and goats. *Australian Journal of Agricultural Research* 28: 501-508.
375. Wilson, J. R. & Kennedy, P. M. 1996. Plant and animal constraints to voluntary feed intake associated with fibre characteristics and particle breakdown and passage in ruminants. *Australian Journal of Agricultural Research* 47(2): 199-225.
376. Wilson, P. R. 1981. Studies in parasitism in red deer. *Proceedings of a Deer Seminar for Veterinarians, Deer Advisory Panel of the New Zealand Veterinary Association* 1: 71-77.
377. Wilson, P. R. 1984. A perspective on internal parasite control. *Proceedings of a Deer Course for Veterinarians. Deer Branch of the New Zealand Veterinary Association*: 89-99.
378. Wilson, P. R. & Collier, A. 1981. Lungworm in deer: A survey of veterinary practices. *Proceedings of a Deer Seminar for Veterinarians. Deer Advisory Panel of the New Zealand Veterinary Association* 1: 78-93.
379. Wilson, P.R. & Pauli, J.V. Blood constituents of farmed red deer (*Cervus elaphus*): I. Haematological values. *New Zealand Veterinary Journal* 30:174-176.
380. Wilson, P. R.; Walker, I. H.; Bond, D. B.; Middleberg, A. & Staples, L. D. 1991. Field evaluation of melatonin implants to advance the breeding season in one year old farmed red deer hinds. *New Zealand Veterinary Journal* 39(1): 23-28.
381. Wilson, P.R.; Audige, L.J.M. & Morris, R.S. 1997. On-farm internal parasite control: luck or design? *Proceedings of a Deer Course for Veterinarians*, Ed. P.R. Wilson. *Deer Branch of the New Zealand Veterinary Association* 14: 115-142.
382. Winch, J. 1986. Internal parasites, their effect on red and rusa deer in southeast Queensland. *Deer Farming into the Nineties*. P. Owen (Ed). Edition 1. Owen Art and Publishing Pty Ltd, Brisbane, Australia: 87-94.
383. Yerex, D. 1982. The farming of deer. World trends and modern techniques. *Agricultural Promotion Associates Ltd, Wellington, New Zealand*.
384. Zucker, W. V. 1983. Tannins: does structure determine function? An ecological perspective. *American Naturalist* 121: 335-365.

**APPENDIX 4.1. APPENDIX TO CHAPTER 4.**

WEEK		0	1	2	3	4	5	6	7	8	9	10	11
<b>TOTAL LEUCOCYTES (x10<sup>9</sup>/l)</b>													
<b>C</b>	mean	4.72	6.86	5.14	5.24	4.80	4.68	4.68	4.68	4.38	4.58	4.62	5.00
	max	6.00	8.70	7.20	6.70	6.00	5.20	5.60	5.60	5.40	5.50	5.80	6.90
	min	3.00	5.00	3.20	3.60	3.30	3.10	2.70	2.70	2.70	3.50	3.20	3.20
<b>LT</b>	mean	4.69	5.74	5.28	4.64	4.50	4.40	4.52	4.52	4.54	4.60	4.90	4.92
	max	6.20	6.50	6.50	5.50	5.00	5.10	5.50	5.50	5.10	4.90	5.70	5.30
	min	3.15	4.40	4.20	4.20	4.10	4.00	3.70	3.70	4.00	4.30	4.50	4.30
<b>MT</b>	mean	4.08	5.64	4.52	4.98	4.38	4.18	4.42	4.42	5.02	4.70	5.26	5.24
	max	5.90	8.00	6.00	7.50	6.60	5.90	6.00	6.00	7.70	6.20	7.10	6.40
	min	2.20	4.10	3.20	2.80	2.70	2.90	2.70	2.70	2.40	2.80	3.50	3.40
<b>HT</b>	mean	4.10	5.48	4.38	4.40	4.30	3.72	4.46	4.46	5.42	4.70	5.12	4.82
	max	6.00	6.50	5.90	6.20	6.20	4.50	5.70	5.70	7.90	5.90	5.50	6.30
	min	2.60	4.60	3.50	3.70	3.30	2.40	3.30	3.30	4.10	4.20	4.80	2.40
<b>EOSINOPHILS DIFFERENTIAL LEUCOCYTE COUNTS (x10<sup>9</sup>/l)</b>													
<b>C</b>	mean	0.01	0.03	0.03	0.01	0.07	0.04	0.01	0.04	0.03	0.01	0.01	0.07
	max	0.04	0.09	0.07	0.05	0.17	0.10	0.05	0.10	0.05	0.04	0.03	0.28
