

## TITLE

THE EPIDEMIOLOGY, IMPACT AND CONTROL OF GASTROINTESTINAL  
NEMATODE INFECTIONS OF GOATS IN KENYA AND SHEEP IN DENMARK.

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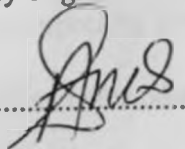
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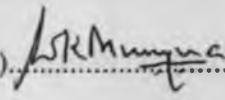
A thesis submitted to the University of Nairobi in partial fulfilment of the  
requirements for the degree of Doctor of Philosophy (Ph.D.).

## DECLARATION

This thesis is my original work and has not been submitted for a degree in any other University.

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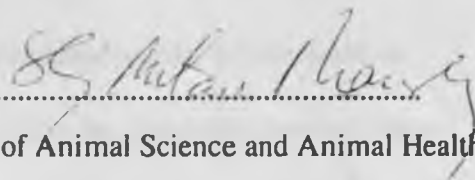
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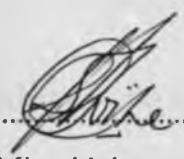
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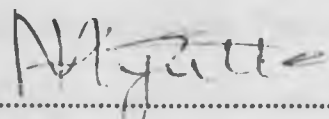
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## Summary

Gastrointestinal nematodes are considered a serious economic problem affecting the livestock industry especially in the tropical and subtropical areas of the world. Control of these gastrointestinal nematodes is best achieved through interventions based on the epidemiology and bionomics of the parasites.

In Kenya, the epidemiology of nematode infections in cattle and sheep has been established in Kiambu and Nyandarua Districts respectively. Information on the epidemiology of these parasites in goats is limited to the work carried out on a mixed sheep and goat farm in the semi arid area of Naivasha. Studies reported in this thesis were undertaken to understand the epidemiology of gastrointestinal nematodes in goat flocks on small scale farms in a marginal area of Thika District. The impact of naturally acquired gastrointestinal nematodes on production in set stocked young goats in this area was also examined.

Two field studies were undertaken to determine the possibility of using an integrated grazing management strategy for the control of gastrointestinal nematode infections in naturally infected weaner lambs and the possibility of using the nematode trapping fungus *Duddingtonia flagrans* in the prevention and control of natural trichostrongyle infections in weaner lambs on pasture in Denmark.

The prevalence and intensity of infection with gastrointestinal nematodes in goats was monitored on 4 small scale farms in a marginal low potential area of Thika District, Kenya over a period of one year (March 1997 - February 1998). The number of nematode eggs per gram (EPG) of faeces varied between farms and with seasons. They were highest during the long rains season (April - June) and also during the heavy short rains (November - February). Differences in EPG levels between age groups of the goats were only observed during the dry season when kids had higher levels than the adults. *Haemonchus contortus* was the main

nematode recovered from coprocultures in all the farms (69 - 100% of all the larva recovered and identified from coprocultures in the farm).

Five flock anthelmintic treatments were given depending on EPG levels of the sampled goats and on the farmers request according to the time they drenched. Four of these treatments were given during the rainy months (April, June, November 1997 and February 1998) and one at the end of the dry month (September). These findings suggested that the prevalence and intensity of gastrointestinal nematode parasite infections in goats in this marginal area of Thika District varied with seasons. The intensity was highest during the rainy season. Differences between age groups only occur during the dry season. *H. contortus* was the main nematode infecting the goats. These findings also suggested that nematode control in goats in this area should be aimed at all ages during the rainy season and kids during the dry season. The number of anthelmintic treatments should be reduced to minimise the cost to the farmer and also delay the development and spread of anthelmintic resistance.

A further study was undertaken for 6 months on two farms where only individual salvage anthelmintic treatments were given to those goats with EPG levels of or over 1500. There were no differences in the age-specific EPG levels nor in the number of goats given salvage treatment in each age group. Parasitic gastroenteritis occurred in kids and lactating does at the end of the dry season with a sudden rise in EPG levels just before the rains. *Haemonchus contortus* was the main nematode recovered from coprocultures.

In conclusion, clinical haemonchosis occurs at the end of the dry season affecting mainly kids and lactating does. This may be as a result of hypobiotic larvae resuming development as has been observed in other studies. Hypobiosis may be occurring in these goats during the dry season and playing a role in the epidemiology of *H. contortus*. Age-specific resistance may not be occurring in goats due to management practices and their

selective feeding habits. Where facilities for laboratory diagnosis are available, individual anthelmintic treatment after sample analysis is recommended as it is cheaper to the farmer and delays the development of anthelmintic resistance. The FAMACHA technique may also be applicable as the main helminth is the blood sucking *H. contortus*. Kids (3 - 6 months) and lactating does should be treated at the end of the dry season to reduce production losses.

The impact of natural gastrointestinal nematodes on production in young goats was investigated using 44 weaner goats (4 - 5 months old) divided into 4 even groups. Each was set - stocked on pasture for 6 months. Goats in two of the groups were suppressively treated every 2 weeks with albendazole at a dosage of 1mg per 10 kg body weight while the other 2 were left as untreated controls. The suppressive treatment prevented weight loss, parasitic gastroenteritis and mortalities in the weaners. Individual goats in the treated groups gained a mean of  $3.1 \pm 0.3$ kg while those in the controls groups gained a mean of  $1.1 \pm 0.2$ kg over the study period of 8 months. Two goats from the control groups died and 2 others from the same group were given salvage anthelmintic treatment after showing signs of parasitic gastroenteritis with EPG levels of between 3500 - 6500 at the end of the dry season. The group-specific EPG levels were significantly higher for the controls. The suppressive treatment did not eliminate faecal egg shedding in the treated groups. A rise reaching 100 EPG at the end of the study was recorded. This may have been due to continuous picking of larvae or selection of resistant nematodes. The number of larvae per kg dry herbage was higher for the paddocks where control groups of goats were kept. The total worm burdens at slaughter after 8 months on pasture were higher for the control groups and *Haemonchus contortus* comprised almost 100% of the total worm burdens in all the goats. Hypobiotic *H. contortus* larvae were recovered at the end of the short rain season (December when the experiment was terminated) in the experimental goats and tracers. Most (70%) were

recovered in the abomasal contents and the rest from mucosal digesta.

Thus, it was shown that natural gastrointestinal nematode infections cause production losses through reduced weight gain, weight losses and mortalities in goats in the study area. The abomasal blood sucking *H. contortus* is the main nematode and undergoes hypobiosis at the end of the rainy season and most of the inhibited larvae are found in the abomasal contents and a few embedded in the mucosa. Regular suppressive anthelmintic treatments prevent production losses but may also lead to selection of resistant nematode populations. Anthelmintic treatments to goats in this area should be given at the end of the dry season and at the beginning of the rainy season to avoid production losses and mortalities.

Alternative control methods of natural gastrointestinal nematode infections in weaner lambs on pasture using integrated grazing management was investigated in Denmark. Sixteen ewes with twin lambs were turned out on naturally infected pastures at the beginning of the grazing season in May. On 1<sup>st</sup> July, the lambs were weaned and randomly allocated to 4 similar groups. Two groups were treated with fenbendazole (dose and move) while the other groups were left untreated (move only). All the groups were moved to clean pasture which had been rested/ spelled for one year and set-stocked in individual group paddocks until slaughter for worm count and identification at the end of September. There was no clinical parasitic gastroenteritis throughout the grazing period. Movement of the lambs to clean pasture without treatment resulted in reduced EPG levels in lambs to less than one third. The move groups had significantly higher EPG levels for the first 6 weeks on the clean pasture compared to the treated and moved groups. The pasture infectivity was also lower in the dose and move paddocks. The weight gains and the serum albumin levels were comparable for all the groups. On total worm counts, significant differences were seen in the number of immature in abomasum - mainly *Ostertagia circumcincta* and small intestinal

*Trichostrongylus vitrinus* ( higher for the move).

Therefore, it was concluded that it is possible to achieve sufficient nematode control and good production levels in weaned lambs without the use of anthelmintics when the lambs are weaned at the beginning of July and moved to a clean pasture for up to 3 months. For a period longer than 3 months, a second move or an anthelmintic treatment may be necessary to avoid production losses and mortalities. This system is applicable in regions where there is a defined grazing period ending with slaughter or housing. In the tropics with no defined grazing season and where transmission and infection especially with *H. contortus* occurs throughout the year, a rapid rotation system of grazing management is recommended as an alternative control method. This is compatible with the shepherding and tethering methods of husbandry common in most tropical areas where small ruminants are raised.

Biological control of natural gastrointestinal nematodes in weaner lambs using the nematode destroying fungus *Duddingtonia flagrans* was studied in Denmark over one grazing period. Four groups of 8 parasite-naïve lambs (3 - 4 months) were turned out on infected pasture in May. Between May and September, 2 groups received *Duddingtonia flagrans* ( $10^6$  chlamydospores per kg body weight per lamb per day) mixed with 100g barley while the other groups only received barley. All groups were set stocked on separate paddocks until slaughter in October. Severe parasitic gastroenteritis occurred in late June affecting all the groups and all the lambs were treated with fenbendazole at a dosage rate of 7.5mg per kg body weight. The EPG levels were comparable for the two treatments throughout the grazing period. Larval development of *Ostertagia/ Trichostrongylus spp* in faecal cultures was 1-28% in the fungi fed groups compared to 60 - 80% in the untreated groups. In September, pasture larval counts were lower in the paddocks grazed by the fungi fed lambs for the *Ostertagia/Trichostrongylus* (930: 4,400 L3/kg) and *Nematodirus spp* (7,200 : 11,600 L3/kg).

At slaughter, the number of immature *Ostertagia spp* was 62% lower in the fungi fed group compared to the untreated groups. Four parasite free lambs introduced into each paddock in October and slaughtered after a 3-week housing period showed significant reduction in total worm burdens in those that were in the paddocks previously grazed by fungi-fed lambs. The total worm burdens were reduced by 86%. The abomasal counts were reduced by 68% ( $P<0.05$ ) while the numbers of *N. spathiger* were reduced by 98% and those for *N. battus* by 97% compared to control group.

In conclusion, dosing sheep on pasture with *D. flagrans* limit the build up of pasture contamination in the late grazing season and subsequently limits the intake of larvae in sheep. This study showed that the strategic use of nematophagous fungi will not eliminate the occurrence of clinical or subclinical disease in early spring due to heavy overwintering infection nor overcome the typical spring nematodiosis. Hence, control in sheep enterprises should be based on an integrated strategy where biological control agents such as *D. flagrans*, grazing management and /or anthelmintics each play a part. The use of a fungal biocontrol agent is a strict preventive measure reducing reinfection with larvae developed from eggs excreted after turnout. This may reduce the otherwise pathogenic worm burdens in the later part of the season to such low but sufficient levels to allow development of natural immunity.

## ACKNOWLEDGEMENT:

To God be the glory for His steadfast love, mercies, grace and favour to me for all these years..

It has taken the support, understanding and facilitation of many people in Kenya and Denmark where this study was carried out between 1995 and 1999 for this work to be accomplished.

My heartfelt gratitude and respect goes to my supervisor the late Professor Peter Nansen (1938 - 26<sup>th</sup> Nov. 1999), who never lived to see the completion of this work. Without his enormous support, facilitation and advice this work would not have been completed. I fully appreciate the warm friendship he extended to me during my stay in Denmark in 1995 and 1999.

I thank my supervisors in Kenya: Prof. W.K. Munyua , Dr N. Maingi and Dr M.N. Kyule for their assistance and advice in the planning and preparation of the project proposals, field studies and writing of the thesis.

I am very grateful for the role played by Prof. S.M. Thamsborg during my studies in Denmark. His advice and suggestions during the planning, his assistance, facilitation and practical help during the field and laboratory studies are greatly appreciated. He also gladly took over official supervision when Professor Nansen passed away. Professor Stig, thank you also for being a friend.

Without the assistance, help and commitment of Dr Michael Larsen, the field studies in Denmark would not have been possible. His help in the planning and coordination of the “fungi group” is greatly appreciated. He worked very hard to make sure that the field and laboratory studies ran smoothly. He was kind enough to introduce me to the intriguing and fascinating world of the nematode destroying fungi. I benefited from his generous guidance

and expertise. Thank you M. Larsen for your interest in my success.

The District Livestock Production officers - Thika District - Mr W.M. Wandai and Mrs M. Waithaka and the livestock extension officer - Ruiru division Mr Kiiru introduced me to the farmers whose farms and animals were used in this study. I thank them most sincerely.

The owners and management of Kionama Estate are also thanked for allowing the use of their farm for construction of experimental facilities. Their understanding and facilitation is greatly appreciated. Mr Kabiru the capable shepherd is thanked for looking after the experimental goats.

The laboratory work in Copenhagen would have been impossible without the assistance and understanding of Lilian Sztuk, Tina Skov and Rikke Anderson. Their help is greatly appreciated.

The field work in Denmark was accomplished with the dedicated help of Niels Midtgaard, Jørgen, O., Helveka, L. (Danish vet. student), Julija and Malija M. (Lithuanian vet. students). It was my pleasure working with such dedicated people and I thank them sincerely.

The skilled technical assistance of Virginia Mumbi Maina (nee Gichohi). Richard Otieno, Mary Mutune and Patrick Wahome who assisted me in the field and laboratory work in Kenya is also very much appreciated. They were “a great people” to work with.

The role of Prof. S.M. Gichovih of the department of Agricultural economics in facilitation of transport to the research sites is acknowledged. I sincerely thank him and his able driver Mr S. M. Njoka.

Without the assistance of friends and colleagues like Saulius Petkevicius, Anders Permin, Shoshana Coates, Nina Stenhard, Toben Schou, Silvina Fernandez, Makundi, A.E. and Kensuke Taira life at the Danish Centre for Experimental Parasitology (DCEP)

“museum” would not have been fruitful and interesting. Their varied inputs are greatly appreciated.

My special thanks go to Helle Kursten, Anne L. Bisp, Prof. S.M. Thamsborg, N.C. Kyvsgaard and A.L. Willingham who took care of my travel arrangements and stay in Denmark. The DANIDA Fellowship Centre (DFC) staff are thanked for facilitating the accommodation.

I thank my employer, the University of Nairobi for granting study leave during the years 1995 and 1999 to undertake the field and laboratory studies in Denmark.

Words fail me to express my gratitude to my helper and companion in life- my wife Catherine Nduta for allowing me to be the “absent” husband and father. Without her support and prayers I would not have made it. I also thank our children Githigia and Wanjiru for their cooperation and agreeing to have an absent father.

The many prayers and encouragement offered by brethren in Kenya and Denmark are greatly appreciated. Your prayers and encouragement went a long way in helping me to accomplish this work. God bless you all.

This study was funded by DANIDA - ENRECA through the Livestock Helminth Research Collaborative Project between University of Nairobi and The Royal Veterinary and Agricultural University Copenhagen, Denmark. The field studies in Denmark were partly funded by the Danish National Research Foundation and the Chr. Hansen Biosystems S/A. All these sources of funds are greatly appreciated and acknowledged.