	min	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<b>LT</b>	mean	0.02	0.01	0.08	0.18	0.23	0.32	0.41	0.37	0.31	0.23	0.24	0.29
	max	0.10	0.04	0.26	0.28	0.40	0.71	0.83	0.77	0.50	0.44	0.41	0.51
	min	0.00	0.00	0.00	0.09	0.04	0.12	0.05	0.05	0.00	0.04	0.05	0.09
<b>MT</b>	mean	0.02	0.00	0.10	0.10	0.25	0.24	0.24	0.29	0.41	0.45	0.37	0.53
	max	0.06	0.00	0.16	0.30	0.55	0.43	0.62	0.36	0.54	0.62	0.65	0.99
	min	0.00	0.00	0.03	0.04	0.00	0.12	0.03	0.11	0.12	0.17	0.11	0.20
<b>HT</b>	mean	0.02	0.06	0.18	0.26	0.36	0.15	0.10	0.34	0.48	0.39	0.41	0.39
	max	0.12	0.24	0.77	1.05	0.87	0.28	0.24	1.03	1.05	0.79	1.10	0.64
	min	0.00	0.00	0.00	0.00	0.07	0.07	0.00	0.10	0.16	0.04	0.15	0.19
<b>NEUTROPHILS</b>													
<b>C</b>	mean	1.36	3.12	1.96	1.72	1.69	1.26	1.53	1.44	1.55	1.70	1.29	1.49
	max	1.74	4.34	2.66	2.35	2.16	1.46	2.09	1.87	1.89	2.42	1.91	2.00
	min	0.89	1.70	1.38	1.19	0.96	0.97	1.03	1.19	1.19	1.20	0.99	0.96
<b>LT</b>	mean	1.78	2.93	2.32	1.51	1.11	1.07	1.46	1.43	1.10	1.37	1.24	1.09
	max	3.84	4.29	3.29	2.21	1.66	1.73	3.03	2.20	2.00	2.06	2.05	1.38
	min	1.04	1.71	1.43	1.16	0.74	0.74	0.92	0.89	0.50	0.94	0.83	0.77
<b>MT</b>	mean	1.19	2.84	1.69	2.18	1.18	0.91	1.48	1.50	1.80	1.68	1.28	1.10
	max	2.54	4.64	2.52	3.98	2.90	1.48	2.07	2.58	2.77	2.94	2.49	1.54
	min	0.29	1.95	1.12	0.56	0.51	0.48	0.74	0.59	0.94	0.67	0.46	0.41
<b>HT</b>	mean	1.43	3.22	1.07	1.50	1.10	1.03	1.61	1.73	2.07	1.64	1.39	1.57
	max	2.94	4.31	1.93	2.54	1.98	1.62	2.34	2.50	3.24	2.66	1.87	2.65
	min	0.29	1.81	0.00	0.84	0.50	0.53	0.69	1.16	0.94	0.60	0.53	0.79
<b>LYMPHOCYTES</b>													
<b>C</b>	mean	3.04	3.32	2.90	3.05	2.52	3.10	2.92	2.95	2.56	2.69	2.85	2.91
	max	4.08	5.13	4.39	3.89	3.18	3.93	3.42	3.92	3.23	3.35	3.54	3.80
	min	1.77	2.03	1.60	2.02	1.39	1.33	1.65	1.43	1.38	1.61	2.08	2.05
<b>LT</b>	mean	2.68	2.56	2.70	2.60	2.61	2.58	2.46	2.56	2.88	2.73	2.94	3.08
	max	3.54	3.47	3.40	3.52	3.40	3.40	3.33	3.20	3.67	3.24	3.45	3.66
	min	1.89	1.76	2.02	1.56	1.89	2.04	1.54	1.85	1.95	2.35	2.34	2.70
<b>MT</b>	mean	2.64	2.57	2.55	2.36	2.55	2.90	2.44	2.40	2.52	2.32	3.14	3.03
	max	4.13	4.10	3.62	3.00	3.69	4.05	3.30	3.66	4.00	3.53	4.16	3.71
	min	1.75	1.39	1.51	1.43	1.89	2.01	1.43	1.64	1.22	1.75	2.38	2.15
<b>HT</b>	mean	2.33	2.03	2.35	2.29	2.46	2.15	2.35	2.18	2.67	2.49	2.88	2.38
	max	3.06	2.79	2.96	2.52	2.93	2.62	2.85	2.45	3.87	3.40	3.35	3.15
	min	1.85	1.36	2.01	2.15	1.82	1.61	1.82	1.88	1.93	1.85	2.45	0.89
<b>MONOCYTES</b>													
<b>C</b>	mean	0.13	0.25	0.18	0.37	0.37	0.19	0.12	0.06	0.11	0.05	0.27	0.44
	max	0.24	0.44	0.32	0.89	0.44	0.42	0.28	0.17	0.26	0.11	0.54	1.10
	min	0.06	0.16	0.07	0.04	0.22	0.00	0.00	0.00	0.05	0.00	0.06	0.16
<b>LT</b>	mean	0.13	0.16	0.16	0.24	0.30	0.31	0.11	0.07	0.08	0.09	0.23	0.33
	max	0.18	0.28	0.25	0.43	0.61	0.48	0.22	0.18	0.15	0.23	0.51	0.60
	min	0.04	0.04	0.04	0.04	0.10	0.17	0.05	0.00	0.00	0.00	0.14	0.14
<b>MT</b>	mean	0.20	0.13	0.18	0.25	0.16	0.13	0.15	0.10	0.11	0.07	0.39	0.44
	max	0.30	0.16	0.39	0.45	0.30	0.30	0.30	0.23	0.20	0.18	0.59	0.61
	min	0.00	0.07	0.03	0.08	0.07	0.00	0.06	0.00	0.02	0.00	0.04	0.20
<b>HT</b>	mean	0.25	0.16	0.14	0.28	0.26	0.35	0.15	0.10	0.13	0.09	0.22	0.33
	max	0.42	0.29	0.25	0.61	0.50	0.86	0.35	0.17	0.28	0.13	0.35	0.43
	min	0.05	0.00	0.00	0.04	0.07	0.12	0.00	0.03	0.05	0.06	0.11	0.17