## Table of contents

|                                                                           |          |
|---------------------------------------------------------------------------|----------|
| Title page .....                                                          | i        |
| Declaration .....                                                         | ii       |
| Summary .....                                                             | iii      |
| Acknowledgements .....                                                    | ix       |
| Table of contents .....                                                   | xii      |
| List of Tables .....                                                      | xviii    |
| List of Figures .....                                                     | xxv      |
| Dedication .....                                                          | xxix     |
| <b>Chapter 1. Introduction and objectives of the study .....</b>          | <b>1</b> |
| 1.1. Introduction .....                                                   | 1        |
| 1.2. Objectives of the study .....                                        | 5        |
| <b>Chapter 2. Literature Review .....</b>                                 | <b>6</b> |
| 2.1. The place of small ruminants in livestock development in Kenya ..... | 8        |
| 2.2. Gastrointestinal nematode infections in small ruminants .....        | 9        |
| 2.3. The role of hypobiosis in the epidemiology of haemonchosis .....     | 12       |
| 2.4. Impact of helminth infection on the host's nutrition .....           | 13       |
| 2.5. Diagnosis of gastrointestinal nematode infections .....              | 14       |
| 2.6. Principles of helminth control .....                                 | 15       |
| 2.7. Availability and distribution of anthelmintics .....                 | 16       |
| 2.8. Alternative dosing methods .....                                     | 16       |
| 2.9. Anthelmintic resistance .....                                        | 17       |
| 2.10. Alternative methods of helminth control .....                       | 19       |
| 2.10.1. Grazing management .....                                          | 19       |

|                                                                                         |    |
|-----------------------------------------------------------------------------------------|----|
| 2.10.1.1. Forms of grazing management .....                                             | 21 |
| 2.10.1.2. Preventive strategies .....                                                   | 21 |
| 2.10.1.3. Evasive strategies .....                                                      | 23 |
| 2.10.1.4. Diluting strategies .....                                                     | 25 |
| 2.10.1.5. The role of nematode epidemiological knowledge in grazing<br>management ..... | 26 |
| 2.10.2. Prospects of a vaccine .....                                                    | 28 |
| 2.10.3. Genetic resistance .....                                                        | 29 |
| 2.10.4. Biological control .....                                                        | 31 |
| 2.10.4.1. Biological control by manipulation of the environment .....                   | 33 |
| 2.10.4.2. Biological control by manipulation of the organisms .....                     | 33 |
| 2.10.4.3. Candidates for biological control .....                                       | 33 |
| 2.10.4.4. Effect of nematode trapping fungi on nematode parasites .....                 | 36 |
| 2.10.4.5. Methods of selecting fungi as biological control agents .....                 | 37 |
| 2.11. Conclusion on non - chemical control methods .....                                | 39 |

### **Chapter 3. Section 1: The epidemiology and prevalence of gastrointestinal**

|                                                                                                                                                                                  |           |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| <b>nematodes in goats in the marginal low potential areas of Thika District .</b>                                                                                                | <b>41</b> |
| 3.1. Introduction to the study area - Thika District .....                                                                                                                       | 41        |
| 3.2. Livestock population in Kakuzi and Ruiru divisions .....                                                                                                                    | 41        |
| 3.3. Goat farm structure and types in Kakuzi and Ruiru divisions .....                                                                                                           | 43        |
| 3.3.1. Management and role of goats in these divisions .....                                                                                                                     | 44        |
| 3.3.2. Studies on the prevalence and intensity of infection with gastrointestinal<br>nematodes in goats in marginal low potential areas Thika District of<br>Central Kenya. .... | 46        |

|                                                                                     |           |
|-------------------------------------------------------------------------------------|-----------|
| 3.3.2.1. Introduction .....                                                         | 46        |
| 3.4. Materials and methods .....                                                    | 47        |
| 3.4.1. Study area and animals .....                                                 | 47        |
| 3.4.2. Sampling and analysis .....                                                  | 47        |
| 3.4.3. Statistical analysis .....                                                   | 48        |
| 3.5. Results .....                                                                  | 48        |
| 3.6. Discussion .....                                                               | 64        |
| <b>3.8. Section 2: Study on the epidemiology of gastrointestinal nematodes</b>      |           |
| <b>of goats when only individual anthelmintic treatments are given...</b>           | <b>68</b> |
| 3.8.1. Introduction .....                                                           | 68        |
| 3.8.2. Materials and methods .....                                                  | 68        |
| 3.8.2.1. Study area and animals .....                                               | 68        |
| 3.8.2.2. Sampling and analysis .....                                                | 69        |
| 3.8.2.3. Statistical analysis .....                                                 | 70        |
| 3.8.3. Results .....                                                                | 70        |
| 3.8.4. Discussion .....                                                             | 76        |
| <b>Chapter 4: Studies on the impact of gastrointestinal helminths on production</b> |           |
| <b>in young goats in the marginal low potential areas of Thika</b>                  |           |
| <b>District of Central Kenya .....</b>                                              | <b>82</b> |
| 4.1. Introduction .....                                                             | 82        |
| 4.2. Materials and methods .....                                                    | 83        |
| 4.2.1. Study area .....                                                             | 83        |
| 4.2.2. Study design .....                                                           | 83        |
| 4.2.3. Statistical analysis .....                                                   | 85        |

|                                                       |     |
|-------------------------------------------------------|-----|
| 4.3. Results .....                                    | 86  |
| 4.3.1. Rainfall data .....                            | 86  |
| 4.3.2. Faecal egg counts .....                        | 86  |
| 4.3.3. Body weights .....                             | 91  |
| 4.3.4. Packed cell volume .....                       | 97  |
| 4.3.5. Total plasma proteins .....                    | 100 |
| 4.3.6. Herbage larval counts .....                    | 104 |
| 4.3.7. Total worm burdens in experimental goats ..... | 106 |
| 4.3.8. Total worm burden in tracer .....              | 106 |
| 4.4. Discussion .....                                 | 110 |

## **Chapter 5: Studies to determine the effectiveness of grazing management**

|                                                                                                  |            |
|--------------------------------------------------------------------------------------------------|------------|
| <b>in controlling gastrointestinal nematodes in weaner lambs on<br/>pasture in Denmark .....</b> | <b>115</b> |
| 5.1. Introduction .....                                                                          | 115        |
| 5.2. Materials and methods .....                                                                 | 116        |
| 5.2.1. Experimental design .....                                                                 | 116        |
| 5.2.2. Meteorological data .....                                                                 | 117        |
| 5.2.3. Sampling and analysis .....                                                               | 117        |
| 5.2.4. Statistical analysis .....                                                                | 119        |
| 5.3. Results .....                                                                               | 119        |
| 5.3.1. Rainfall and temperature .....                                                            | 119        |
| 5.3.2. Feeding, clinical observations and weight gains .....                                     | 119        |
| 5.3.3. Faecal egg counts .....                                                                   | 124        |
| 5.3.4. Pasture larval counts .....                                                               | 129        |

|                                                                                          |            |
|------------------------------------------------------------------------------------------|------------|
| 5.3.5. Serum albumin levels .....                                                        | 129        |
| 5.3.6. Faecal larval cultures .....                                                      | 133        |
| 5.3.7. Worm counts .....                                                                 | 134        |
| 5.4. Discussion .....                                                                    | 138        |
| <br><b>Chapter 6: Studies on the preventive effect of the fungus <i>Duddingtonia</i></b> |            |
| <b><i>flagrans</i> on trichostrongyle infections of lambs on pasture in Denmark.</b>     | <b>144</b> |
| 6.1. Introduction .....                                                                  | 144        |
| 6.2. Materials and methods .....                                                         | 145        |
| 6.2.1. Experimental design .....                                                         | 145        |
| 6.2.2. Fungal material .....                                                             | 146        |
| 6.2.3. Meteorological data .....                                                         | 146        |
| 6.2.4. Sampling and analysis .....                                                       | 146        |
| 6.2.5. Statistical analysis .....                                                        | 148        |
| 6.3. Results .....                                                                       | 148        |
| 6.3.1. Rainfall and temperature .....                                                    | 148        |
| 6.3.2. Faecal egg counts and clinical data .....                                         | 150        |
| 6.3.3. Faecal larval cultures .....                                                      | 154        |
| 6.3.4. Pasture larval counts .....                                                       | 154        |
| 6.3.5. Serum albumin levels .....                                                        | 159        |
| 6.3.6. Worm counts of the groups C 1 +2 and F 1 +2 .....                                 | 159        |
| 6.3.7. Tracer lambs .....                                                                | 161        |
| 6.4. Discussion .....                                                                    | 162        |
| <br><b>Chapter 7: General discussion, conclusions and significance of the study ....</b> |            |
| 7.1. General discussion and conclusions .....                                            | 166        |

7.2. Significance of the study ..... 174

**References** ..... 177

## List of Tables

### Chapter 2:

|                                                                                                              |    |
|--------------------------------------------------------------------------------------------------------------|----|
| Table 2.1. Distribution of major nematodes of small herbivores and their<br>relative ranking by region ..... | 10 |
|--------------------------------------------------------------------------------------------------------------|----|

### Chapter 3:

|                                                                                                                                                                                                                                                                                              |    |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Table 3.1. Estimated livestock population in Kakuzi and Ruiru Divisions<br>- Thika district in 1997 .....                                                                                                                                                                                    | 43 |
| Table 3.2. Repeated measures analysis of variance for the overall effect of age<br>groups on the EPGs between the months of May 1997 and February 1998 .                                                                                                                                     | 51 |
| Table 3.3. Repeated measures analysis of variance on the effect of age group on<br>EPGs on monthly basis .....                                                                                                                                                                               | 52 |
| Table 3.4. Repeated measures analysis of variance for the effect of age groups<br>on the EPGs during the long rains season (May - June), dry season<br>(July August and September) and short rains season (October,<br>November and December 1997, January and February 1998) of the study . | 53 |
| Table 3.5. Repeated measures analysis of variance for the overall effect of<br>the four farms on the EPGs between the months of May 1997<br>and February 1998 .....                                                                                                                          | 53 |
| Table 3.6. Repeated measures analysis of variance for the effect of farm on<br>EPGs during each month of the study .....                                                                                                                                                                     | 54 |
| Table 3.7. Repeated measures analysis of variance for the effect of farms<br>on the EPGs during the long rains (May and June), dry season                                                                                                                                                    |    |

|                                                                                |    |
|--------------------------------------------------------------------------------|----|
| (July, August and September) and the short rains season                        |    |
| (October, November and December 1997, January and February 1998) ..            | 54 |
| Table 3.8. Summary of larvae identified from cultures in June, September and   |    |
| December 1997 and February 1998 .....                                          | 55 |
| Table 3.9. Repeated measures analysis of variance on the overall effect of age |    |
| groups on the PCV levels between the months of April 1997 and                  |    |
| February 1998 .....                                                            | 57 |
| Table 3.10. Repeated measures analysis of variance on the effect of age        |    |
| groups on the PCV levels on monthly basis .....                                | 57 |
| Table 3.11. Repeated measures analysis of variance on the effect of            |    |
| age groups on the PCV levels during the long rains (April, May                 |    |
| and June), Dry season (July, August and September) and the                     |    |
| short rains season (October, November and December 1997,                       |    |
| January and February 1998) .....                                               | 58 |
| Table 3.12. Repeated measures analysis of variance on the overall effect of    |    |
| farm on the PCV levels between April 1997 and February 1998 .....              | 58 |
| Table 3.13. Repeated measures analysis of variance on the effect of farms      |    |
| on PCV levels on monthly basis .....                                           | 59 |
| Table 3.14. Repeated measures analysis of variance for the effect of farm      |    |
| on the PCV levels during the long rains season (April, May and June),          |    |
| dry season (July, August and September) and the short rains season             |    |
| (October, November and December 1997, January and February 1998) .             | 59 |
| Table 3.15. Repeated measures analysis of variance on the overall effect of    |    |

|                                                                                                                                                                                                                                                                                                                          |    |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| age groups on the total plasma protein levels between April 1997<br>and February 1998 .....                                                                                                                                                                                                                              | 60 |
| Table 3.16. Repeated measures analysis of variance for the effect of age groups<br>on the total plasma protein values on monthly basis .....                                                                                                                                                                             | 62 |
| Table 3.17. Repeated measures analysis of variance for the effect of age groups<br>on total plasma protein levels during the long rains season (April, May<br>and June), dry season (July, August and September) and the short rains<br>season (October, November and December 1997, January and<br>February 1998) ..... | 62 |
| Table 3.18. Repeated measures analysis of variance for the overall effect of farms<br>on the total plasma protein levels between April 1997 and February 1998.                                                                                                                                                           | 63 |
| Table 3.19. Repeated measures analysis of variance on the effect of farm on the<br>total plasma protein values on monthly basis .....                                                                                                                                                                                    | 63 |
| Table 3.20. Repeated measures analysis of variance for the effect of farm on the<br>total plasma protein levels during the long rains season (April, May and<br>June), dry season (July, August and September) and the short rains season<br>(October, November and December 1997, January and February 1998) ..         | 64 |
| Table 3.21. Distribution of salvage treated goats among age groups during the<br>rainy months (May, June, November and December) and dry season<br>(July, August, September and October) during the 8 months<br>epidemiological study period .....                                                                       | 72 |

## Chapter 4:

|                                                                                                                                                                                                                                                          |    |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Table 4.1. Repeated measures analysis of variance for the overall effects<br>of suppressive treatment on the change in EPGs following suppressive<br>treatment over 11 sampling occasions (22 weeks after start of study) .....                          | 89 |
| Table 4.2. Repeated measures analysis of variance on the effect of suppressive<br>treatment at each of the 11 sampling occasions .....                                                                                                                   | 90 |
| Table 4.3. Repeated measures analysis of variance for the overall effect of<br>group on the change of EPG (DEPG) over the 11 sampling occasions ....                                                                                                     | 90 |
| Table 4.4. Repeated measures analysis of variance for the effect of group<br>on LEPG at each sampling for the 11 sampling occasions .....                                                                                                                | 91 |
| Table 4.5. Repeated measures analysis of variance of the overall effect<br>of suppressive treatment on the weight gains (change in weight)<br>for the 11 sampling occasions .....                                                                        | 93 |
| Table 4.6. Repeated measures analysis of variance of the effect of suppressive<br>treatment and treatment nested in group on the weight gain on each<br>of the 11 sampling occasions (change in bwt 1 minus change in bwt 11)<br>(Dbwt 1 - Dbwt11) ..... | 94 |
| Table 4.7. Repeated measures analysis of variance on the overall effect of<br>group on the weight gains (change in weight - Dbwt 11) for the 11<br>sampling occasions .....                                                                              | 95 |
| Table 4.8. Repeated measures analysis of variance of the effect of suppressive<br>treatment on body weights at each of the 11 sampling occasions .....                                                                                                   | 95 |
| Table 4.9. Repeated measures analysis of variance of the overall effects of<br>suppressive treatment on change of PCV levels for the 11 sampling<br>occasions (change in PCV levels from 1 <sup>st</sup> sampling - DPCV 11) .....                       | 97 |

|                                                                                                                                                                                  |    |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Table 4.10. Repeated measures analysis of variance of the effect of suppressive treatment nested in group on PCV during each of the 11 sampling occasions (PCV 1 - PCV 11) ..... | 99 |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|

|                                                                                                                                                              |     |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Table 4.11. Repeated measures analysis of variance on the overall effect of group on the change in PCV levels over the 11 sampling occasions (DPCV 11) ..... | 100 |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|

|                                                                                                                                             |     |
|---------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Table 4.12. Repeated measures analysis of variance on the effect of each group on the PCV levels at each of the 11 sampling occasions ..... | 100 |
|---------------------------------------------------------------------------------------------------------------------------------------------|-----|

|                                                                                                                                                                                        |     |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Table 4.13. Repeated measures analysis of variance of the overall effect of suppressive treatment on the change in total protein levels over the 11 sampling occasions (DTPP 11) ..... | 101 |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|

|                                                                                                                                                                              |     |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Table 4.14. Repeated measures analysis of variance on the effect of suppressive treatment on total plasma protein at each of the 11 sampling occasions TPP 1 - TPP 11) ..... | 103 |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|

|                                                                                                                                                                      |     |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Table 4.15. Repeated measures analysis of variance for the overall effect of group on the change in total plasma protein values over the 11 sampling occasions ..... | 103 |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|

|                                                                                                                                                       |     |
|-------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Table 4.16. Repeated measures analysis of variance for the effect of group on the total plasma protein values at each of the 11 sampling occasions .. | 104 |
|-------------------------------------------------------------------------------------------------------------------------------------------------------|-----|

### Chapter 5:

|                                                                                                                                                                                                           |     |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Table 5.1. Repeated measures analysis of variance for the effect of anthelmintic treatment before moving to clean pasture (dose and move) on weight gains of the weaner lambs over the study period ..... | 121 |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|

|                                                                                  |  |
|----------------------------------------------------------------------------------|--|
| Table 5.2. Repeated measures analysis of variance for the effect of anthelmintic |  |
|----------------------------------------------------------------------------------|--|

treatment before moving to clean pasture on accumulated weight gains  
over the study period ..... 121

Table 5.3. Repeated measures analysis of variance for the effect of anthelmintic  
treatment before moving to clean pasture on log-transformed (LEPG)  
of the weaner lambs during the study period ..... 124

Table 5.4. Repeated measures analysis of variance for the effect of different  
groups on the log-transformed EPG of the weaner lambs during the  
study period ..... 125

Table 5.5. Repeated measures analysis of variance for the effect of treatment  
before moving to clean pasture on the serum albumin levels of the  
weaner lambs during the study period ..... 133

Table 5.6. Repeated measures analysis of variance for the overall effect of  
anthelmintic treatment before moving to clean pasture on the serum  
albumin levels of the weaner lambs ..... 133

Table 5.7. Arithmetic mean total worm counts ( $\pm$  SD) and the mean ( $\pm$  SD)  
for the species identified ..... 136

Table 5.8. Non-repeated measures analysis of variance for the effect of  
anthelmintic treatment before moving to clean pasture on the log-  
transformed immature abomasal worm counts, adult abomasal counts,  
small intestinal counts and the total counts of the weaner lambs ..... 137

Table 5.9. Non-repeated measures analysis of variance for the effect of  
anthelmintic treatment before moving to clean pasture on the log-  
transformed counts of various species of worms recovered from  
the weaner lambs ..... 138

Chapter 6:

Table 6.1. Worm burdens for the fungi treated and control animals at  
slaughter (geometric means and range). Speciation based on  
4 lambs per treatment group ..... 159

Table 6.2,. Worm burdens for tracer lambs grazing paddocks of previously  
untreated (group TC 1 + 2) and fungi treated lambs (group TF1 + 2)  
(geometric means and range) ..... 162