<b>BASOPHILS</b>													
<b>C</b>	mean	0.18	0.15	0.06	0.12	0.15	0.10	0.11	0.19	0.13	0.13	0.20	0.10
	max	0.56	0.21	0.32	0.47	0.22	0.25	0.19	0.55	0.49	0.35	0.56	0.30
	min	0.06	0.09	0.00	0.00	0.06	0.00	0.00	0.05	0.00	0.00	0.03	0.00
<b>LT</b>	mean	0.08	0.09	0.03	0.13	0.25	0.11	0.09	0.10	0.16	0.15	0.24	0.12
	max	0.16	0.33	0.13	0.28	0.33	0.17	0.14	0.28	0.40	0.24	0.51	0.18
	min	0.00	0.00	0.00	0.00	0.08	0.04	0.00	0.00	0.04	0.00	0.09	0.05
<b>MT</b>	mean	0.03	0.10	0.00	0.10	0.24	0.08	0.12	0.13	0.19	0.17	0.09	0.15
	max	0.09	0.30	0.00	0.20	0.53	0.12	0.36	0.23	0.41	0.35	0.19	0.22
	min	0.00	0.00	0.00	0.00	0.11	0.03	0.00	0.06	0.07	0.09	0.00	0.06
<b>HT</b>	mean	0.06	0.00	0.02	0.06	0.14	0.12	0.15	0.11	0.10	0.10	0.17	0.15
	max	0.12	0.00	0.07	0.15	0.19	0.22	0.35	0.19	0.26	0.18	0.30	0.27
	min	0.00	0.00	0.00	0.00	0.09	0.03	0.05	0.05	0.00	0.04	0.10	0.00

WEEK		0	1	2	3	4	5	6	7	8	9	10	11
<b>TOTAL ERYTHROCYTES (x10<sup>12</sup>/l)</b>													
C	mean	13.38	12.10	11.65	11.63	11.56	12.03	11.28	10.99	10.38	10.42	10.51	10.26
	max	14.38	13.49	12.96	13.07	13.21	13.39	12.49	12.18	10.90	10.88	12.25	10.88
	min	11.97	10.86	9.71	9.73	9.46	11.25	9.85	9.71	10.01	9.94	9.87	9.75
LT	mean	12.50	12.43	12.42	13.37	12.79	12.41	12.52	12.45	11.49	10.92	10.67	11.17
	max	12.89	14.78	15.14	15.93	15.07	14.08	14.66	16.61	13.35	12.77	12.53	12.63
	min	11.87	10.55	10.79	11.79	11.64	11.46	11.25	10.83	10.11	9.74	9.67	9.97
MT	mean	12.37	12.78	12.67	12.21	11.79	12.04	12.05	12.05	11.73	10.58	10.99	10.35
	max	13.60	13.35	13.85	13.00	12.71	13.00	12.89	12.85	12.32	12.46	12.22	11.32
	min	11.79	12.39	11.31	11.61	10.37	11.00	10.34	11.43	10.92	9.47	9.84	9.40
HT	mean	12.93	13.14	12.01	13.57	12.63	13.05	12.20	12.81	11.64	11.20	11.49	11.29
	max	14.78	14.49	13.35	15.39	13.64	14.70	14.59	15.47	13.85	13.99	15.40	12.98
	min	10.45	10.04	9.25	11.84	11.14	11.50	10.58	10.90	9.84	9.47	9.54	9.74
<b>PACKED CELL VOLUME (%)</b>													
C	mean	47.8	44.8	42.6	42.6	42.8	45.4	42.6	41.4	40.2	40.4	41.8	42.4
	max	51.0	47.0	47.0	46.0	48.0	49.0	47.0	45.0	46.0	44.0	51.0	47.0
	min	45.0	40.0	37.0	39.0	38.0	42.0	38.0	40.0	37.0	36.0	37.0	36.0
LT	mean	45.6	45.4	45.8	48.8	46.6	46.0	46.8	47.0	43.6	42.0	41.8	46.2
	max	51.0	50.0	52.0	54.0	51.0	48.0	51.0	60.0	47.0	45.0	45.0	55.0
	min	43.0	37.0	42.0	42.0	44.0	43.0	42.0	41.0	42.0	39.0	37.0	39.0
MT	mean	44.8	45.2	44.6	42.8	42.4	43.0	43.4	43.6	42.4	39.2	41.2	40.2
	max	47.0	47.0	46.0	46.0	46.0	47.0	46.0	45.0	45.0	41.0	46.0	45.0
	min	41.0	42.0	43.0	38.0	38.0	38.0	39.0	42.0	39.0	36.0	37.0	32.0
HT	mean	46.4	47.2	43.8	49.0	45.8	48.0	45.0	47.2	43.6	42.0	43.2	44.8
	max	52.0	50.0	50.0	53.0	48.0	52.0	50.0	52.0	47.0	48.0	54.0	50.0
	min	40.0	39.0	36.0	46.0	43.0	43.0	41.0	42.0	38.0	37.0	36.0	38.0
<b>HAEMOGLOBIN CONCENTRATION (g/l)</b>													
C	mean	161.4	154.2	149.2	155.8	155.4	161.2	148.8	154.6	149.8	151.6	155.0	153.0
	max	176.0	165.0	162.0	168.0	173.0	174.0	161.0	166.0	175.0	170.0	192.0	169.0
	min	150.0	134.0	129.0	143.0	136.0	146.0	130.0	147.0	138.0	138.0	137.0	133.0
LT	mean	158.8	158.0	158.8	178.0	169.8	163.0	165.6	168.2	163.4	156.4	155.8	162.6
	max	177.0	175.0	180.0	198.0	188.0	174.0	178.0	195.0	183.0	169.0	166.0	180.0
	min	148.0	124.0	143.0	150.0	158.0	147.0	147.0	145.0	157.0	144.0	141.0	143.0