## LIST OF FIGURES

### Chapter 3:

|                                                                                                                                                              |    |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Figure 3.1. Thika District Agro-ecological zones .....                                                                                                       | 42 |
| Figure 3.2. Total monthly rainfall recorded at Thika meteorological station<br>5km from the study sites for the years 1996, 1997 and 1998 .....              | 49 |
| Figure 3.3. Arithmetic mean monthly group faecal egg counts (EPGs) for the<br>kids, young and adults between March 1997 and February 1998 .....              | 50 |
| Figure 3.4. Arithmetic mean monthly group packed cell volume (PCV) for the<br>kids, young and adults between March 1997 and February 1998 .....              | 56 |
| Figure 3.5. Arithmetic mean monthly group total plasma proteins for the<br>kids, young and adults between March 1997 and February 1998 .....                 | 61 |
| Figure 3.6. Total monthly rainfall recorded at Thika meteorological station<br>12km from the study farm during the study period May - December<br>1998 ..... | 71 |
| Figure 3.7. Arithmetic mean faecal egg counts (EPGs) for the kids, young and<br>adults at each sampling during the study period May - December 1998 ...      | 73 |
| Figure 3.8. Arithmetic mean packed cell volume (PCV) for the kids, young<br>and adults during the study period May - December 1998 .....                     | 74 |
| Figure 3.9. Arithmetic mean total plasma protein(TPP) for the kids, young<br>and adults during the study period May - December 1998 .....                    | 75 |

### Chapter 4:

|                                                                                                                                                                                     |    |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Figure 4.1. Total monthly rainfall recorded at Thika meteorological station<br>12km from experimental site for the months of May - December<br>during the years 1997 and 1998 ..... | 87 |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|

|                                                                                                                                                                                                                |     |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Figure 4.2. Arithmetic mean group strongyle faecal egg per gram (EPG) counts for the suppressively treated and control goats during the study period May to December 1998.....                                 | 88  |
| Figure 4.3. Arithmetic mean group weights for the suppressively treated and control goats during the study period May to December 1998.....                                                                    | 92  |
| Figure 4.4. Accumulated weight gains for the suppressively treated and control goats for the first 11 sampling occasions .....                                                                                 | 96  |
| Figure 4.5. Arithmetic mean group packed cell volume (PCV) for the suppressively treated and control goats during the study period .....                                                                       | 98  |
| Figure 4.6. Arithmetic mean group total plasma proteins (TPP) for the suppressively treated and control goats during the study period .....                                                                    | 102 |
| Figure 4.7. Arithmetic mean infective larvae (L3) per kg dry herbage for the paddocks grazed by the suppressively treated and control goats during the study period May to December 1997 and January 1998..... | 105 |
| Figure 4.8. Arithmetic mean group total worm burdens for the suppressively treated and control goats at slaughter .....                                                                                        | 107 |
| Figure 4.9. Arithmetic mean ( $\pm$ SEM) group abomasal immature worm counts for the suppressively treated and control goats at slaughter .....                                                                | 108 |
| Figure 4.10. Arithmetic mean total worm counts for the tracers introduced into the paddocks at the end of the dry and short rains seasons .....                                                                | 109 |
| <b>Chapter 5:</b>                                                                                                                                                                                              |     |
| Figure 5.1. Monthly rainfall and mean temperatures obtained from a meteorological station within 1 km from experimental paddocks .....                                                                         | 122 |

|                                                                                                                                                                                              |     |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Figure 5.2. Accumulated mean group weight gains from moving to clean<br>pasture to slaughter .....                                                                                           | 123 |
| Figure 5.3. Mean group faecal egg counts (EPG) over the study period from 2<br>weeks before moving to clean pasture until slaughter .....                                                    | 126 |
| Figure 5.4. Arithmetic mean strongyle faecal egg per gram(EPGs) counts from<br>2 weeks before moving to clean pasture up to slaughter .....                                                  | 127 |
| Figure 5.5. Arithmetic mean Nematodirus faecal egg per gram(EPGs) counts<br>from 2 weeks before moving to clean pasture up to slaughter .....                                                | 128 |
| Figure 5.6. Arithmetic mean infective larvae (L3) per kg dry herbage in the<br>paddocks grazed by the groups from moving to clean pasture to one<br>month after slaughter of the lambs ..... | 130 |
| Figure 5.7. Arithmetic mean strongyle infective larvae(L3) per kg dry herbage<br>in the paddocks from date of moving to 3 weeks after slaughter of the<br>lambs .....                        | 131 |
| Figure 5.8. Arithmetic mean Nematodirus infective larvae(L3) per kg dry<br>herbage in the paddocks from date of moving to 3 weeks after<br>slaughter of the lambs .....                      | 132 |
| Figure 5.9. Arithmetic mean group worm counts (+ SEM) for the abomasum<br>(adults and immature), small intestines and the total worm counts of the<br>lambs at slaughter .....               | 135 |

**Chapter 6:**

|                                                                                                                                          |     |
|------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Figure 6.1. Monthly rainfall and mean temperatures obtained from a<br>meteorological station within 1km from experimental paddocks ..... | 149 |
|------------------------------------------------------------------------------------------------------------------------------------------|-----|

|                                                                                                                                                                                                                                 |     |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Figure 6.2. Arithmetic means strongyle (mainly <i>Ostertagia</i> and <i>Trichostrongylus</i> species) faecal egg counts of lambs dosed with fungi (groups F1 + 2) and untreated lambs (groups C 1 + 2) .....                    | 151 |
| Figure 6.3. Arithmetic means <i>Nematodirus</i> species faecal egg counts of lambs dosed with fungi (groups F1 + 2) and untreated lambs (groups C 1 +2) ...                                                                     | 152 |
| Figure 6.4. Percentage composition of various species of <i>Nematodirus</i> excreted by the lambs during the study as identified by egg morphology .                                                                            | 153 |
| Figure 6.5. The proportion of eggs of <i>Ostertagia</i> and <i>Trichostrongylus</i> developing to infective stages in group faecal cultures of lambs dosed with fungi (groups F1 + 2) and untreated lambs (group C1 + 2) ...    | 155 |
| Figure 6.6. The proportion of eggs of <i>Nematodirus</i> (mainly <i>N. spathiger</i> ) developing to infective stages in group faecal cultures of lambs dosed with fungi (groups F1 + 2) and untreated lambs (group C1 + 2) ... | 156 |
| Figure 6.7. Arithmetic means pasture larval counts of <i>Ostertagia</i> / <i>Trichostrongylus</i> species in paddocks grazed by lambs dosed with fungi (Paddocks F1 + 2) and untreated lambs (paddocks C1 + 2) .....            | 157 |
| Figure 6.8. Arithmetic means pasture larval counts of <i>Nematodirus</i> species in paddocks grazed by lambs dosed with fungi (Paddocks F1 + 2) and untreated lambs (paddocks C1 + 2) .....                                     | 158 |
| Figure 6.9. Arithmetic means serum albumin levels of lambs dosed with fungi (groups F1 + 2) untreated lambs (group C1 +2) .....                                                                                                 | 160 |

### **DEDICATION:**

This work is dedicated to the special three: My wife Catherine Nduta Maina and our children Githigia Titus Maina and Wanjiru Christine Maina.

## Chapter 1

### **Introduction and objectives of the study**

#### 1.1. Introduction

Helminthosis is of considerable significance in a wide range of agro-climatic zones in the world. It constitutes one of the most important constraints to small ruminant production. The widespread occurrence of infection at sub-clinical level with internal parasites in grazing animals, the associated loss of production, the cost of anthelmintics and death of infected animals are some of the major concerns (Preston and Allonby, 1978). It has become increasingly apparent that losses due to helminth infection play a significant and in some instances a greater role than the acute diseases (FAO, 1991). This is because of the fact that compared to the essentially temporary nature of acute disease, where if animals survive, recovery is rapid and they are often immune for life, nearly all grazing animals are infected all the time with some parasites and pickup from pasture is continuous. By far the great losses in production associated with helminths are due to sub-clinical infections. These losses largely go unnoticed, or are accepted as the norm by the farmer. The profound impact of chronic conditions such as helminth infections on long term animal productivity is great (Waller, 1997a).

Normally, parasitic gastroenteritis (PGE) is the result of simultaneous infection by several parasite species. In Uruguay, it is estimated that helminth infections account for losses in cattle of up to 50 kg body weight per animal resulting in a total loss of US \$ 2020 million per year. In Australia, helminth infections in sheep cause losses of about Australian \$ 300 million per year (FAO, 1991). In Kenya, *H. contortus* infection in sheep was estimated to cause an annual loss of US \$ 26 million in the agriculture sector in 1976 (Preston and Allonby, 1979).

Several workers have reported on the epidemiology of gastrointestinal nematodes of goats

in Kenya. Lutu, (1983), reported on the epidemiology in dairy goats in a high potential high rainfall area farm of the Kenya highlands. Shavulimo and Semenye, (1990), reported on helminthosis in goats in some high, medium and low rainfall areas of Western Kenya covered by the Small Ruminant -CRSP project. Maingi, *et al.*, (1993), reported on the point prevalence of gastrointestinal nematodes in indigenous goats on four farms in four districts (Kiambu, Kajiado, Nairobi and Muranga Districts). Gatongi, (1996), and Gatongi *et al.*, (1998), reported on the epidemiology of gastrointestinal nematodes of sheep and goats and the role of hypobiosis in Naivasha, a semi arid area of Kenya. Njanja *et al.*, (1987), had reported on anthelmintic resistance of gastrointestinal nematodes in goats on this farm. Githigia *et al.*, (1996), reported on the prevalence and levels of infection with helminths in goats on an institutional farm at Machanga in a semi arid area of Kenya over a period of one year. No reports are available on the impact of gastrointestinal nematodes on goats in Kenya.

All the above reports are limited in the number and type of farms involved, period of study and the sampling intervals. There is therefore a need to understand the epidemiology, prevalence, control and impact of gastrointestinal nematodes in goats through a detailed study in areas where goats play a major role in the economy especially among small holders.

The problems associated with the current excessive dependence on anthelmintics especially in small ruminants have stimulated research in alternative control methods. Various strategies of integrated grazing management have been devised as alternative to or in addition to anthelmintic

treatments in sheep and goats. These include preventive, evasive and diluting strategies (Michel, 1985; Barger, 1997). Success has been reported in the use of these strategies in sheep and/ or goats in Australia (Barger and Southcott, 1978; Barger, 1999; Nielzen, *et al.*, 1996), Fiji Islands (Barger, *et al.*, 1994), Britain (Coles and Roush, 1992), South Africa (VanWyk and Van

Schalkwyk, 1990) and Malaysia (Chandrawathani, 1997). Presently there are no reports on the use of such integrated strategies of grazing management as alternatives for control of gastrointestinal nematodes of sheep in Denmark.

Numerous reports from Denmark have shown the effect of the nematode destroying fungus *Duddingtonia flagrans* in the field on the pre-parasitic larval stages of nematodes in cattle (Grønvold *et al.*, 1993; Larsen *et al.*, 1995; Nansen *et al.*, 1995), in horses (Larsen *et al.*, 1996; Fernandez *et al.*, 1997) and in pigs (Nansen *et al.*, 1996). There are no reports on the effect of *Duddingtonia flagrans* on naturally acquired gastrointestinal nematodes in sheep on pasture in Denmark. Several workers have reported on the ability of this fungus to reduce the number of parasitic nematode larvae in sheep in faecal cultures under laboratory conditions after passage through the gastrointestinal tract (Larsen *et al.*, 1994; Waller and Faedo, 1993; Waller *et al.*, 1994). There is need for a field experiment to investigate the effect of *D. flagrans* on acquisition of gastrointestinal nematodes in sheep grazing naturally infected pastures in Denmark

Among the options available for control of the helminths is the use of anthelmintics. Recently, there has been an increasing awareness of environmental issues which may influence anthelmintic usage through consumer demand for animal products and pastures free from chemical residues (Woolaston and Baker, 1996). There is also the emergence of anthelmintic resistance which is threatening the small ruminant production (Waller, 1997b). In the developed countries, helminth control is by strategic anthelmintic treatments and /or controlled grazing. In Africa, these control methods are limited by the high costs of anthelmintics, their uncertain availability, increasing frequency of drug resistance and limited scope for controlled grazing (Waller, 1997b). Despite the recent increase in research for helminth vaccine, no new vaccine has been introduced in the market apart from the irradiated larval vaccines against *Dictyocaulus viviparus* and *D. filaria* infection in cattle and sheep respectively (reviewed by Armour, 1987).

Apart from anthelmintic resistance, there are other concerns to the use of chemical drugs such as ecotoxicity problems related to some anthelmintics like avermectins which affect non-targeted organisms colonizing the faeces which are beneficial for activities such as soil aeration and nutrient recycling. Such chemicals lead to death of larvae and adults of dung breeding insects or reduce the number and viability of eggs produced by adults. These lead to delayed dung dispersal or degradation in the pasture (Reviewed by Herd, 1995). There is also the realization that residues of anti - parasitic chemicals accumulate in the tissues of the host animals and ultimately have adverse consequences, if not for human health, then possibly for international trade (Woolaston and Baker, 1996). There is growing consumer demand for food free from chemical residues leading to increased adoption of ecological or organic food production (Thamsborg *et al.*, 1999). Such production avoids the use of chemical products. Therefore, there is need to develop and test alternative and cheaper methods of control.

These considerations provide an urgency to develop alternative or complimentary control strategies which eliminate or lessen the reliance on anthelmintics. Exploiting genetic variations in host resistance to endo-parasites is one option both in controlling endo-parasites and improving efficiency of production in small ruminants (Waller, 1997a). Another option would be strategic treatments only when endo-parasites are likely to be transmitted. Future strategies to delay anthelmintic resistance will rely on a combination of immunological, mechanical and other non- chemical approaches (Sutherst, 1986). Biological control could be such a non - chemical control method. Sutherst, (1986) further pointed out that the most important target for biological control must be the dung pats which harbour helminth parasite eggs as well as immature stages of several arthropod parasites. The possibility of biological control of animal parasitic nematodes by use of nematode destroying microfungi has received increasing attention (Nansen *et al.*, 1986; 1995, Larsen *et al.*, 1994; 1995). For practical use of nematode destroying

fungi as biological agents, deposition of the fungal material should preferably be in the dung pats, where the entrapment and the killing of the nematode larvae take place ( Nansen, 1993).

Another non - chemical control method is grazing management / pasture management based on the epidemiology of the nematode parasites. This includes mixed grazing, alternate grazing and rotational grazing practices.

## 1.2. **Objectives of the study**

The research was undertaken in Kenya and Denmark between 1995 and 1999. It involved goats in Kenya and sheep in Denmark.

The Kenyan studies were mainly on the epidemiology, control and impact of gastrointestinal nematodes in goats in the marginal low potential areas of Thika District, Central Kenya.

The Danish studies were undertaken during two grazing seasons and had two main aims, namely to examine use of grazing management for control of nematode infections in weaner lambs and the possibility of biological control of *Trichostrongyle* infections of lambs on pasture. Specifically the main objectives of the studies reported in this thesis were as follows:

1. To study the epidemiology of gastrointestinal nematodes in goats in the marginal low potential areas of Thika District, Central Kenya.
2. To study the impact of naturally acquired gastrointestinal nematodes on the production of young goats in the marginal low potential areas of Thika District, Central Kenya.
3. To study the effectiveness of grazing management in controlling gastrointestinal nematodes in weaner lambs on pasture in Denmark.
4. To study the effect of feeding the nematode - trapping fungus, *Duddingtonia flagrans*, on naturally acquired trichostrongyle infections of weaner lambs on pasture in Denmark.

### 2.0 Literature Review

The epidemiological patterns of helminths are influenced by rainfall, temperature, availability of relevant hosts, management systems of livestock production, regulation of worm population through resistance and the physiological status of the animal (Barger, 1999). The infective larvae of most nematodes develop directly from eggs passed out in faeces as long as they get the required temperature, oxygen and moisture ( Rossanigo and Gruner, 1994) while those of certain nematodes pass through different intermediate hosts to reach the infective stages. The dynamics of endo-parasites and their transmissions in the habitat can be studied by monitoring their presence in their respective hosts and in pastures. The infective nematode stages should be available on pasture for transmission during grazing.

There are seasonal variations of endo-parasite infections depending on whether eggs passed out in faeces develop into infective stages or not. Fast development has been recorded in shade and warm moist environment (Rossanigo and Gruner, 1994). The larvae of most nematodes are found attached on blades of grass in pasture. Animals become infected by ingesting these larvae during grazing or feeding. These larvae are not evenly distributed on the blades of grass. Most are found near the edges while few are found near the soil (Peacock, 1996). They also vary in distribution with the period of the day.

In addition, the distribution also varies with the distance from where the faeces were dropped. More larvae are found near the faeces and decrease with increasing distance away from the pellet. Estimation of levels of pasture contaminations helps to determine to what degree animals are exposed to the risks of parasitic infections in different seasons ( Dinaburg, 1944; Dinnik and Dinnik, 1961; Fabiyi *et al.*, 1988).

Development of nematode eggs to infective larvae and their survival on pasture are also

influenced by temperature, rainfall and other environmental conditions. Investigation carried out on nematode larvae ecology in some parts of Africa by Dinnik and Dinnik (1958), in Iraq by Altaif and Yakoob (1987), in Europe by Southcott *et al.*, (1976) , Australia by Banks *et al.*, (1990) and in Mexico by Fernandez-Ruvalcaba *et al.*, (1994) have shown that the rate of development and longevity of the eggs vary at different temperatures, humidity and in different geo - ecological regions. The seasonal availability and abundance of the free living stages are the key factors in the occurrence and severity of parasitic infections, and are influenced by these environmental conditions. For Example, Farnandez - Ruvalcaba *et al.*, (1994) suggested that humidity is the limiting factor for the off-host development phase of *H. contortus*.

Aumont and Gruner (1989) working on population evolution studies of gastrointestinal nematodes on herbage under tropical conditions in Guadeloupe, observed a marked depressive effect by the dry season on egg hatching and L3 development. Thus, the climatic changes and the amount of dry matter on pasture during the grazing period are the main important factors which could interfere with the overall evolution of the infective L3 population size.

In the tropics and sub tropics, the environment is favorable more - or- less continuously throughout the year for free living stages of nematode parasites to hatch and develop (Waller, 1997a). In these regions, the limiting environmental variable controlling the translation of worm eggs to infective larvae is rainfall, because temperatures are always high enough to facilitate this process. Consequently, there is a direct correlation between the severity of helminth problems and rainfall in these regions (Waller, 1997a).

An infected host may react in various ways after the infection. This also depends on the amount of infective larvae ingested, species of nematode, nutritional status of the hosts as well as the physiological status.

Goats and sheep with their small body size, high reproductive capacity and rapid growth

rates, are ideally suited to production by resource poor small holders (ILCA, 1992). They can be integrated into the overall production system, absorbing surplus labor and consuming small amounts of otherwise unused feed. The low capital requirement for starting or expanding small ruminant production means that risks are low and that the enterprise is well suited to low input systems (ILCA, 1992).

Small ruminants provide almost 30% of the meat consumed and around 16% of the milk produced in sub-Saharan Africa (ILCA, 1992). Increased production of these ruminants will boost farm income and generate cash with which to purchase inputs for other farm production activities. In Nigeria, the livestock industry generates a revenue of up to US \$ 2 billion and provides variable animal protein and occupation for over one million families engaged in livestock trade (Fabiya, 1987). In Botswana (Carmichael, 1972), the country's economy is dependent on livestock population.

## 2.1. The place of small ruminants in livestock development in Kenya.

It has been estimated that over 80% of 20 million sheep and goats in Kenya are found in the arid and semi- arid areas where they provide important sources of dietary proteins primarily in the forms of meat and milk (Gatongi, 1996). They also provide employment and are considered as means of socio - economic status. In maximizing the meager resources in the arid and semi - arid areas, livestock production has been on the fore front (Gatongi, 1996). In the past, beef cattle production has been a success but currently it has become non - viable due to the changing land tenure system that has led to the subdivision of formerly vast ranches into small plots (Gatongi, 1996). Consequently, small ruminants have become the best choice of livestock enterprise in these areas. The small stature of goats requiring low feed intakes and their feeding habits that enable them access green shoots from poor quality shrubs make them suitable for small farmer situations. However, the farmer has to contend with husbandry problems such as bacterial, viral

and parasitic diseases. Gastrointestinal parasitism is one of the most prevalent problems and its chronic nature steadily eats away the farmer's profits (Costa and Vieira, 1983; Lutu, 1983; Gatongi, 1996; Silva *et al.*, 1998).

## 2.2. Gastrointestinal nematode infections in small ruminants

Gastrointestinal helminths impose severe economic constraints on sheep and goats production worldwide. Losses occur due to mortalities, reduced production due to sub - clinical parasitism and direct cost in managing the infection (Barger, 1982; ILCA, 1992; Carles, 1993; Gatongi, 1996).

*Haemonchus contortus* is the most important nematode of small ruminants in South America, Africa, Asia, Australia and the Pacific Islands (FAO, 1991; Barger, 1997) (Table 2.3).

*Haemonchus contortus* is a trichostrongylid nematode with a direct life cycle. Infective larvae (L<sub>3</sub>) on pastures are ingested by grazing animals and pass to the abomasum where they develop to L<sub>4</sub>, L<sub>5</sub> and finally adult worms. Sexual maturity is attained three weeks post - infection when female worms start laying eggs. These are passed out in feces. Under suitable conditions of effective herbage cover, moisture and warmth, eggs hatch to L<sub>1</sub> which molt to L<sub>2</sub> and finally to L<sub>3</sub> to complete the cycle.

Table 2.1: Distribution of major nematodes of small herbivores and their relative ranking by region

| region                   | (Source FAO, 1991) |     |     |     |     |     |     |     |     |
|--------------------------|--------------------|-----|-----|-----|-----|-----|-----|-----|-----|
| Nematode species         | Region             |     |     |     |     |     |     |     |     |
|                          | 1                  | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   |
| <i>Haemonchus</i>        | (1)                | (1) | (1) | (1) | (1) | (1) | (1) | (1) | (1) |
| <i>contortus</i>         |                    |     |     |     |     |     |     |     |     |
| <i>Trichostrongylus</i>  | (2)                | (2) | (2) | (2) | (2) | (2) | (2) | -   | -   |
| <i>spp</i>               |                    |     |     |     |     |     |     |     |     |
| <i>Oesophagostomum</i>   | (3)                | (3) | -   | -   | (3) | (3) | (4) | -   | -   |
| <i>spp</i>               |                    |     |     |     |     |     |     |     |     |
| <i>Ostertagia spp</i>    | -                  | -   | (3) | (4) | -   | -   | (5) | -   | -   |
| Lungworms                | -                  | -   | -   | (2) | -   | -   | (3) | -   | -   |
| <i>Dictyocaulus</i>      |                    |     |     |     |     |     |     |     |     |
| <i>Strongyloides spp</i> | (4)                | (4) | (4) | (5) | (4) | (4) | -   | -   | -   |

() = relative ranking

Region 1 = Central and South America

Region 2 = Equatorial South America

Region 3 = Southern South America

Region 4 = Northern Africa

Region 5 = Equatorial Africa

Region 6 = South Africa

Region 7 = Southwestern Asia and India

Region 8 = Central East Asia

Region 9 = South East Asia

In Kenya, *Haemonchus contortus* has been reported as the most common and economically important nematode in small ruminants. It causes high morbidities as well as mortalities in sheep and goats (Allonby and Urquhart, 1975; Preston and Allonby, 1978; Maingi, 1991; Mwamachi *et al.*, 1995; Baker *et al.*, 1998). Ulvund *et al.*, (1984) found that ewes and lambs grazing contaminated pastures were heavily infected with *Haemonchus contortus* and needed to be treated every 3 - 4 weeks throughout the year. These animals lost considerable weight. In a humid tropical environment in Kenya, Diani Estate farm lost 81 out of 335 lambs due to helminthosis within a period of 6 weeks after weaning (ILCA, 1992). Helminthosis caused 50.6% of the deaths. Severe losses in sheep and goats also occur in non humid areas including the semi arid areas. This has been attributed to the resumption of development of large numbers of hypobiotic larvae during the rainy season (Gatongi *et al.*, 1998)

High mortalities from haemonchosis in young lambs during the rainy season has been reported in Nigeria (Eysker and Ogunsusi, 1980). Other important nematodes include *Trichostrongylus spp*, *Oesophagostomum spp*, *Strongyloides spp*, *Cooperia spp* and *Nematodirus spp*.

The nodular worm *Oesophagostomum spp* also causes additional losses, rendering the intestines useless for production of surgical suture and sausage making (Stewart and Gasbarre, 1989). It is a major cause of condemnation at slaughter of the large and small intestines because they cause localized nodules on the walls of the intestines (Githigia *et al.*, 1995). Such losses may not be quantified due to the ceremonial and cultural value placed on roasted intestines in some communities. The tapeworm *Moniezia expansa* is a problem of young lambs and kids below the age of 6 months. Research has shown that it is not associated with any tangible production losses. In the Pacific Island of Fiji, death through worm infections emerged as the most serious threat to a sound goat industry to a point where the country was ready to abandon intense goat farming

in the mid 1980s (Lawrence, 1992). However, assistance from UNDP/FAO and Australian Centre for International Agricultural Research (ACIAR) helped to save the situation. The main helminths identified there were *Haemonchus contortus* and *Trichostrongylus colubriformis* which increased with all attempts to intensify grazing systems for goats (Barger *et al.*, 1994). Following some basic epidemiological survey, it was found that the generation interval of *Haemonchus contortus* was very short (4 -7 days) in these tropical environments. With this information, the farmers were able to practice pasture rotation at intervals of 4 days and this helped to solve the problem (Barger *et al.*, 1994).

### 2.3. The role of hypobiosis in the epidemiology of haemonchosis

Hypobiosis in *H. contortus* may be induced by the host resistance and environmental changes (Eysker, 1981; Gibbs, 1986). In the temperate regions of the world, hypobiosis is the primary means of overwintering for *H. contortus* (Gibbs, 1967). Because the infective stage of this nematode is highly susceptible to winter conditions the nematode survives the winter in the hypobiotic forms and resumes development when climatic conditions become favorable for transmission (Anderson, 1972; Michel, 1974).

Studies of hypobiosis in *H. contortus* in the tropical and sub- tropical regions of the world show variable results. No hypobiosis was detected in Brazil (Charles, 1989) and Egypt (El-Azazy, 1990). Low levels of hypobiosis was observed in Zimbabwe (Pandey, 1990; Pandey *et al.*, 1994), South Africa ( Horak *et al.*, 1991), Nigeria (Chiejina *et al.*, 1988), Saudi Arabia (El - Azazy, 1995), Mauritania (Jacquiet *et al.*, 1995), Malaysia (Ikeme *et al.*, 1987) and in a Merino flock in Kenya (Allonby and Urquhart, 1975). High levels of hypobiosis were reported in Nigeria ( Ogunsusi and Eysker, 1979) and in Kenya (Gatongi *et al.*, 1998). In the Kenyan study, it was reported that hypobiotic larvae and the adult worm populations co - existed in proportions that varied with seasons in sheep and goats (Gatongi *et al.*, 1998). Levels of hypobiosis increase

during the dry months and decrease during the wet months. The adult worm population increases during the wet months and decrease during the dry months (Gatongi *et al.*, 1998). This has also been reported in goats in Zimbabwe (Pandey *et al.*, 1994) and in sheep and goat flocks in Mauritania (Jacquiet *et al.*, 1995).

Experience by farmers and veterinarians in semi arid areas in Kenya has shown that acute haemonchosis occurs in sheep and goats (especially young stock) immediately after the onset of the long rains (Gatongi *et al.*, 1998). This occurs so quickly that the source of the acute infection is unlikely to be fresh infection from the pastures. There have been instances when acute haemonchosis has been reported during the dry season even before the onset of the rains (Gatongi *et al.*, 1998). The source of this disease has been attributed to resumption of development of hypobiotic larvae. This suggested that hypobiosis is advantageous to the survival of the parasite under harsh conditions of dry months. This facilitates successful transfer from infected hosts to susceptible hosts in subsequent rainy seasons (Gatongi *et al.*, 1998).

#### 2.4. Impact of helminth infection on the host's nutrition:

Numerous investigations have been carried out to determine the impact of helminth infections on the host nutrition (Nesheim, 1984; Keusch and Solomons, 1985; Crompton, 1986). Common features of infection with intestinal helminths are: a reduction in voluntary feed intake, reduction of digestibilities of dry and organic matter, a decrease in efficiency of feed utilization, significantly higher nitrogen output and a rise in plasma urea concentration (Armour *et al.*, 1987; Parkins and Holmes, 1989; Blackburn *et al.*, 1991; Knox *et al.*, 1994). Host food intake is reduced depending on either the infective dose given to the host or the number of established parasites present (Crompton, 1984). Work done in goats has shown that feed intake in infected goats is depressed in the first 3 months but is subsequently followed by compensatory reaction (Blackburn *et al.*, 1991). These investigators also showed that increased levels of

nutrition can compensate for the detrimental effects of *H. contortus*. Nutrition was found to be more important in counteracting the consequences of parasitic infection than in counteracting the establishment of the same infection.

Detailed investigations on the mechanisms of gastrointestinal dysfunction of the parasitized host have shown that the increased endogenous loss of protein into the gastrointestinal tract is a key feature, partly as a result of leakage of plasma proteins and also from increased exfoliation of gut epithelial cells and muco-protein secretion (Poppi *et al.*, 1986; Brown *et al.*, 1991). The presence of gastrointestinal nematodes generally increases abomasal fluid flow rate (Hennessy *et al.*, 1993).

Anthelmintic efficacy against resistant parasite strains can be increased when food type (Ali and Chick, 1992) or food intake (Ali and Hennessy, 1993) slow the gut flow rate, presumably by prolonging the exposure of the worms to anthelmintic.

## 2.6. Diagnosis of gastrointestinal nematode infections

The diagnosis of gastrointestinal nematodes is based on direct examination of faecal samples. The McMaster technique (Anon., 1986) is the most widely used. It is simple and quick but has low sensitivities especially where worm eggs are few or unevenly distributed in the faecal samples. Furthermore, egg counts are greatly affected by consistency of faeces, duration and degree of infection, fecundity and maturity of the worms, appetite and resistance of the host (Lutu, 1983). Although it is possible to derive a correlation between faecal egg counts and total burden of parasitic nematodes in young, this is poor in older animals (FAO, 1991). The clinical importance of hypobiotic larvae in ruminants is now well recognized and their isolation and counting depends on standard procedures for making post mortem mucosal analysis (Anon. 1986; Gatongi, 1996; Gatongi *et al.*, 1998).

## 2.6 . Principles of helminth control:

Effective helminth control is a major element in ensuring the sustainability of animal production (Waller, 1997a). Eradication of most helminth infections is not practical. Thus, the major aim is to ensure that parasite populations do not exceed levels compatible with economic production. This objective is achieved by three interrelated approaches: grazing management, use of anthelmintics, and utilization of natural or artificially induced immunity (Waller, 1997a). Potentially the most efficient control requires the complete integration of all the three facets. This is possible only on the basis of a full understanding of the epidemiology of helminth infections (Brunsdon, 1980).

In developing countries, there are poorly / or ineffective set plans of prophylactic control of gastrointestinal helminths. Anthelmintics are utilized in intensive livestock operations, such as government and commercial farms. In Kenya, their use in small scale production farms is mainly in dairy enterprises (Kinoti *et al*, 1994) while in other enterprises it is mainly irregular and haphazard (Mbaria *et al*, 1995). Their use in nomadic systems is limited or absent in some places. Treatments are carried out when the animals show clear clinical signs of helminthosis (Kinoti *et al*, 1994). Most control measures recommended revolve around regular anthelmintic treatment which in the wet season range from monthly ( Ethiopia-Bergeon, 1968; Kenya-Ulvund *et al*, 1984) to six weeks ( Nigeria- Lee, 1955), and treatment throughout the year if the dry season is not longer than 3 months. In the wet tropics, in the North Queensland, Copeman and Hutchinson, (1980) recommended anthelmintic treatment for calves during the period of weaning for 5 - 8 months when the calves are most susceptible to the effects of gastrointestinal strongyle parasites. They gave optimum frequency as 3 -4 times each two months apart. In Diani Mombasa, lambs are treated at weaning and monitored every week until they are one year (ILCA, 1992). All animals are treated when the monitor lambs have a mean EPG of 1500 - 2000 (ILCA, 1992). In

Fenbendazole, treatment for lambs was recommended at an interval of 3 - 4 weeks both during the dry and wet seasons (Ulvund *et al.*, 1984). Although other methods have been recommended, they are rarely applied by traditional pastoralists who are generally reluctant to deviate from their customs. These other methods include movement of animals after dosing to a clean pasture (unstocked for some months) as recommended for Zimbabwe, Zambia and Malawi (Le Roux, 1957) and rotational grazing as recommended by Lee (1955) for Western Nigeria, Griffiths (1957) for Burma and Lawrence (1992) for goats in Fiji. This practice aims at breaking the multiplication cycle of the nematodes.

## 2.7. Availability and distribution of anthelmintics

Although a lot of effort has been put on sustainable control programs to reduce dependence on chemicals, protection of the effectiveness and availability of modern anthelmintics is critical as they have a role to play in the control of acute and chronic worm infections in developing countries. Availability of these chemicals at affordable prices and marketability of animal and animal products vary from country to country and from one husbandry practice to another.

Anthelmintic use is faced with two major obstacles, one being the cost of the drug and the other proper use at farm level. The first problem may be solved by manufacturing and marketing of cheaper formulations of the efficient anthelmintics like benzimidazoles (introduced from the 1960's and no longer protected by the original invention patent). The second problem is linked with levels of education, community infrastructures and activities within the agricultural and veterinary extension services.

## 2.8. Alternative dosing methods

Several alternatives to direct treatment of individual animals are being promoted in certain developing regions. Fenbendazole incorporated into medicated blocks of feed supplement

is being marketed in S.E. Asia especially in Malaysia, Pacific Islands of Fiji and India (Knox *et al.*, 1994). The use of medicated blocks in free range conditions is not recommended due to variable intake. Anthelmintics can also be incorporated into cubes or loose mineral premix given as a supplement to livestock. There is increased interest in the new technologies that provide sustained *in vivo* release of anthelmintics. These controlled release devices allow a high degree of control of the rate of drug delivery and represent an important new technology in the management of gastrointestinal parasitism (Donald, 1985). These have been used extensively in cattle with promising results ( McKellar, 1988).