MT	mean	155.0	152.0	153.8	152.2	153.2	150.6	153.2	157.6	155.2	145.8	149.4	143.6
	max	167.0	161.0	160.0	165.0	169.0	165.0	164.0	168.0	161.0	155.0	164.0	162.0
	min	142.0	136.0	150.0	137.0	134.0	133.0	140.0	141.0	141.0	134.0	137.0	113.0
HT	mean	161.8	162.4	153.8	177.8	166.2	171.8	159.4	173.6	160.0	157.6	160.6	162.2
	max	181.0	173.0	172.0	190.0	174.0	185.0	170.0	190.0	176.0	180.0	189.0	181.0
	min	138.0	137.0	126.0	168.0	157.0	157.0	146.0	151.0	142.0	141.0	140.0	138.0
<b>FIBRINOGEN (g/l)</b>													
C	mean	2.6	3.8	3.6	2.2	1.4	1.4	1.0	1.8	1.8	1.0	1.2	2.0
	max	4.0	5.0	6.0	4.0	2.0	3.0	1.0	5.0	4.0	1.0	2.0	3.0
	min	1.0	2.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
HT	mean	2.8	3.4	3.4	2.2	2.2	2.8	1.0	3.2	1.8	1.6	1.6	2.0
	max	5.0	6.0	7.0	3.0	5.0	5.0	1.0	6.0	3.0	2.0	3.0	4.0
	min	2.0	2.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
<b>SERUM TOTAL PROTEIN (g/l)</b>													
C	mean	65.02	68.04	65.78	65.28	71.10	66.68	68.06	64.32	58.98	63.36	63.82	63.30
	max	67.80	72.80	69.60	67.90	76.30	68.20	73.30	67.70	62.70	65.20	68.30	67.50
	min	64.10	65.30	62.00	63.20	66.20	64.30	64.70	61.10	51.00	61.70	59.70	57.80
LT	mean	60.40	64.60	63.50	62.46	65.94	63.62	66.84	62.38	54.86	59.74	60.12	63.68
	max	63.40	66.20	66.10	65.70	70.40	65.20	69.00	64.90	59.20	61.90	63.30	75.30
	min	55.00	63.20	61.10	58.90	61.20	62.70	64.50	58.80	47.40	56.40	56.70	51.00
MT	mean	62.88	65.66	64.98	61.36	65.14	64.98	70.92	64.30	60.32	62.42	65.22	60.38
	max	66.70	69.80	66.90	67.20	68.90	67.70	72.60	66.00	65.20	65.20	70.00	64.00
	min	58.60	56.80	63.10	53.10	59.50	61.70	68.70	60.00	54.00	59.00	62.70	55.20
HT	mean	65.48	72.88	65.28	68.52	66.32	68.94	71.54	67.20	62.32	64.58	61.28	63.76
	max	69.30	90.80	70.70	71.30	71.30	73.10	74.80	74.10	66.70	69.50	67.20	66.30
	min	63.40	66.80	59.60	63.00	62.60	63.10	67.50	59.40	60.40	59.80	48.30	61.10
<b>SERUM ALBUMIN (g/l)</b>													
C	mean	41.30	44.10	41.04	41.70	45.48	43.54	43.44	43.32	36.56	39.26	40.46	40.22
	max	44.60	49.50	44.00	46.90	48.80	47.20	46.40	46.10	39.30	41.00	42.30	42.80
	min	37.20	37.30	35.50	36.70	43.70	39.10	37.90	37.20	32.90	35.70	39.00	37.60
LT	mean	39.80	42.90	39.88	40.86	43.88	42.70	41.88	39.08	33.62	36.14	37.28	38.92
	max	42.30	47.20	42.20	45.60	47.60	46.70	43.00	42.00	37.80	37.10	38.50	48.10
	min	36.60	39.10	38.90	36.80	39.90	39.50	41.10	37.10	27.50	35.10	35.50	33.30
MT	mean	40.80	41.78	41.16	40.12	42.02	41.22	41.76	38.30	33.56	34.80	35.30	34.92
	max	42.70	46.50	44.10	43.90	46.00	44.90	45.90	43.20	39.70	38.10	39.70	39.40
	min	37.40	33.50	38.40	34.80	36.70	35.10	36.50	33.10	25.50	30.50	29.10	29.10
HT	mean	40.78	45.40	40.56	42.22	41.26	41.08	38.82	37.94	32.72	34.46	35.18	35.68
	max	44.70	53.00	45.10	46.20	44.60	47.10	42.20	42.50	36.50	38.30	40.30	40.30
	min	37.10	37.50	35.70	38.40	38.60	36.80	35.70	31.90	28.30	28.90	30.70	31.30
<b>SERUM GLOBULIN (g/l)</b>													
C	mean	23.72	23.94	24.74	23.58	25.62	23.14	24.62	21.00	22.42	24.10	23.36	23.08
	max	26.90	30.60	27.10	28.30	31.80	28.40	28.80	25.20	29.60	29.50	29.00	25.20
	min	19.50	21.00	19.70	19.90	19.90	19.20	21.20	17.30	16.60	20.70	20.70	19.00
LT	mean	20.60	21.70	23.62	21.60	22.06	20.92	24.96	23.30	21.24	23.60	22.84	24.76
	max	24.40	24.70	25.00	24.60	25.30	23.20	27.80	27.10	23.20	26.70	26.50	28.10
	min	17.80	18.20	22.20	20.10	19.80	18.50	21.70	19.50	19.00	21.30	19.30	17.70
MT	mean	22.08	23.88	23.82	21.24	23.12	23.76	29.16	26.00	26.76	27.62	29.92	25.46
	max	25.70	27.00	26.30	23.60	26.30	29.00	36.10	30.40	28.50	34.70	37.90	30.40
	min	17.00	22.00	20.40	15.10	18.90	20.30	25.30	22.10	24.60	24.70	23.30	23.00
HT	mean	24.70	27.48	24.72	26.30	25.06	27.86	32.72	29.26	29.60	30.12	26.10	28.08
	max	27.10	37.80	29.60	31.00	30.00	31.90	37.90	35.00	34.80	34.60	31.50	32.90
	min	19.30	21.30	18.80	20.20	19.20	19.50	29.90	25.70	23.90	25.80	17.60	20.80

WEEK		0	1	2	3	4	5	6	7	8	9	10	11
<b>SERUM PEPSINOGEN (mU Tyrosine/I)</b>													
C	mean	139	90	81	160	102	100	94	111	186	84	142	124
	max	291	178	217	377	167	195	150	283	274	217	274	217
	min	17	17	17	16	16	17	17	49	86	16	34	17
LT	mean	198	360	189	146	180	260	243	175	181	228	264	213
	max	349	1336	380	211	285	491	428	365	308	618	682	555
	min	34	16	16	95	86	103	32	16	16	16	32	34
MT	mean	355	202	150	254	320	268	242	234	322	254	302	280
	max	1062	357	260	523	808	602	476	476	650	422	444	444
	min	137	51	51	137	84	16	16	120	69	137	227	137
HT	mean	123	335	134	186	484	608	482	377	473	454	454	474
	max	301	1463	285	333	808	1157	682	713	697	745	697	840
	min	16	16	16	34	200	67	235	95	50	16	101	185
<b>SERUM GASTRIN (pmol)</b>													
C	mean	73	83	68	58	70	55	45	55	49	59	50	64
	max	131	135	139	102	106	74	63	68	79	69	74	73
	min	46	43	40	42	36	35	32	32	33	50	28	41
LT	mean	44	56	52	77	71	68	64	61	67	62	68	59
	max	58	77	94	137	105	97	77	82	88	107	114	96
	min	35	36	31	35	40	45	50	49	50	36	32	36
MT	mean	38	43	51	96	80	131	95	92	91	91	97	107
	max	42	49	75	144	106	273	138	135	172	179	170	194
	min	33	30	39	38	50	81	62	56	57	63	43	49
HT	mean	57	48	61	80	79	83	72	84	90	103	91	100
	max	92	78	86	124	112	118	97	108	166	130	138	161
	min	19	24	29	53	48	42	56	70	41	63	57	74
<b>FAECAL EGG COUNT (epg)</b>													
C	mean	0	0	0	0	0	0	0	0	0	0	0	0
	max	0	0	0	0	0	0	0	0	0	0	0	0
	min	0	0	0	0	0	0	0	0	0	0	0	0
	No. >0	0	0	0	0	0	0	0	0	0	0	0	0
LT	mean	0	0	0	20	40	190	50	50	70	90	0	10
	max	0	0	0	100	100	400	150	200	300	350	0	50
	min	0	0	0	0	0	0	0	0	0	0	0	0
	No. >0	0	0	0	1	2	4	3	2	2	2	0	1
MT	mean	0	0	0	60	100	90	90	30	50	0	40	10
	max	0	0	0	200	150	150	250	50	150	0	150	50
	min	0	0	0	0	0	50	50	0	0	0	0	0
	No. >0	0	0	0	3	4	5	5	3	3	0	2	1
HT	mean	0	0	0	40	40	140	120	60	90	60	30	10
	max	0	0	0	100	100	350	350	150	300	200	150	50
	min	0	0	0	0	0	0	0	0	0	0	0	0
	No. >0	0	0	0	3	2	3	4	3	3	3	1	1
<b>FAECAL LARVAE COUNT (Ipg)</b>													
C	mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	max	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	min	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	No. >0	0	0	0	0	0	0	0	0	0	0	0	0
LT	mean	0.0	0.0	0.0	3.0	16.9	36.3	20.5	58.3	23.5	17.7	15.3	10.4
	max	0.0	0.0	0.0	9.0	55.3	119.3	46.8	258.0	97.5	58.8	59.3	27.3
	min	0.0	0.0	0.0	0.3	3.5	4.8	1.3	0.0	0.0	0.3	0.8	0.3
	No. >0	0	0	0	5	5	5	5	3	4	5	5	5
MT	mean	0.0	0.0	0.0	18.8	58.5	122.9	112.2	48.7	26.3	20.6	5.0	2.6
	max	0.0	0.0	0.0	34.8	164.0	263.0	338.0	147.0	63.8	47.0	12.8	5.3
	min	0.0	0.0	0.0	5.0	5.8	4.5	1.8	1.3	0.0	3.0	1.3	0.3
	No. >0	0	0	0	5	5	5	5	5	4	5	5	5
HT	mean	0.0	0.0	0.0	25.1	50.5	81.5	115.0	115.0	72.9	95.2	55.1	23.8