In sheep, the effectiveness of a controlled release intra-ruminal device containing albendazole (Profil sheep captec, Smithkline Beecham Animal Health Ltd) has been proved in Britain (Bell and Thomas, 1992), and are becoming widely used in Australia and New Zealand. Treatment with the controlled release bolus eliminated the existing worm burdens of lambs and protected against reinfection for up to 3 months. Similar results have been reported from the Kenyan highlands where the bolus eliminated the existing worm burden among ewes and protected them against re-infection for 120 days ( Munyua *et al.*, 1997). The animals with the bolus had better weight gains. Boisvenue *et al.*, (1988) demonstrated the effectiveness of continuous low dose intra-ruminal infusion of fenbendazole against benzimidazole resistant *H. contortus*. This suggests that such boluses may play an important role in dealing with anthelmintic resistance.

## 2.9. Anthelmintic resistance

Prichard *et al.* (1980) defined resistance as “ a significant increase in the ability of individuals within a population to tolerate doses of a compound which would otherwise prove lethal to the majority of individuals in a normal population.” Resistance is a heritable character (Waller, 1986) hence the problem of anthelmintic resistance needs to be recognized globally as

one of the greatest threats to grazing livestock production and a major threat to the small ruminant industries in many countries of the world (Waller, 1997b). The potentials for development of resistance to drugs in helminths are high where anthelmintics are used frequently and where management and environmental factors favor selection for resistance (Waller, 1987). This explains the widespread occurrence of resistance recorded in U.S.A., Brazil., Australia, (Kelly and Hall, 1979), U.K. (Cawthorne and Cheong, 1984) and the Republic of South Africa (Van Wyck and Malan, 1988). Reported field cases of anthelmintic resistance are mainly confined to the trichostrongylid nematodes ( *Haemonchus*, *Ostertagia* and *Trichostrongylus* species) in sheep and goats (Prichard *et al.*, 1980; Waller, 1986; Maingi, 1991 ).

Anthelmintic resistance in sheep and goats has been reported in various areas of the tropics where anthelmintics have been administered for several years like Sri Lanka (Van Ajken, *et al.*, 1989), Kenya (Njanja *et al.*, 1987; Waruiru *et al.*, 1991; Mwamachi *et al.* 1995) and Tanzania (Bjorn, *et al.*, 1991).

Multiple, high level anthelmintic resistance in nematode parasites of sheep and goats is common throughout the tropics and sub-tropics (Waller 1997b). There is no doubt that the development of widespread, high- level anthelmintic resistance represents the greatest single threat to the control of gastrointestinal parasitism of small ruminants in the humid regions of the developing world (Waller, 1997b). Attempts to expand sheep and goat production by replacing the traditional village production systems, which rarely involve anthelmintic treatment, with large scale commercial enterprises induces complete reliance on anthelmintics to control nematode parasites. Thus, with increased intensification of small ruminant operations, anthelmintic treatments have become frequent and haphazard (Waller, 1997b). These are the two factors responsible for development of drug resistance in any target pest species (Waller, 1997b). This has been worsened by the proliferation in the number of generic anthelmintic products of poor

quality offered to the farmers at reduced prices, together with the unethical practices of drug substitution and adulteration (Waller, 1997b).

No new anthelmintic is expected especially for sheep and goats in the tropical and sub tropical areas. The anthelmintic market has now shifted to cattle in North America and Europe where there is no resistance (Waller, 1997b).

Alternative approaches to nematode control other than intensive anthelmintic treatment, have received greater attention in recent years. Some can provide immediate benefits whilst others hopefully will prove valuable in the short term. For any nematode parasite control scheme to be sustainable, it requires carefully planned use of effective anthelmintics (Waller, 1997a).

Care should be taken to dose animals properly and to utilize safe grazing strategies based on knowledge of the epidemiology of the parasites (Michel, 1969) and importantly, alternating between anthelmintic groups on a yearly basis (FAO, 1991).

A number of methods are used to evaluate efficacy of anthelmintics. The best method for survey purposes is the faecal egg count reduction test (FECRT) (Presidente, 1985; Waller, 1986). The method compares faecal worm egg counts of groups of animals before and after treatment (Waller, 1986; Johansen, 1989).

## 2.10. Alternative methods of helminth control

### 2.10.1. Grazing management

Grazing management is often referred to as integrated or clean grazing system. It includes mixed grazing, alternate grazing and rotational grazing practices. Several factors influence the success of integrated grazing systems. These may include host specificity of the parasites, the longevity of the free living stages and the duration of pasture resting or alternate use. Longevity of the infective larvae depends on the weather, soil and herbage cover. The pasture resting will be influenced by survival of infective L3 in soil and faeces (Bairden *et al.*, 1995).

Pastures provide the link between the free living and parasitic phases of nematode parasites of grazing livestock. At different stages of growth, pasture species may facilitate or impede the survival of the free living population, the establishment of parasite burdens and lessen or intensify the effects of parasitism in livestock. In temperate regions of the world, a large number of recommendations to improve efficiency of parasite control are based on combining anthelmintic treatment with some form of grazing management (Barger, 1997). This is absent in the tropics despite a greater potential for grazing management. This is because despite the fact that development of free living stages is generally much faster and more successful than in the temperate regions, their longevity is much shorter. Studies in the wet tropical climates show that peak larval concentrations occur on pasture within one week after contamination, but fall to barely detectable levels within 4- 6 weeks (Banks, *et al*, 1990; Barger *et al.*, 1994; Sani *et al.*, 1995). Grazing systems have been developed for the Pacific Island countries (Barger *et al.*, 1994) and Malaysia (Sani *et al.*, 1995) to exploit these finding. These involved the subdivision of pasture into small plots and allowing sheep or goats to graze for not more than 4 days before movement to a new plot. Animals were moved through the sub - plots returning to the original sub plot after 30 days. This avoided auto-infection while managing pasture productivity. The need for anthelmintic treatment is drastically reduced and can be even eliminated (Barger *et al.*, 1994).

Mixed grazing of cattle and sheep is commonly practiced. In Australia alternate grazing of sheep with cattle (Barger and Southcott, 1978; Donald, *et al.*, 1987) and lambs with dry adult sheep have achieved levels of worm control similar to that obtained by monthly drenching. Initially the recommended worm control program for summer rainfall regions of Australia (Worm kill: Dash, 1986) suggested ewes receive two broad spectrum and three closantel treatment during the grazing season. Later these recommendations were revised as it was found

that ewes only needed a single drench at weaning and two closantel treatments before housing. Lambs, under the Wormkill programme, are given three broad -spectrum drenches, two closantel treatments ( to control *Haemonchus* and liver flukes) and moved at weaning to a low risk paddock( a paddock that has not been grazed by lactating ewes or lambs within the last 16 weeks, Taylor, 1994). This has led to good worm control in ewes and lambs and the elimination of *Haemonchus* in some areas (Barger *et al.*, 1991) while reducing the use of broad -spectrum anthelmintics.

#### 2.10.1.1. Forms of grazing management

The simplest and most effective forms of grazing management which limit obstacles to animal production raised by helminth parasitism are those governed by geographic location and / or choice of production system to avoid specific helminthoses. Examples include the use of relatively insusceptible wethers for wool production in semi arid grazing systems, the reservation of pastures infested with liver flukes (*Fasciola hepatica*) for grazing by cattle rather than the more susceptible sheep and the location of sheep and goat industries in the drier inland regions of continents rather than in the wetter coastal fringes. These procedures avoid rather than control helminths (Barger, 1997).

In the management to control parasitism, there are strategies which reduce the number of, or effects of, helminth parasites in animal production systems where their economic viability would otherwise be threatened by parasitism. These strategies were classified by Michel (1985) as 'preventive', 'evasive' and 'diluting' strategies.

#### 2.10.1.2. Preventive strategies

These rely on putting worm free animals on clean pasture or suppressing egg output by anthelmintic treatment in the early part of the grazing season until the initial population of infective larvae on pasture has declined to safe levels.

These include the short intensive treatment regimes using controlled- release devices or carefully timed sequences of conventional anthelmintic treatments for calves in their first half of the grazing season. The devices are given at turnout and the anthelmintic treatments are given at turnout, at 8 weeks and at 13 weeks (Barger, 1997). Among the Australian sheep producers, there are recommended worm control programs (Barger, 1995) which use a similar strategy to control nematode infections in weaner lambs. The lambs are drenched at weaning and moved to safe pastures prepared by spelling or grazing with non- susceptible hosts, with a further 2 or 3 anthelmintic treatments at 8-week intervals.

Exploitation of host specificity has also been used as a form of preventive strategy where cattle and sheep are alternated over the same pasture commonly referred to as alternate grazing. This method was used by all sheep producers and 92% of cattle herds in Scotland (Gettinby *et al.*, 1987). Parasite species pathogenic to one species either do not infect the alternative host or are less pathogenic and prolific. *Ostertagia spp* show little cross infectivity between cattle and sheep while *Trichostrongylus* and *Cooperia spp* can survive and reproduce in both host species (Roberts, 1942; Porter, 1953). *H. placei* is pathogenic to cattle, sheep and goats (Barger, 1999) and alternate grazing may not be viable in the tropics where this parasite is prevalent. This has been used as a way of preparing clean pastures for young sheep by pre-grazing with cattle and for young cattle by pre-grazing with sheep. Barger and Southcott, (1975), found that after grazing sheep on cattle pastures, tracer calves picked up fewer infective larvae. Inderbitzin *et al.*, (1981) reported that grazing cattle on sheep pastures for the second part of the grazing season reduced faecal egg counts over a 4 year study. The benefits were enhanced when calves were given an anthelmintic before grazing the sheep pastures. The best results have been obtained with an alternation period of 6 months and a drench at the time of pasture swapping (Barger and Southcott, 1978). The level of control obtained in this system was equivalent in terms of

parasitological and production parameters to suppressively treated sheep over a period of 3 years. A 3 -year study in New Zealand demonstrated some benefit to the alternate grazing of sheep and cattle. Lower parasite burdens were found in sheep compared to cattle. This lowered parasitism in sheep may have been due, in part to the use of helminth resistant rams in the breeding programmes (Niezen *et al.*, 1996).

Despite the initial successes of alternate grazing of sheep with cattle to control nematodes in both species, there are reports of *Trichostrongylus axei* causing death and weight loss in sheep grazing cattle pastures, an indication that it may evolve increased ability to infect sheep in response to alternation of sheep and cattle. O'callaghan *et al.*, (1992) reported clinical disease in sheep caused by the purely cattle parasite *Ostertagia ostertagi*. Bairden *et al.*, (1995) reported initial success but eventual failure of sheep / cattle alternate grazing system over a 4- year period possibly through selection of strains of *O. ostertagi* and *C. oncophora* that were capable of extended survival time on pasture.

#### 2.10.1.3. Evasive strategies

These rely on anthelmintic treatment and movement of livestock to safe pastures just before the larvae resulting from the eggs are likely to appear in significant numbers in the original pasture. The goal is to keep pasture larval populations as low as possible. This was devised by Michel (1969) for control of gastrointestinal nematodes in calves and by Thomas and Boag for ewes and lambs (Thomas, 1982). The anthelmintic removed the worms in calves or lambs that might have contaminated the safe pasture. The move enabled the young animals to escape exposure to mid-summer larval peak resulting from hatching of eggs passed earlier in the season. Variation in the dose and move method has been extended to the control of lung worm in cattle in Europe (Jørgensen, 1981) and *Ostertagia* and *Cooperia* in young cattle in Australia by escaping late Winter and early Spring peak larval availability resulting from Autumn and Winter

contamination (Smeal *et al.*, 1980).

Barger *et al.*, (1994) described a further and successful evasive strategy which involved rotational grazing system for goats in a wet tropical environment. The wet tropical climates favor rapid and continuous egg hatching and larval development (Waller, 1997a). These also result in extremely high death rates of infective larvae on pasture. Barger *et al.*, (1994) found out that larvae survived in heavily contaminated pastures for less than two months and peak concentration occurred one week post contamination. Larvae were detected as early as 4 days after eggs were deposited. The survival times for the major nematode species encountered were similar (*H. contortus*, *T. colubriformis* and *O. columbianum*). A grazing system was developed to exploit these findings consisting of 10 paddocks each grazed in sequence for 3.5 days, then spelled for 31.5 days. Egg counts of the rotationally grazed goats were less than half those of similar set-stocked goats on an adjacent paddock which required over 3 times anthelmintic treatments in a year compared to the rotationally grazed goats. There were indications that the rotationally grazed goats could have done without anthelmintic treatment even for the lactating and pregnant does (Barger, 1997). The longer a pasture is allowed to remain fallow, the lower the pasture larval burdens will be when it is grazed next (Stromberg and Auerbeck, 1999).

Another simple evasive strategy was developed by Eysker *et al.*, (1998a&b). They investigated the repeated moves to clean pasture on the build up of gastrointestinal nematodes in calves. Three groups of 4 - 5 month old calves were turned out on pasture in May and moved to clean pasture monthly from the beginning of July over two grazing seasons. One group was moved only in the beginning of July, the second in the beginning of July and August while a third in the beginning of July, August and September. During the last month before housing, moderate to high infection were acquired by the groups which were moved once or twice while low to moderate infections were maintained in the group that was moved three times. Their results

demonstrated that moving calves at monthly intervals to clean pasture could be an effective method for the control of parasitic gastroenteritis. Also, their results cautioned that the last move should not occur more than a month before the end of the grazing season. They observed that when calves graze more than one month on the same pasture after July, nematode infections build up rapidly. They simplified the system developed by Oostendorp and Harmsen (1968) which required moves of every 2 - 3 weeks throughout the grazing season, to only three from July onwards when the calves are housed in October. This limited number of moves makes the system applicable for farmers.

#### 2.10.1.4. Diluting strategies:

These exploit the concurrent grazing of susceptible animals with a greater population of helminthologically inert animals, of the same or different species, in order to reduce the herbage infestation resulting from their combined faecal output of worm eggs. They are widely used and aimed at giving the younger animals a greater choice in diet selection. If susceptible animals graze with a greater number of non-susceptible animals, the average rate of the pasture contamination with worm eggs will be substantially reduced and this is reflected in reduced larval uptake by susceptible stock. This dilution effect may be explained by the fact that calves do not develop clinical helminthosis before weaning when they graze with the cows. Mixed grazing of cattle and sheep has also been examined. Jordan *et al.*, (1988) examined the effect of grazing ewes and lambs or cows and calves, either alone, or mixed with other species. They found that mixed grazing resulted in reduced sheep parasitism and increased productivity of lambs, but also increased cattle parasitism and reduced productivity of calves.

A special type of dilution strategy based on the revision of resistance by introduction of susceptible worms was designed by Van Wyk and Van Schalkwyk, (1990). It involved thoroughly deworming sheep infected with a resistant strain of *H. contortus* at a time when

pastures were expected to have low levels of infectivity. This was followed by artificially infecting sheep with infective larvae from a susceptible strain. The sheep were closely monitored to ensure that the introduced worms do not lead to clinical disease. The susceptible worms contaminate and thereby dilute the resistant *H. contortus* in the pasture.

This technique of diluting resistant with susceptible worms has particular relevance to a new sheep grazing system called the 50 / 50 system promoted in South Africa (Kirkman and Moore, 1995). In this system, all the animals on a given farm are concentrated on half the pasture, while the other half is rested, with these treatment being rotated annually. In this way, practically worm free pastures are available annually and it is possible to obtain high levels of dilution of resistant worms, on the condition that special precautions are taken to prevent exposure to resistant larvae surviving in places of common access on the farm like surroundings of handling pens, passageways for herding sheep between paddocks.

All these strategies reduce the number of anthelmintic treatments. Each system carries the seeds of its own destruction as seen in the failure of some of the otherwise earlier successful strategies. It is not possible to completely eliminate anthelmintic use and grazing management should be geared at ensuring that the efficacy of the available anthelmintics is maintained and the development of anthelmintic resistance is slowed down. This will require a thorough understanding of the role of epidemiology in grazing management (Barger, 1999).

#### 2.10.1.5. The role of nematode epidemiological knowledge in grazing management:

Epidemiology of ruminant helminths is the foundation on which strategic parasite control programmes are designed (Stromberg and Averbeck, 1999). The design of a strategic parasite control programme requires a knowledge of the dynamics of egg shedding from the host and the resulting pasture larval populations. It is important to know if larvae are available when animals are turned out on to pasture, when larvae populations reach their maximum numbers and when

they are induced to become hypobiotic. The major epidemiological variable influencing worm burdens of grazing animals is the infection rate or the number of infective larvae ingested from pasture each day. This is usually estimated as the concentration of infective larvae per unit weight of herbage on offer. As daily intake of pasture by ruminants is relatively constant on a day to day basis, daily intake of infective larvae will be proportional to the concentration of larvae on herbage. Similarly, worm burdens of previously worm free “tracer” animals grazed for short periods (2 - 3 weeks) also reflect current larval availability. Such measurements taken at regular intervals for at least one year will show the seasonal availability of infective larvae. Such patterns may show a repeating seasonal pattern of trough and peaks as in most temperate environments or a relatively constant level of larval availability throughout the year as seen in wet tropical environments. In these tropical environments, temperatures and rainfall show little or no seasonal variation and are always favourable for helminth egg hatching and larval development throughout the year.