	max	0.0	0.0	0.0	83.3	133.3	221.8	198.0	229.8	240.3	323.3	128.8	86.3
	min	0.0	0.0	0.0	1.0	13.0	6.0	45.0	8.8	2.0	5.0	0.3	0.0
	No. >0	0	0	0	5	5	5	5	5	5	5	5	4
<b>NEMATODE COUNTS</b>													
		Lungworm			GI	Abo	SI	LI	Abo	Abo			
		Total	Adult	Immature	Total	Total	Total	Total	Ost-types	Trich			
		Total	Total	Total	Total	Total	Total	Total	Total	Total			
C	mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
	max	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
	min	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
LT	mean	30.8	28.6	2.2	439.2	435.4	0.0	3.8	239.3	194.1			
	max	51.0	50.0	4.0	998.0	991.0	0.0	9.0	446.0	545.1			
	min	10.0	7.0	1.0	269.0	260.0	0.0	0.0	154.6	59.8			
	No. >0	5	5	5	5	5	0	3	5	5			
MT	mean	32.0	30.0	2.0	612.4	593.2	14.0	5.2	340.2	250.8			
	max	59.0	57.0	4.0	1118.0	1081.0	30.0	9.0	454.0	627.0			
	min	19.0	15.0	0.0	95.0	80.0	0.0	1.0	80.0	0.0			
	No. >0	5	5	4	5	5	3	5	5	2			
HT	mean	73.0	68.6	4.4	806.4	786.8	14.0	5.6	547.7	239.2			
	max	161.0	159.0	8.0	1544.0	1532.0	50.0	14.0	934.5	597.5			
	min	7.0	7.0	0.0	213.0	200.0	0.0	2.0	200.0	0.0			
	No. >0	5	5	4	5	5	3	5	5	4			

**APPENDIX 4.2. APPENDIX TO CHAPTER 4. NATURALLY REARED DEER (MT INFECTION RATE)**

WEEK		0	1	2	3	4	5	6	7	8	9	10	11
<b>TOTAL LEUCOCYTES (x10<sup>9</sup>/l)</b>													
NR	mean	5.04	6.18	5.02	4.76	4.98	4.62	4.62	4.40	5.40	4.80	5.15	5.65
	max	5.90	7.80	6.40	6.30	6.70	6.20	5.70	5.80	6.40	5.80	5.80	6.80
	min	4.00	5.10	4.20	3.60	2.90	3.10	2.60	1.90	4.00	4.20	3.90	4.30
<b>EOSINOPHILS DIFFERENTIAL LEUCOCYTE COUNTS (x10<sup>9</sup>/l)</b>													
NR	mean	0.03	0.06	0.20	0.19	0.39	0.29	0.21	0.18	0.18	0.29	0.49	0.44
	max	0.08	0.13	0.45	0.38	0.77	0.68	0.40	0.34	0.25	0.44	0.74	0.61
	min	0.00	0.00	0.00	0.04	0.11	0.00	0.00	0.07	0.10	0.17	0.12	0.30
<b>NEUTROPHILS</b>													
NR	mean	1.41	3.26	1.48	1.21	0.75	0.76	1.22	1.18	1.40	1.34	0.58	1.04
	max	1.82	4.21	1.98	2.02	1.07	1.30	1.82	1.97	2.11	2.11	0.70	1.56
	min	1.09	2.70	0.88	0.80	0.41	0.46	0.60	0.59	0.80	0.69	0.47	0.69
<b>LYMPHOCYTES</b>													
NR	mean	3.21	2.50	3.02	2.88	3.28	3.03	2.48	2.76	3.31	2.82	3.17	3.66
	max	4.03	4.29	3.71	3.21	4.15	3.73	3.19	4.10	4.10	3.71	3.89	4.62
	min	2.52	1.48	1.94	2.23	2.12	1.43	0.65	0.49	2.56	1.96	2.38	3.01
<b>MONOCYTES</b>													
NR	mean	0.22	0.25	0.27	0.35	0.41	0.42	0.35	0.14	0.20	0.14	0.75	0.41
	max	0.30	0.45	0.47	0.41	0.58	0.62	0.56	0.20	0.32	0.22	0.91	0.61
	min	0.17	0.11	0.10	0.29	0.15	0.23	0.03	0.04	0.05	0.06	0.58	0.22
<b>BASOPHILS</b>													
NR	mean	0.18	0.10	0.05	0.14	0.16	0.13	0.37	0.14	0.31	0.22	0.16	0.10
	max	0.65	0.20	0.13	0.32	0.26	0.37	0.80	0.23	0.51	0.38	0.23	0.24
	min	0.00	0.00	0.00	0.00	0.04	0.00	0.08	0.05	0.06	0.12	0.10	0.04
<b>TOTAL ERYTHROCYTES (x10<sup>12</sup>/l)</b>													
NR	mean	12.40	11.92	11.23	11.62	11.24	11.56	10.62	10.91	10.57	10.63	10.28	10.23
	max	13.49	12.86	11.94	12.92	11.89	12.00	11.46	12.10	11.66	12.77	11.74	11.06
	min	11.73	11.44	10.83	11.04	10.37	10.81	9.67	10.02	9.76	9.00	8.47	9.34
<b>PACKED CELL VOLUME (%)</b>													
NR	mean	0.46	0.45	0.42	0.44	0.43	0.45	0.41	0.43	0.42	0.42	0.42	0.42
	max	0.52	0.50	0.46	0.51	0.47	0.47	0.45	0.46	0.45	0.45	0.46	0.46
	min	0.43	0.43	0.40	0.42	0.39	0.42	0.38	0.39	0.39	0.41	0.38	0.38
<b>HAEMOGLOBIN CONCENTRATION (g/l)</b>													
NR	mean	162.6	155.6	151.4	162.0	154.8	158.2	145.0	155.2	158.0	158.0	158.8	152.3
	max	183.0	174.0	167.0	185.0	173.0	172.0	156.0	166.0	166.0	169.0	169.0	168.0
	min	150.0	148.0	142.0	151.0	145.0	147.0	132.0	140.0	149.0	149.0	147.0	137.0
<b>SERUM TOTAL PROTEIN (g/l)</b>													
NR	mean	68.20	68.80	66.37	66.48	68.68	68.06	69.50	62.84	61.55	66.05	66.60	67.05
	max	78.80	69.60	68.20	69.60	73.20	69.40	74.20	69.60	65.80	68.40	69.80	70.10
	min	62.20	67.70	64.50	64.70	60.50	66.50	63.80	47.90	56.90	61.70	63.40	63.80
<b>SERUM ALBUMIN (g/l)</b>													
NR	mean	41.90	38.80	38.13	38.10	39.23	37.74	37.26	34.88	32.20	34.78	34.65	38.15
	max	53.50	43.40	41.40	43.20	40.20	40.80	42.00	39.60	34.90	38.70	37.40	41.60
	min	37.20	35.50	36.00	35.80	38.70	35.60	32.40	22.10	27.30	33.00	33.40	34.50
<b>SERUM GLOBULIN (g/l)</b>													
NR	mean	26.55	30.00	27.53	28.38	29.45	30.88	32.93	29.13	29.35	31.28	31.95	28.90
	max	30.40	32.90	29.60	32.20	34.00	31.90	36.70	34.60	32.30	34.30	36.40	35.60
	min	24.30	24.30	25.00	23.50	20.30	28.90	27.60	25.80	26.70	28.40	27.80	23.80
<b>SERUM PEPSINOGEN (mU Tyrosine/l)</b>													
NR	mean	305.0	250.2	322.2	311.8	376.8	414.6	476.2	445.4	454.3	535.5	578.3	629.8
	max	617.0	463.0	583.0	531.0	617.0	805.0	942.0	788.0	737.0	788.0	822.0	640.0
	min	120.0	86.0	171.0	137.0	154.0	171.0	154.0	154.0	137.0	291.0	240.0	86.0
<b>SERUM GASTRIN (pmol)</b>													
NR	mean	60.2	65.8	54.0	75.0	89.8	86.6	87.4	113.0	161.3	121.8	165.8	132.3
	max	125.0	90.0	69.0	132.0	176.0	207.0	162.0	237.0	405.0	249.0	437.0	306.0
	min	40.0	34.0	41.0	45.0	42.0	32.0	41.0	57.0	44.0	56.0	60.0	65.0
<b>FAECAL EGG COUNT (epg)</b>													
NR	mean	0.0	0.0	0.0	12.5	162.5	187.5	87.5	75.0	37.5	50.0	12.5	62.5
	max	0.0	0.0	0.0	50.0	300.0	300.0	150.0	150.0	100.0	100.0	50.0	200.0
	min	0.0	0.0	0.0	0.0	50.0	100.0	50.0	50.0	0.0	0.0	0.0	0.0
	No. >0	0	0	0	1	5	5	5	4	2	3	1	2
<b>FAECAL LARVAE COUNT (lpg)</b>													
NR	mean	0.0	0.0	0.0	23.4	74.6	181.5	86.9	43.6	56.1	51.4	7.6	62.5
	max	0.0	0.0	0.0	42.8	161.5	569.3	224.8	116.0	85.8	115.5	17.3	200.0
	min	0.0	0.0	0.0	6.3	12.5	16.8	11.5	8.5	13.3	17.0	1.3	0.0
	No. >0	0	0	0	4	5	5	5	4	4	4	4	4
<b>NEMATODE COUNTS</b>													
		Lungworm			GI	Abo	SI	LI	Abo	Abo			
		Total	Adult	Immature	Total	Total	Total	Total	Ost-types	Trich			
NR	mean	43.0	40.8	2.3	530.0	488.5	37.5	4.0	477.5	11.0			
	max	111.0	106.0	5.0	1197.0	1193.0	150.0	7.0	1169.1	23.9			
	min	7.0	6.0	1.0	187.0	180.0	0.0	1.0	169.2	0.0			
	No. >0	5	5	4	5	5	1	4	5	3			

**APPENDIX 6.1.**

**APPENDIX TO CHAPTER 6.**

WEEK		0	1	2	3	4	5	6	WEEK		0	1	2	3	4	5	6
<b>TOTAL EOSINOPHILS (x10<sup>9</sup>/l)</b>									<b>TOTAL LEUCOCYTES (x10<sup>9</sup>/l)</b>								
L	mean	0.00	1.00	2.00	3.00	4.00	5.00	6.00	L	mean	3.69	3.83	3.39	3.47	3.70	3.84	4.05
	max	0.00	1.00	2.00	3.00	4.00	5.00	6.00		max	4.20	5.80	4.50	5.00	5.00	5.00	4.80
	min	0.00	1.00	2.00	3.00	4.00	5.00	6.00		min	3.00	2.70	2.60	2.40	3.00	2.70	3.00
B	mean	0.00	1.00	2.00	3.00	4.00	5.00	6.00	B	mean	3.40	3.81	3.83	3.57	3.40	3.85	3.77
	max	0.00	1.00	2.00	3.00	4.00	5.00	6.00		max	4.60	5.30	6.60	5.70	4.60	7.00	6.70
	min	0.00	1.00	2.00	3.00	4.00	5.00	6.00		min	2.60	2.20	2.40	2.50	2.30	2.40	1.50