Clinical parasitic gastroenteritis in small ruminants usually occurs at or shortly after times of peak larval availability. Even with the absence of clinical disease, the timing of peak larval availability on pasture is of crucial importance in understanding the population dynamics of parasite populations as this is when the largest worm burden is acquired. It is against these seasonal peaks that control strategies must be directed.

The origin of peak larval availability may arise from recently deposited eggs which are induced to hatch and develop by recent weather events such as rainfall or favourable temperature as occurs in cases of *Haemonchus contortus* infection outbreaks or larvae derived from eggs deposited over many weeks or months as the case with *Trichostrongylus colubriformis* and *Ostertagia circumcincta*. Once the origin of peak larval availability is known, consideration is made at either preventing or avoiding them as part of the control programme. In temperate areas,

peak larval availability occurs in spring, summer or autumn from eggs deposited in winter, spring or early summer respectively depending on the temperature and rainfall relationship. Eggs deposited on wet tropical pastures hatch and develop to infective larvae rapidly resulting in peak larval availability and infectivity on pasture within a week.

The relative ability of infective larvae to survive on pasture at different times of the year is important in formulation of a control programme. This determines how long a pasture remains dangerous following high levels of larval concentration. Cool dry weather prolongs larval survival while hot, wet weather shortens it. Infective larvae do not feed and survive on stored energy. Low temperatures and dry conditions prevent active movement by the larvae and minimize their energy expenditure.

The aim of a strategic anthelmintic treatment is to remove a worm burden which if untreated, would later produce sufficient eggs to lead to an escalation in pasture infestation with larvae. To be long term, precautions against re- infection such as movement of animals to relatively uncontaminated pasture immediately after treatment must be taken. Another precaution would be timing the treatment just before naturally occurring seasonal discontinuities in infection like extreme climatic conditions of either very dry or very low temperatures or intense drought which can drastically lower the infectivity of pastures in some environment. Larvae of *Haemonchus contortus* cannot survive extreme cold as occurs in northern continental winters, so treatment of housed sheep or goats in winter is effective in controlling this species.

The role of grazing management in worm control programmes based on epidemiological knowledge is to provide clean pastures on which stock may safely graze, usually but not always after a strategic anthelmintic treatment.

#### 2.10.2. Prospects of vaccines:

Immunization against helminths have generally not been successful. Apart from vaccines

against bovine and ovine lung worm (*Dictyocaulus* spp) and canine hookworm, the use of either attenuated live vaccines or “dead” vaccines against naturally acquired infections have proved disappointing. However, recent advances in immunology, molecular genetics and recombinant DNA technology, may perhaps lead to a breakthrough (Dineen, 1984). Promising results have recently been reported for *H. contortus* (Jasmer and McGuire, 1991; Newton, 1995) and against *Cysticercus ovis* infection in sheep (FAO, 1991; Lightowlers and Rickard, 1993).

After discovery of the irradiated vaccine against *Dictyocaulus* in the 1960's, attempts were immediately made to extend this control principle to gastrointestinal helminths. The method worked well under experimental conditions for *Haemonchus contortus* and *Trichostrongylus colubriformis* in sexually mature sheep. Unfortunately the protective effect was found to be either too weak or too variable in field infections to be a practical proposition especially in young lambs (Smith, 1999). These are the most highly susceptible to infection on pasture.

A major handicap in all efforts to develop vaccines against helminths is the poor knowledge base on the nature and regulation of the immune response of the infected host. The use of vaccines will require careful cost benefit analysis, based on thorough epidemiological knowledge prior to instituting a vaccine - based control program (FAO, 1991).

Control of liver flukes and gastrointestinal nematodes will continue to be nearly always based on a combination of anthelmintic drugs and pasture management. This is not sustainable due to the threat by the advent of strains of worms resistant to anthelmintics (Smith, 1999),

#### 2.10.3. Genetic resistance:

Another potential source for additional helminth parasite control is the enhancement of genetically based resistance to infection in the host. Although the tropically adapted breeds lack the productivity and performance capacity of their temperate counterparts in the temperate regions, they possess great advantage of being able to thrive in their environment and to tolerate

or resist disease. Examples may include resistance of *Bos indicus* cattle to the cattle tick *Boophilus microplus*, trypanotolerance of the N'dama and West African Short horn cattle, nematode resistance in the East African Red Maasai, Florida and Louisiana Native, Barbados Blackbelly and St. Croix breeds of sheep, and the trematode resistance in Javanese thin - tailed sheep (Waller, 1997b). Amarante *et al.* (1999) showed that cross breeding Rambouillet and Florida Native sheep resulted in the production of F1 ewes resistant to gastrointestinal nematode infections. These are the few which have been chosen for study leaving a huge genetic resource throughout the tropics and sub- tropics which have evolved characteristics based on the Darwinian principle of “survival of the fittest”. The extreme selection pressure induced by the combination of environmental stress, malnutrition, continuous massive parasite challenge with no remedial treatment have been imposed on these for many years (Waller, 1997b).

Many livestock improvement projects in the tropics are based on introduction of fast growing high yielding European breeds. This has resulted in a large amount of indiscriminate cross- breeding, without any attempt to compare the exotic breeds or their cross with indigenous breeds although in some case it has been extremely difficult to keep the exotic breeds alive and reproducing (Baker, 1996). Not only do the exotic breeds have reduced tolerance to disease and heat stress, greater nutrient maintenance requirements because of generally larger frames, but in sheep produce a definitely un-desirable product in the wet tropics mainly wool. They require regular shearing to dispose off the worthless fleece which predisposes the sheep to heat stress, dermatitis, fleece rot and myiasis ( Manuelli, 1997). Progress has been made in breeding tropically adapted breeds. In Fiji, breeding strategies for selecting sheep suited to the tropical environment also includes selection for natural resistance to parasites (Woolaston *et al.*, 1995). Breeds of sheep with resistance to nematode infection often display a reduced peri-parturient rise in faecal egg counts compared to susceptible sheep (Woolaston, 1992). The expression of genetic

resistance to gastrointestinal nematodes is accompanied by a fall in the worm egg counts due to a reduction in the fecundity of female parasites or a reduction in the percentage of invading larvae that become adults (Gruner *et al.*, 1986). It is also accompanied by the ability of the host to maintain unchanged production level (resilience) under moderate levels of infection (Albers *et al.*, 1987).

There is evidence that genetic variation in resistance to nematode infections within breeds (Gray *et al.*, 1987) can be as great as that between breeds (Barger, 1989). Because of natural evolution, breeding programs in the tropics should aim at genetic improvement of the local indigenous breeds. Exploiting genetic resistance of livestock disease in general and to parasites in particular, represents the ultimate approach towards sustainable parasite control especially for poor resource farmers.

Genetic variation can be exploited to increase resistance of sheep populations to parasitic nematodes. Breeding alone is not a viable option for worm control and interaction between resistant genotypes and other methods of modifying the resistance of the host, principally nutritional enhancement of resistance and improvement of immunological status by vaccination needs to be investigated (Gray, 1997).

Commercial breeding programmes for sheep which include resistance of gastrointestinal roundworms are now operating in Australia and New Zealand ( Woolaston and Baker, 1996).

#### 2.10.4. Biological control:

Biological control has been defined as pest suppression with biotic agents, excluding the process of breeding for resistance to pests, sterility techniques and chemical modification of pest behavior (Papavizas and Levis, 1988). The biotic agents are natural enemies, deliberately introduced to reduce the pest population. The bio-control agent may be artificially modified (e.g. genetic engineering), naturally modified (selection programs) or remain unmodified as the

nematophagous fungi. In the applied sense, biological control has been defined as an ecological method designed by man to lower pest or parasite population to acceptable sub-clinical densities or to keep these populations at a non-harmful level using living antagonists (Grønvold *et al.*, 1996).

Parasite control has been almost exclusively focused on the parasitic phase within the host - particularly in the use of anthelmintics, attempts to develop vaccines and breeding animals with natural resistance / resilience to worms. However, in most situations and for most of the time, the greatest proportion of the biomass of the parasite population is not in the host but on pasture. Research activities should pay attention to control of the parasites in the free living stage for a sustainable parasite control (Waller, 1998). Biological control agents rarely eliminate the target organism, but reduce the numbers to acceptable levels and maintain a balance between the pathogen and the antagonist. In contrast to chemical control which is directed to the parasite in the host, biological control is focused on the free living stages on pasture.

Biological control can be divided into natural and applied biological control. Natural biological control is produced by native (or co- evolved) natural enemies in the normal environment. Although such organisms exist against worm parasites they have little impact. Intensification of livestock grazing systems have favored parasites by providing an abundance of susceptible hosts and favorable pasture micro -environment for free living stages. Applied biological control is produced by human intervention. This may be further divided into classical biological control, which is effected by introduction of exotic natural enemies, or augmentative biological control which is brought about by the enhancement of natural enemies already present in a given environment.

One activity of man which could be a form of biological control although almost incidental is the practice of collecting bovine dung for use as fuel, building materials or manure.

Examples of classical biological control include the introduction of the *Cactoblastis* moth to control the prickly pear and *Myxomatosis* to control rabbits both in Australia. These have been successful but disasters may occur when the enemies outnumber the parasites and destroy even the beneficial organisms as was the case with the cane toad and the cane beetles in Australia. Control of nematode parasites of livestock is likely to be by augmentative approach, either by manipulation of the environment or of the existing natural enemies of parasites (Waller, 1998).

#### 2.10.4.1. Biological control by manipulation of the environment

There are several examples of environmental manipulation for biological control of insects such as changes of land use, habitat provision reducing natural enemies of beneficial species and improved pesticide utilization. In livestock there is evidence that organic farming practices increase the abundance and variety of dung dwelling microorganisms, particularly fungi which may include nematophagous species (Bell, 1983). This may partly contribute to the good parasite control in organically reared lambs in New Zealand (Niezen *et al.*, 1996). There is also some evidence that the type of plant species used in pastures can influence the species and type of fungi that colonize the dung of the livestock that graze on the pastures (Hay and Nielzen, 1995). The “green manuring” of land by ploughing in of various crops as a replacement of synthetic fertilizers encourages the proliferation of earthworms which influences the free living stages of parasites.

#### 2.10.4.2. Biological control by manipulation of the organisms

This consist of production and field release of a given species of natural enemies of parasite larvae. This may be inoculative where a small dose is released with the assumption that they will multiply and their progeny will provide long term pest suppression or inundative where a large dose is released with the aim of providing immediate pest suppression.

#### 2.10.4.3. Candidates for biological control

Dung removers: These include mainly beetles and earthworms and to a lesser extent birds that feed on coprophilous arthropods. Dung beetles are found throughout the world and these are often capable of rapid and complete dung removal and thus are indirectly responsible for significant reductions in the number of free - living stages of parasites (Waller and Faedo, 1996). This is however, dependent on ideal weather conditions. Earthworms are the main dung degraders in the cool, moist regions of the world. In northern Europe earthworms play an important role in the removal of cattle dung from pastures and can be responsible for significant reduction of infective larvae on pasture (Grønvold, 1987).

Parasite antagonists: These include micro arthropods, protozoa, predacious nematodes, viruses, bacteria and fungi which exploit the free - living stages of parasites as a food source (Waller and Faedo, 1996). Among the bacteria is *Bacillus penetrans* which is a promising candidate for the control of parasitic nematodes of plants. It produces highly resistant spores which attach to the cuticle and invade the nematodes.

Fungi that exhibit anti - nematode properties have been known for a long time. They consist of a great variety of species which include nematode trapping (predacious) fungi, endo-parasitic fungi that invade nematodes by spores, fungal parasites of cyst and root-knot nematodes also called egg parasitic fungi (Barron, 1977; Nordbring-Hertz, 1988). The most important groups of nematophagous fungi are the predacious and the endo-parasitic. Fungi from these two classes are found in all environments throughout the world, but are particularly abundant in rich agricultural soils.

The nematode trapping fungi are a group of microfungi which are able to differentiate their mycelia in specialized organelles to catch and destroy nematodes such as adhesive networks, knobs and constricting or non - constricting rings. Fungi in this class may also produce chemo-attractant and / or chemotoxic substances (Waller and Faedo, 1993). Within a short time

following capture of the nematode, the fungus penetrates the worm and destroy it. The endo-parasitic fungi invade the nematode from adhesive spores that stick to the cuticle, from spores that are ingested by the nematode, or from motile spores in water. The egg parasitic fungi attack eggs especially of cyst and root - knot nematodes of plants (Nordbring-Hertz, 1988).

Majority of the approximately 200 species of nematophagous fungi described today belong to the class Deuteromycetes, the imperfect fungi. Many of the fungi in the predacious group belong to the Deuteromycetes whereas the endo-parasitic and egg - parasitic fungi belong to the classes Chytridiomycetes, Oomycetes, Zygomycetes, Basidiomycetes and Deuteromycetes respectively.

In a study to isolate nematode trapping fungi acquired and able to survive gut passage. 23 examples of nematophagous fungi were noted from approximately 2500 rectal faecal samples collected from Fijian islands where most small ruminants are kept. Twelve pure isolates of *Arthrobotrys* were made (Manueli *et al.*, 1999). Nematophagous fungi were only isolated from sheep faeces. This is likely due to differences in feeding behavior with goats spending most of the time browsing on trees and shrubs while sheep are almost exclusive grazers. The pasture / soil / mat micro environment is the preferred growing location for nematophagous fungi. Grazing sheep rather than browsing goats are better suited for isolation of these fungi in the field (Manueli *et al.*, 1999).

In a survey of nematophagous fungi in fresh faeces of cattle over a period of one year in Minas Gerais state Brazil, Saumell *et al.*, (1999) found 10 genera of nematophagous fungi. Six of the genera were predacious and one was endoparasitic. 3.8% of the samples examined had nematophagous fungi and *Arthrobotrys oligospora* was one of the isolates. This shows that grazing animals are exposed to nematode destroying fungi naturally and several genera are able to survive gut passage.

Hay *et al.*, (1997), deposited sheep faeces on bare ground, rye grass, brown top and white clover. They found that in all these ground cover 71% of the faeces was colonized by nematophagous fungi in summer and 58% in Autumn. Eight three percent of the faeces were found to contain nematophagous fungi 3 days after deposition in summer and 58% of the faeces in autumn. These results suggest that there are a number of species of nematophagous fungi in the environment that are able to enter the deposited faeces soon after deposition on a variety of types of ground cover. *Athrobotrys oligospora*, *Monacrosporium candidum* and *Nematoctonus spp* (nematode trapping) and *Harposporium leptospira* (endoparasitic) were identified in the deposits.

#### 2.10.4.4. Effect of nematode trapping fungi on nematode parasites:

Compared to the amount of research on biological control of plant parasitic nematodes, only few attempts have been made in order to control parasitic nematodes in animals. Most work has been done in Denmark, Australia, USA, Mexico and Malaysia. Biological control by means of nematophagous fungi targets the free living stages of parasitic nematodes present in animal dung, with the purpose of lowering larvae populations on pasture. By reducing the number of the infective stages of the parasitic nematodes to a minimum, the transmission of the parasite to a new host is expected to be restricted to levels where production losses can be avoided. Although direct application of fungal spores to animal beddings has potential to control some nematodes such as *Strongyloides papillosus* (Chandrawathani *et al.*, 1998), it seems that for most circumstances in which nematode- destroying fungi can be used in the prophylactic control of nematode parasites of livestock, is when the fungal material is incorporated in the diet of animals. Several genera of fungi have been tested and the nematode- trapping fungi, *Duddingtonia flagrans* have proved the ideal choice. *Duddingtonia flagrans* was first isolated by Duddington in 1949, who gave it the name *Trichothecium flagrans*. In 1969, Cooke relocated

the fungus in a newly created genus, *Duddingtonia*. *D. flagrans* is the only known species of this genus.

The fungus, *D. flagrans* traps nematodes by means of adhesive three dimensional network of hyphae (filaments forming the fungal thallus or mycelium). The most important feature of this fungus is the abundant production of resting spores called chlamydospores which have superior ability to survive passage through the gastrointestinal tract of livestock. This stage can survive the harsh environmental conditions of gut passage (anaerobic, enzymatic and thermal) in livestock (Larsen *et al.*, 1991; 1992; Larsen, 1999; Mendoza de Gives *et al.*, 1998), to which fungi that produce thin - walled conidia are unable to survive (Faedo *et al.*, 1997).

#### 2.10.4.5. Methods of selecting fungi as biological control agents:

An important principle for selecting candidate fungi as possible biological control agents is to obtain isolates from the field in the region, or country, where this work is to be performed. This has the advantage of overcoming the stringent requirements regarding importation and field release of exotic living organisms in most countries and loss of fungal attributes associated with continuous laboratory passage. The fungal species that have evolved to survive under local environmental conditions are much better strains to work with than those derived from centralized fungal collections.

The most relevant sites for sampling would be the environment where the fungi are expected to exert their effects, notably fresh faecal deposits although in intensive animal production systems, animal bedding may also be appropriate. This is because the fungi will be expected to survive passage through the gastrointestinal tract of animals and then to trap nematodes in freshly deposited faeces. A large number of nematophagous fungi from other sources such as soil and pasture would fail this test. An exception of this would be those for direct application of the fungi on bedding in intensive forms of animal production where internal

parasitism is a problem. Example are the intensive calf rearing units in the southern Island of Japan where *Strongyloides papillosus* can cause sudden death in massively infected animals in the hot summer months (Taira and Ura, 1991). A practical solution to this problem may be the direct application of fungal elements to the bedding (Chandrawathani *et al.*, 1998). The fungi would quickly colonize the bedding and reduce the overwhelming numbers of *S. papillosus* leaving enough survivors to provoke the normal, rapid acquisition of immunity against the parasite.

Previous experiments (Grønvold *et al.*, 1987; 1988; Nansen *et al.*, 1986; 1988a) have shown that nematophagous fungus *Arthrobotrys oligospora*, when mixed with cattle faeces, is capable of reducing the number of infective larvae of *Ostertagia ostertagi* and *Cooperia oncophora* in dung and surrounding herbage. This species has however been found to be unable to survive passage through the gastrointestinal tract, to germinate and trap infective larvae in dung (Faedo *et al.*, 1997). This may be due to the fact that it produces only conidia unlike *D. flagrans* which produces conidia and the resistant chlamydospores. Using *D. flagrans*, it is possible to reduce both the number of infective larvae on herbage and the subsequent nematode populations establishing in grazing animals (Grønvold *et al.*, 1993; Wolstrup *et al.*, 1996; Nansen *et al.* 1996; Larsen *et al.*, 1995; 1996; Farnandez *et al.*, 1997).

Daily supplement with barley grains supporting the growth of *D. flagrans* reduce parasitism and increase productivity in grazing cattle, pigs and sheep (Larsen *et al.*, 1996). These results are especially exciting as they demonstrate that the principle of biological control of nematode parasites using nematophagous fungi is applicable to many production systems where daily supplementation is practical. Daily feeding of animals with a supplement containing fungus can be applicable in intensive grazing systems, in systems where daily gathering of animals is part of the daily farm routine activities such as milking time in dairy farms, night housing or the

common practice of daily feeding of horses.

Recent work in Brazil has shown that the endoparasitic fungus *Harposporium anguillulae* is able to reduce infective larvae of *Haemonchus contortus* in sheep faecal cultures (Charles *et al.*, 1996). The fungi which are nematode invaders (endoparasitic) rather than trappers (predacious) are found in abundance in cattle dung three days after deposition on pasture. This provides a window of opportunity for these fungi to invade the actively feeding first and second stage larvae.

A review and overview of the current position and possibilities of biological control using the net forming *D. flagrans* has been done by Larsen (1999). It is evident from the review that biological control by means of predacious fungi is moving from being a promising possibility to a reality. The increased involvement of multinational agencies in addition to national and industrial interests will keep the momentum and there is hope that a biological control product may surface at the turn of the century (Larsen, 1999). The fact that free living larvae and nematode trapping fungi share time and space in that they are present in the faecal mass and develop there at the same time, present a unique possibility to exploit nematode trapping fungi as biological control agents.

The incorporation of chlamydospores of *D. flagrans* into blocks to control gastrointestinal nematodes in sheep has been tried in Fiji with encouraging results (Waller, 1998). An attempt has also been made to incorporate the fungus into slow release devices (Waller, 1998). Chlamydospores of *D. flagrans* can withstand tableting pressure required for manufacture of these devices (Waller, 1998).

#### 2.11. Conclusions on use of non - chemical control methods:

The urgency to develop and implement these control strategies stems from the severe problems of anthelmintic-resistant worm populations developing and spreading across the world,

in particular in small ruminants(Waller, 1997a).

Of the novel approaches to worm control which are currently under consideration, breeding for resistance ( Gray and Gill, 1993), nutritional supplementation (Knox and Steel, 1996) and improved grazing management (Barger, 1996) offer immediate prospects of application to complement the strategic use of anthelmintics. Vaccination against ruminant gastrointestinal nematodes remains elusive although the earlier irradiated live vaccines and more recent killed vaccines had some experimental success (Urquhart, 1980; Newton, 1995).

None of the strategies of grazing management, worm vaccines, breeding for resistant hosts or biological control in isolation will provide a solution to anthelmintic resistance. A combination of strategies and methods are required to reduce the problem of anthelmintic resistance (Waller, 1997b). Integrated Pest Management (IPM) is the only way in which sustainable parasite control of livestock can be achieved. This has been defined as the planned integration of a range of methods and techniques to minimize the use of drugs and at the same time, maximize the profitability of the farming enterprise. The use of these non - chemotherapeutic means of parasite control must become incorporated into grazing livestock systems as soon as they become practicable.

Epidemiologically based grazing management systems have been established and principles of selecting for host resistance to worms have proved practically possible especially in sheep. There are still hurdles to be cleared before the “new generation worm vaccines” are developed against important nematodes. Biological control has made exciting progress and promises great potential in the control of the entire range of the nematode parasites across the complete spectrum of livestock species in almost all management systems in both developed and developing countries (Waller, 1997b). These have shown the real possibility of using non chemical control methods in the integrated livestock nematode control.

## CHAPTER 3: SECTION 1

### 3.0 The epidemiology of gastrointestinal nematodes in goats in the marginal low potential areas of Thika District.

#### 3.1. Introduction to study area- Thika District:

Thika District is one of the 7 districts of Central Province. It borders Kiambu, Machakos, Maragwa, Nairobi and Muranga districts. It is located between Longitudes 30° 45' and 37° 30' E and Latitudes 0° 45' S and 1° 15'S. The district covers a total area of 1634 square kilometers. It is divided into six divisions namely Gatanga, Gatundu, Kamwangi (which cover the upper part of the district in agro- ecological zones 1 and 2) and Thika municipality, Kakuzi and Ruiru (which cover the lower medium and low potential area of the district in agro - ecological zones 3, 4 and 5). The research was conducted in Ruiru and Kakuzi divisions in the low potential agro - ecological zone 3 ( marginal coffee- UM3 and Sunflower /maize/sisal-UM4) (Figure 3.1). In this area coffee, pineapples, floricultural and horticultural crops are grown under irrigation.

Goats in this area are kept for local sale, slaughter and for provision of milk and meat at homes. They are also kept as easily disposable assets. They are usually sold in times of need such as sickness, death and for payment of school fees. They may also be used for ceremonies as payment of bride price (Ministry of Agriculture and Livestock development Annual Report, 1997). Most of the goats are of the Small East African breed, with crosses of Galla goats. Inbreeding has been said to be a major problem (Ministry of Agriculture and Veterinary Development Annual Report, 1997). The Small Ruminant Livestock Project is currently involved in breed improvement by cross breeding the local does with Alpine bucks.

#### 3.2. Livestock population in Kakuzi and Ruiru divisions, Thika District

The estimated population of livestock in the two divisions in 1997, as well as the

AGRO-ECOLOGICAL ZONES IN THIKA DISTRICT

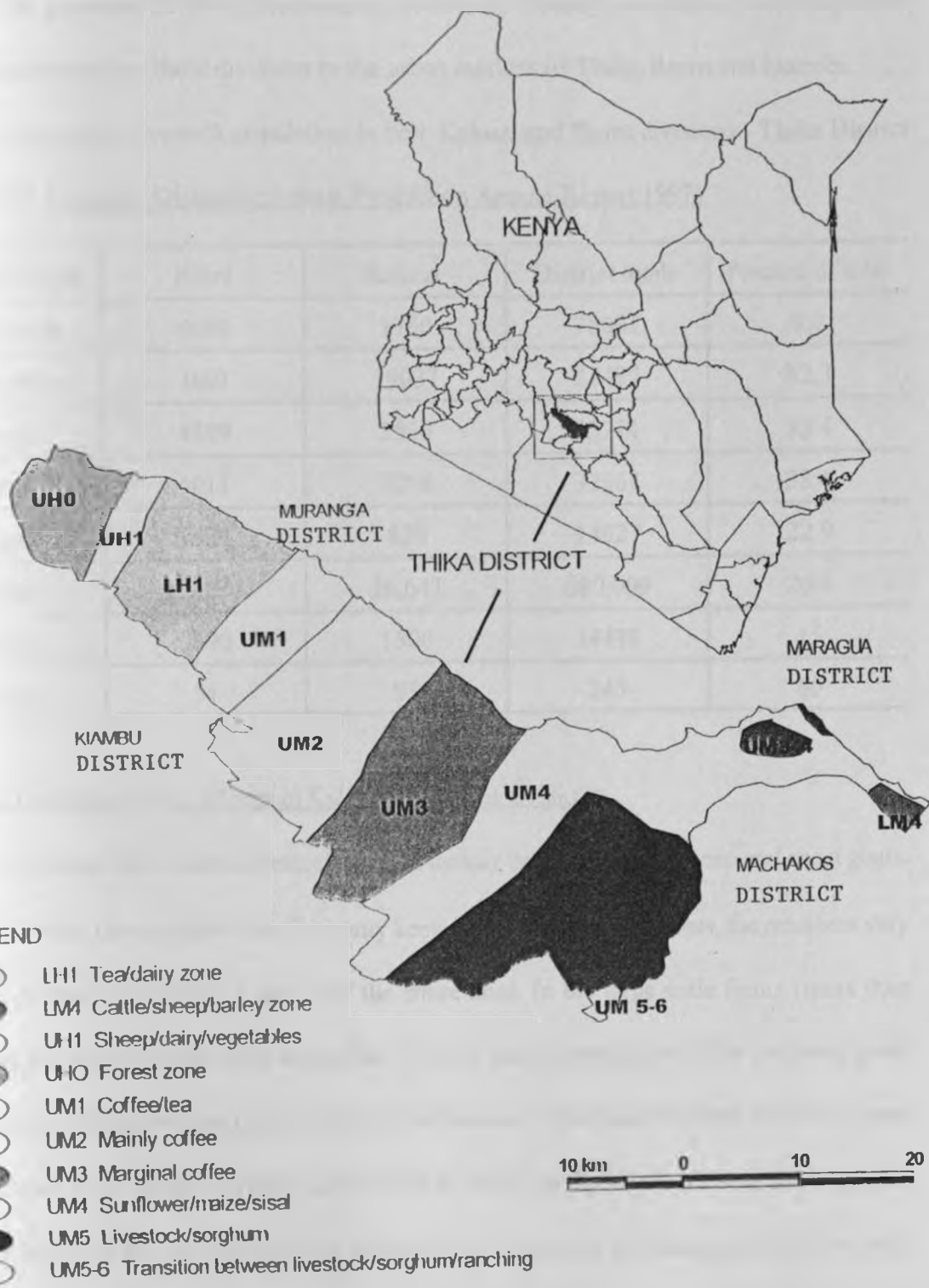


Figure 3.1: Agro-ecological zones in Thika District.  
The study was done in Agro-ecological zones UM3 and UM3-4

percentage contributions are shown on Table 3. 1. These divisions have 82.3% of the beef cattle, 38.4% of the goats and 33.4% of the sheep in the district. Poultry production is also important due to the proximity of these divisions to the urban markets of Thika, Ruiru and Nairobi.

Table 3.1. Estimated livestock population in both Kakuzi and Ruiru divisions - Thika District in 1997. ( Source: District Livestock Production Annual Report 1997)

| Livestock type | Ruiru   | Kakuzi | District totals | Percent of total |
|----------------|---------|--------|-----------------|------------------|
| Dairy cattle   | 4680    | 1750   | 78081           | 8.2              |
| Beef cattle    | 3603    | 19037  | 27497           | 82.3             |
| Sheep          | 4889    | 3550   | 25271           | 33.4             |
| Goats          | 5013    | 7284   | 32064           | 38.4             |
| Pigs           | 2526    | 820    | 14627           | 22.9             |
| Poultry        | 112,353 | 26,643 | 680,009         | 20.4             |
| Rabbits        | 3690    | 1500   | 34498           | 15               |
| Donkeys        | 54      | 93     | 245             | 60               |

### 3.3. Goat farm structure and types in Kakuzi and Ruiru divisions.

Most farms have mixed livestock species mainly beef cattle, hair sheep and meat goats. Some small scale farmers (less than 5ha) only keep goats. In the mixed farms, the numbers vary and goats compose between 1/3 and ½ of the entire herd. In the large scale farms (more than 50ha) goat keeping is not the main enterprise. Most of these farms grow coffee and keep goats to supplement their earning and also utilize the idle labor of retired and old farm workers. There are more small scale farms than large scale farms in these two divisions. Most of the improved breeds are found in the large scale farms which act as a source of breeding stock for the small scale farms.

### 3.3.1. Management and role of goats in these divisions:

These divisions supply most of the goat meat consumed in the district and are popular with livestock traders. A large number of goats are sold in December and January for the Christmas festivities and payment of school fees. They are sold for slaughter mostly within the district with a few going for breeding in the high potential areas of the district.

Most goats are kept under extensive free range management systems and grazed together with cattle and sheep. They leave the homestead in the morning and return in the evening except in farms where cattle are milked when they return in the early afternoon. Supplementation with crop residues or improved pastures is practiced by some small scale farmers mainly to goats destined for sale, lactating does or kids are usually left around the homestead. Communal grazing and use of cut roadside pastures are common among the small scale farmers especially during the dry season. Goats may also have access to harvested fields in the small scale farms at this time. Zero grazing units for goats are rare. Few units are found on peri-urban farms around Thika Town which specializes in dairy cattle. Goat units are part of the dairy cattle unit and goats are occasionally fed on dairy cattle feeds and cut herbage. Milk from goats is normally sold to hotels for cheese making or pooled with the milk from cattle. These units have pure breed Toggenbergs, German Alpines or their crosses. The owners of such farms are hobby goat keepers and beneficiaries of earlier goat improvement projects (Ministry of Agriculture and Livestock Development annual report, 1997).

Anthelmintic treatments for goats vary with the farms and individual farmers. In the large scale farms (more than 50ha), goats are drenched together with cattle and sheep. This may vary from monthly during the wet season, six times to four times in a year. The benzimidazole group of anthelmintics are the most commonly used due to their availability in the market in various cheap brands. Some of the brands have been found to be ineffective (Wanyangu, *et al.*, 1996).

In the small scale farms (less than 5ha) anthelmintic treatment for goats is rare and goats are only drenched if there is any left drench after drenching cattle and sheep. Such drenches are usually under dosages, irregular and haphazard. These farms are characterized by high kid mortalities especially immediately after the rains when the kids start accompanying their mothers. The “urban” goat keepers who work in town and go home over the weekend tend to overuse anthelmintics and drenchings can be even weekly or fortnightly. These farms are characterized by high levels of anthelmintic resistance.

### 3.3.2 Studies on the prevalence and intensity of infection with gastrointestinal nematodes in goats in the marginal low potential areas of Thika District in Central Kenya.

#### 3.3.2.1. Introduction:

Goats have been shown to play a major role in rural economies ( Peacock, 1996). They are able to utilize idle labor and consume herbage which other livestock are unable to utilize (Gatongi, 1996). They are highly adaptable to a broad range of climatic and geographic conditions and are more widely distributed than any other mammalian livestock. Goats are also managed under almost all production systems like feral, trans-human, extensive, intensive and under zero grazing systems (Peacock, 1996). Goats provide their owners with a vast range of useful products and services including milk, meat, skins, horns and manure. They are cheap to buy and flocks can be built up until they contain many goats, which spreads the risk inherent in livestock ownership. They have been used by many development agencies as the first step up and out of poverty. In the arid and semi-arid areas, goats are the principal source of income (Gatongi, 1996; Silva *et al.*, 1998) and play a major role in human survival during droughts and famines (Peacock, 1996).

Gastrointestinal helminths have been recognized as one of the major factors that limit goat production in a wide range of agro-climatic zones (Costa and Vieira, 1983; Lutu, 1983; Shavulimo, *et al.*, 1988; Shavulimo, 1993; Joshi, 1994; Jacquet *et al.*, 1995; Gatongi, 1996). They reduce productivity on clinical and subclinical levels. They cause production losses such as reduced weight gains, lower milk and meat production and mortalities especially in the young.

Effective control of gastrointestinal nematodes in goats is only possible after a clear understanding of their epidemiology in the various regions. The epidemiology of gastrointestinal nematodes of goats in the marginal low potential areas of Thika District, Central Kenya has not been established. This study was undertaken to establish the prevalence and intensity of

gastrointestinal helminth infections in goats on 4 farms in a marginal low potential area of Thika District in Central Kenya.

### 3.4. Materials and Methods:

#### 3.4.1. Study area and animals:

The study was undertaken in the marginal low potential area (Ecozone 3) of Thika District, Central Kenya between March 1997 and February 1998. This area receives an average annual rainfall of 1100mm which is bimodal in distribution. The long rains fall between March and May while the short rains fall between October and December. The amount and duration of rainfall varies from year to year and from season to season. Four small scale farms (< 5ha) were purposively selected for the study with the help of the district livestock production extension officer. Subsistence crops were the main farming activity on these farms. The Small East African goats is the main breed kept with a few Galla crossbreeds. The goats on these farms are mainly kept for meat and local slaughter. Goats of various ages ( kids, young and adults) were ear-tagged for identification with the consent of the owners ( 25 goats in each farm). Each age group was represented in each farm. All the goats in the flock were treated with albendazole at the dosage of 3.8mg per kg body weight at the beginning of the study (faecal samples prior to treatment showed that the goats were infected with gastrointestinal nematodes at various levels) and whenever the Eggs Per Gram (EPG) of faeces in 25% of the ear-tagged goats was more than 600.