S	mean	0.00	1.00	2.00	3.00	4.00	5.00	6.00	S	mean	3.59	3.45	4.33	3.67	3.72	3.70	3.59
	max	0.00	1.00	2.00	3.00	4.00	5.00	6.00		max	4.80	5.00	6.70	6.60	6.10	5.20	4.50
	min	0.00	1.00	2.00	3.00	4.00	5.00	6.00		min	2.80	2.00	2.70	2.30	2.60	3.00	3.00
<b>TOTAL NEUTROPHILS (x10<sup>9</sup>/l)</b>									<b>TOTAL ERYTHROCYTES (x10<sup>12</sup>/l)</b>								
L	mean	0.00	1.00	2.00	3.00	4.00	5.00	6.00	L	mean	12.53	11.91	11.49	11.51	11.24	11.10	11.65
	max	0.00	1.00	2.00	3.00	4.00	5.00	6.00		max	14.06	13.28	12.78	13.48	12.42	12.47	13.20
	min	0.00	1.00	2.00	3.00	4.00	5.00	6.00		min	11.02	10.69	10.27	9.86	9.97	9.94	10.04
B	mean	0.00	1.00	2.00	3.00	4.00	5.00	6.00	B	mean	12.78	12.04	11.36	11.07	10.73	11.10	11.27
	max	0.00	1.00	2.00	3.00	4.00	5.00	6.00		max	14.82	12.97	13.16	12.52	11.90	12.81	13.20
	min	0.00	1.00	2.00	3.00	4.00	5.00	6.00		min	11.19	10.96	10.01	10.13	9.57	9.94	10.17
S	mean	0.00	1.00	2.00	3.00	4.00	5.00	6.00	S	mean	13.25	12.09	11.96	11.28	11.01	11.45	12.14
	max	0.00	1.00	2.00	3.00	4.00	5.00	6.00		max	15.20	14.44	14.51	13.77	12.94	13.40	13.23
	min	0.00	1.00	2.00	3.00	4.00	5.00	6.00		min	11.86	10.46	10.98	10.34	8.71	10.20	10.96
<b>TOTAL LYMPHOCYTES (x10<sup>9</sup>/l)</b>									<b>FAECAL EGG COUNT (epg)</b>								
L	mean	0.00	1.00	2.00	3.00	4.00	5.00	6.00	L	mean	0	7.5	65.0	140.0	142.5	150.0	210.0
	max	0.00	1.00	2.00	3.00	4.00	5.00	6.00		max	0	25.0	150.0	450.0	350.0	300.0	550.0
	min	0.00	1.00	2.00	3.00	4.00	5.00	6.00		min	0	0.0	25.0	25.0	25.0	50.0	50.0
B	mean	0.00	1.00	2.00	3.00	4.00	5.00	6.00	B	No. >0	0	7	10	10	10	10	
	max	0.00	1.00	2.00	3.00	4.00	5.00	6.00		mean	0	60.0	87.5	87.5	195.0	240.0	140.0
	min	0.00	1.00	2.00	3.00	4.00	5.00	6.00		max	0	500.0	450.0	250.0	550.0	600.0	450.0
S	mean	0.00	1.00	2.00	3.00	4.00	5.00	6.00	S	min	0	0.0	25.0	25.0	50.0	50.0	25.0
	max	0.00	1.00	2.00	3.00	4.00	5.00	6.00		No. >0	0	5	10	10	10	10	
	min	0.00	1.00	2.00	3.00	4.00	5.00	6.00		mean	0	32.5	87.5	162.5	230.0	275.0	167.5
									max	0	250.0	350.0	550.0	700.0	1200.0	450.0	
									min	0	0.0	25.0	25.0	25.0	50.0	25.0	
									No. >0	0	4	10	10	10	10	10	





## ADDENDA

Page 1.	Line 9.	Replace <u>Pere Davids deer</u> with <u>Pere David's deer</u> .
Page 3.	Table 1.1.	Replace <u>22 kg/hd</u> with <u>50 kg/hd</u> for 90/91 season.
Page 11.	Line 25.	Replace <u>Pere Davids deer</u> with <u>Pere David's deer</u> .
Page 14.	Line 26.	Replace <u>Edu</u> with <u>Adu</u> .
Page 29.	Line 5.	Replace <u>defense</u> with <u>defence</u> .
Page 29.	Line 7.	Replace <u>defense</u> with <u>defence</u> .
Page 31.	Line 16.	Replace <u>lotus</u> with <u>Lotus</u> .
Page 56.	Line 1.	Replace <u>diet selected</u> with <u>estimated diet selected</u> .
Page 65.	Line 1.	Replace <u>diet selected</u> with <u>estimated diet selected</u> .
Page 65.	Line 12.	Replace <u>diet selected</u> with <u>estimated diet selected</u> .
Page 92.	Title.	Replace <u>drenched</u> with <u>treated</u> .
Page 162.	Line 27.	Replace <u>36hrs)</u> with <u>36hrs</u> .
Page 225.		Insert reference: Randall, R.W. & Gibbs, H.C. 1981. Effect of clinical and sub-clinical gastrointestinal helminthiasis on digestion and energy metabolism in calves. American Journal of Veterinary Research 42: 1730-1734.