#### 3.4.2. Sampling and analysis:

Rectal faecal samples were taken fortnightly. The EPG of faeces was determined by the modified McMaster technique with a lower limit of detection of 100epg (Anon.,1986). Ethylene-diamine-tetraacetic-acid (EDTA) blood was taken monthly by jugular venipuncture and packed cell volume (PCV) and Total Plasma Protein (TP) were determined using the microhaematocrit and refractometry methods respectively. Faecal samples were pooled for all the goats on each

farm during all the sampling occasions. The pooled samples were incubated at 27°C for 14 days and recovered larvae were counted and identified according to the published guidelines (Anon., 1986; Hansen and Perry, 1994).

Rainfall data were recorded at Thika meteorological station.

#### 3.4.3 Statistical analysis:

The faecal egg count data were logarithmically transformed and used for analysis of variance according to the expression  $Y = \log(X + 50)$  in order to stabilize the variances. The log-transformed faecal egg count data, PCV data and total plasma protein data were analyzed using the repeated measures analysis of variance (ANOVA) in the GLM procedure of SAS (SAS, 1990). The data for the months of March and April were not included as the EPG counts were zero. Comparisons were made between age groups (kids, young and adults), seasons (long rains, dry season and short rains) and the different farms (Farm number) using repeated measures ANOVA. For ease of overview, the results of each age group was pooled in the graphs and figures across the farms. The fortnight data for faecal egg count (EPG) were pooled and averaged to give a mean monthly value that was used for analysis. This made the comparisons possible with the PCV and Total plasma protein data on the graphs and figures.

#### 3.5. Results:

The total monthly rainfall (mm) recorded in the study area during the period 1996, 1997, and 1998 is shown on Figure 3.2. The amount of rainfall received towards the end of 1997 and early 1998 was higher than the long term average for these areas. The duration of the rainfall was also longer than the normal expected period of the short rains between October and December.

The arithmetic mean strongyle faecal egg counts for the 3 groups of goats (kids, young and adults) are shown on Figure 3.3. The counts were higher in all the age groups during the long rains between April and August and during the heavy short rains between November and

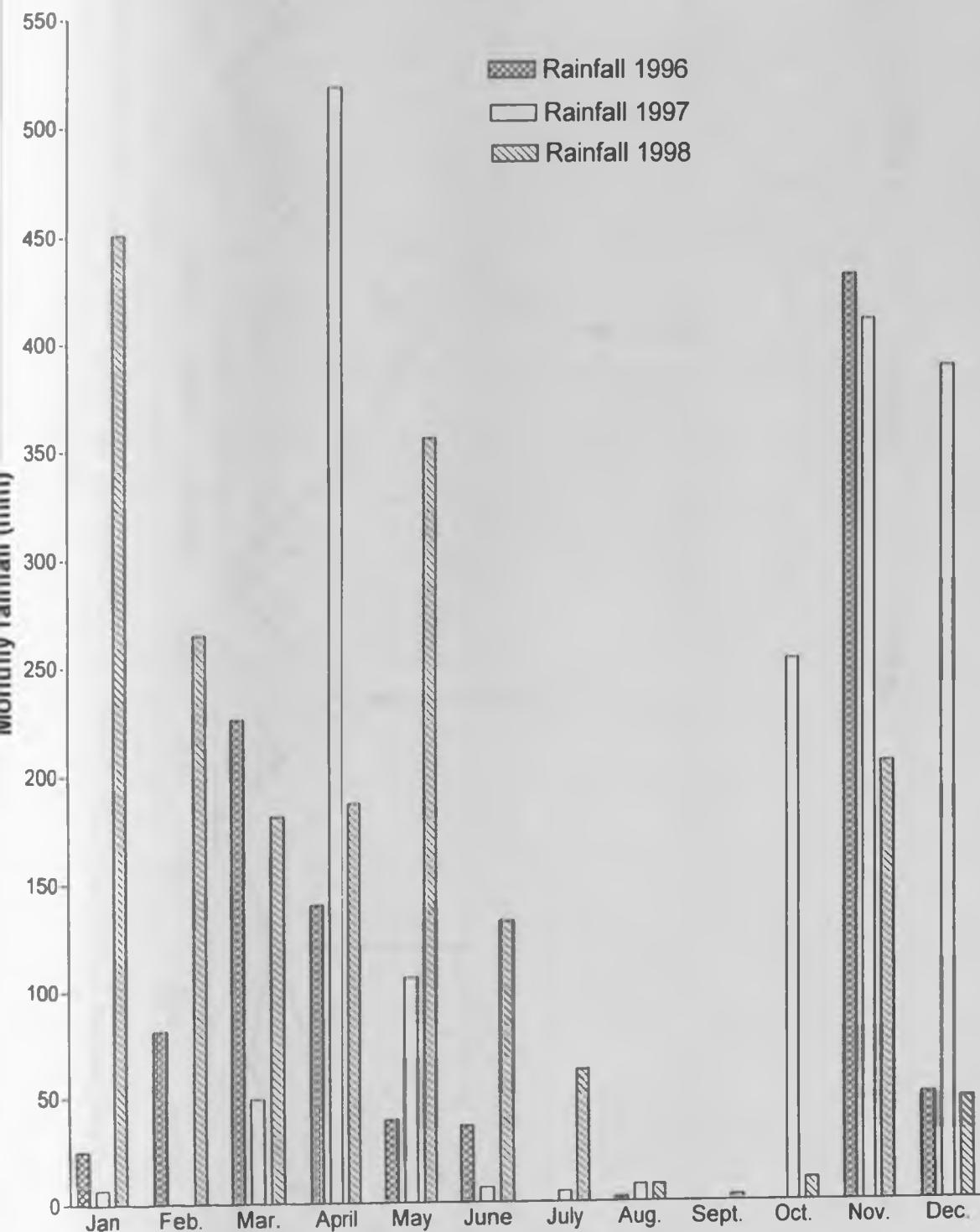


Figure 3.2: Total monthly rainfall recorded at Thika meteorological station 5km from the study sites for the years 1996, 1997 and 1998.

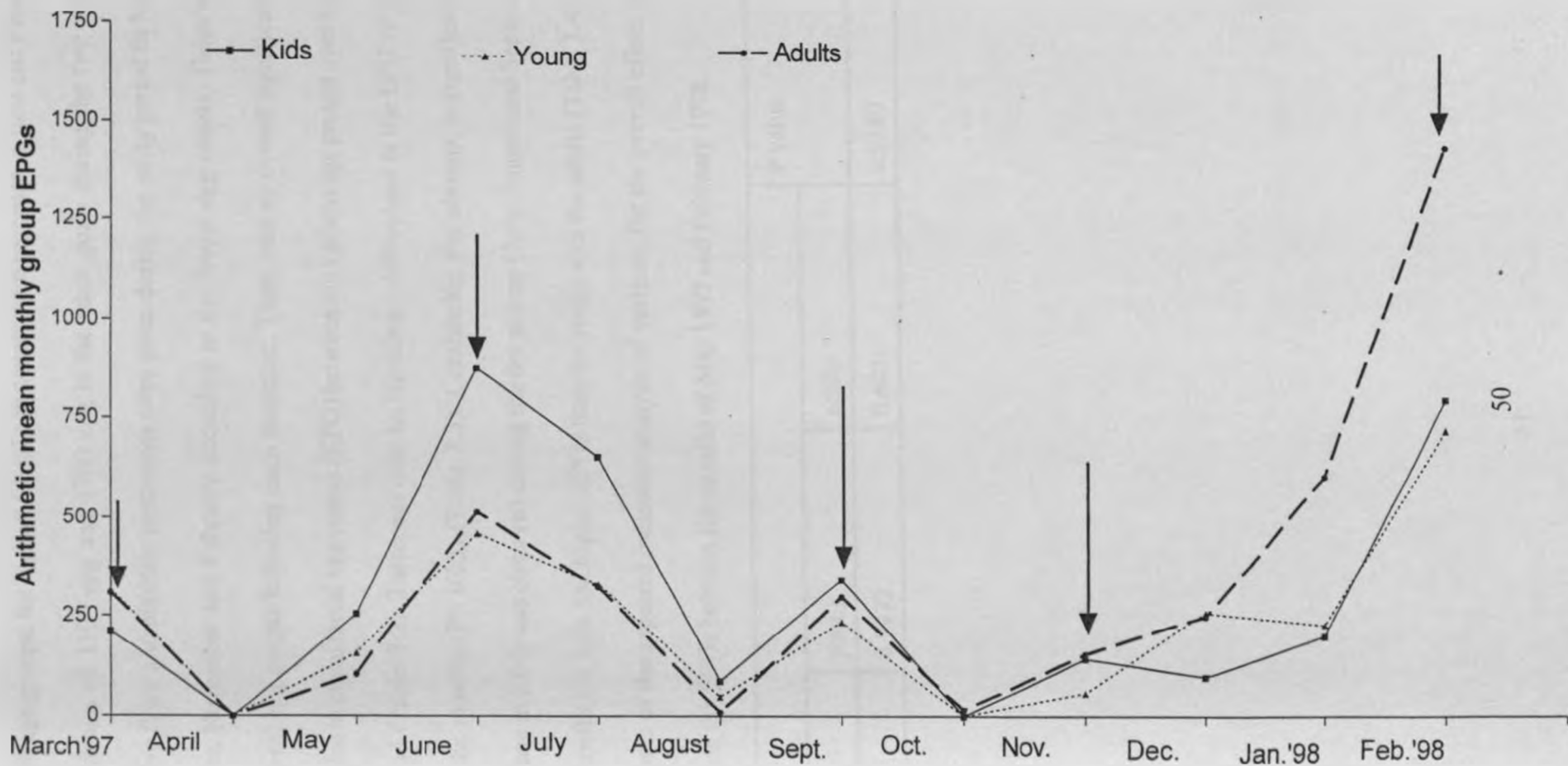


Figure 3.3: Monthly arithmetic mean group faecal egg counts (EPGs) for the kids, young and adults between March 1997 and February 1998. Arrows indicate flock anthelmintic treatments.

February. For all age groups, the highest faecal egg counts were recorded in June and February reaching a maximum of 1100 epg and 1700 epg in the adult goats during the two months respectively. Five flock anthelmintic treatments were given during the study period in March, June, September, November and February according to the faecal egg counts. There was a reduction in faecal egg counts following each treatment. There was no overall significant ( $P=0.5349$ ) difference in mean faecal egg counts (EPG) between the various age groups over the one year study period (Table 3.2). There was also no significant differences in the EPG of faeces during any of the months ( $P> 0.05$ ) (Table 3.3). Considering the seasons, a significant ( $P=0.0242$ ) difference in EPG was observed during the dry season (July, August and September). During these months the kids had higher EPGs than the young and the adults (Table 3.4).

Table 3.2: Results of the repeated measures analysis of variance for the overall effect of age groups on the EPGs between the months of May 1997 and February 1998.

| Lsmeans |        |        | P value |
|---------|--------|--------|---------|
| Kids    | young  | adults |         |
| 1.3321  | 0.9972 | 0.6071 | 0.5349  |

Table 3.3:Results of the repeated measures analysis of variance on the effect of age group on the EPGs on monthly basis:

| Month        | Lsmeans |       |        | P values |
|--------------|---------|-------|--------|----------|
|              | Kids    | Young | Adults |          |
| May          | 5.361   | 4.927 | 4.902  | 0.4240   |
| June         | 6.444   | 4.967 | 5.106  | 0.2271   |
| July         | 6.085   | 5.576 | 5.802  | 0.3508   |
| August       | 4.574   | 4.384 | 4.148  | 0.2367   |
| September    | 6.025   | 5.307 | 5.178  | 0.1433   |
| October      | 3.975   | 3.391 | 4.093  | 0.2482   |
| November     | 5.023   | 4.604 | 4.845  | 0.3208   |
| December     | 4.938   | 5.06  | 5.013  | 0.9618   |
| January '98  | 5.66    | 5.241 | 5.505  | 0.5110   |
| February '98 | 6.693   | 5.924 | 5.512  | 0.1201   |

Table 3.4: Results of the repeated measures analysis of the variance for the effect of age group on the EPGs during the long rains season (May - June), Dry season (July, August, September) and the short rains season (October, November, December 1997 , January and February 1998) of the study.

| Seasons     | Lsmeans |       |        | P Value |
|-------------|---------|-------|--------|---------|
|             | Kids    | Young | Mature |         |
| Long rains  | 5.013   | 5.078 | 5.217  | 0.4002  |
| Dry season  | 5.561   | 5.089 | 5.043  | 0.0242  |
| Short rains | 5.259   | 4.952 | 4.993  | 0.4844  |

At farm level, there was a significant ( $P= 0.009$ ) difference in EPG among the age groups within the farms during the long rains season months of May and June (Table 3.5). The farms had a significant( $P= 0.009$ ) effect on the EPG levels (Table 3.5). The farm effects were significant ( $P< 0.05$ ) during the months of May and November 1997 and February 1998 (Table 3.6). The effect of the farm on the EPG was significant during the long rains and the dry season ( $P< 0.05$ ) (Table 3.7). The effect was not significant ( $P= 0.2224$ ) during the short rains season.

Table 3.5: Results of the repeated measures analysis of variance for the overall effect of the four (4) farms on the EPGs between the months of May 1997 and February 1998

| Lsmeans |        |        |        | P value |
|---------|--------|--------|--------|---------|
| Farm 1  | Farm 2 | Farm 3 | Farm 4 |         |
| 0.7503  | 0.4292 | 0.3398 | 0.3960 | 0.009   |

Table 3. 6: Results of the repeated measures analysis for the effect of farm on EPGs during each month of the study.

| Month        | Lsmeans |        |        |        | P Value |
|--------------|---------|--------|--------|--------|---------|
|              | Farm 1  | Farm 2 | Farm 3 | Farm 4 |         |
| May          | 5.195   | 4.421  | 5.102  | 5.539  | 0.0044  |
| June         | 6.159   | 5.952  | 6.181  | 6.586  | 0.212   |
| July         | 5.584   | 5.477  | 5.957  | 6.265  | 0.1553  |
| August       | 4.042   | 4.307  | 4.468  | 4.657  | 0.1086  |
| September    | 5.913   | 5.361  | 5.054  | 5.686  | 0.1228  |
| October      | 4.213   | 3.909  | 3.922  | 3.955  | 0.053   |
| November     | 5.336   | 4.236  | 4.926  | 4.799  | 0.0023  |
| December     | 5.414   | 5.207  | 4.736  | 4.658  | 0.3552  |
| January '98  | 5.61    | 5.967  | 5.106  | 5.196  | 0.1674  |
| February '98 | 5.945   | 6.85   | 5.44   | 5.935  | 0.029   |

Table 3.7: Results of the repeated measures analysis of variance for the effect of farm on the EPGs during the long rains (May and June), Dry season (July, August and September) and the short rains season (October, November, December 1997, January and February 1998) of the study.

| Seasons     | Lsmeans |        |        |        | P value |
|-------------|---------|--------|--------|--------|---------|
|             | Farm 1  | Farm 2 | Farm 3 | Farm 4 |         |
| Long rains  | 5.072   | 4.752  | 5.199  | 5.367  | 0.0008  |
| Dry season  | 5.18    | 5.048  | 5.16   | 5.536  | 0.0288  |
| Short rains | 5.303   | 5.234  | 4.826  | 4.909  | 0.2224  |

*Haemonchus contortus* was the main nematode recovered from the pooled farm coprocultures throughout the study period. A few *Oesophagostomum* spp and *Chabertia ovina*

were recovered periodically (Table 3.8).

Table 3.8: Summary of larvae identified from cultures in June, September and December 1997 and February 1998

| Month         | Species of larvae identified | Percentage composition |
|---------------|------------------------------|------------------------|
| June          | <i>Haemonchus contortus</i>  | 69                     |
|               | <i>Chabertia ovina</i>       | 24                     |
|               | <i>Oesophagostomum</i>       | 7                      |
| September     | <i>Haemonchus contortus</i>  | 90                     |
|               | <i>Chabertia ovina</i>       | 5                      |
|               | <i>Oesophagostomum</i>       | 5                      |
| December      | <i>Haemonchus contortus</i>  | 98                     |
|               | <i>Chabertia ovina</i>       | 2                      |
| February 1998 | <i>Haemonchus contortus</i>  | 100                    |

The mean monthly PCVs for various age groups are shown on Figure 3.4. The mean monthly levels in all the age groups and the farms were within the normal range of 25 - 45% documented for goats (Radostits *et al*, 1997). There was no overall significant ( $P=0.0754$ ) differences in PCV levels between the age groups although the PCV levels in kids increased slightly over the study period compared to the young and the adults (Table 3.9). Considering each month, there was a significant ( $P=0.0187$ ) difference only in the first month of study i.e during April when the kids had lower PCV values than the other groups. There were no significant ( $P>0.05$ ) differences between the age groups in the other months (Table 3.10). When considering the seasons, significant differences were only evident during the long rains season when the kids had lower PCV levels compared to the young and the adults ( $P<0.05$ ). There were no significant differences in the other seasons (Table 3.11).

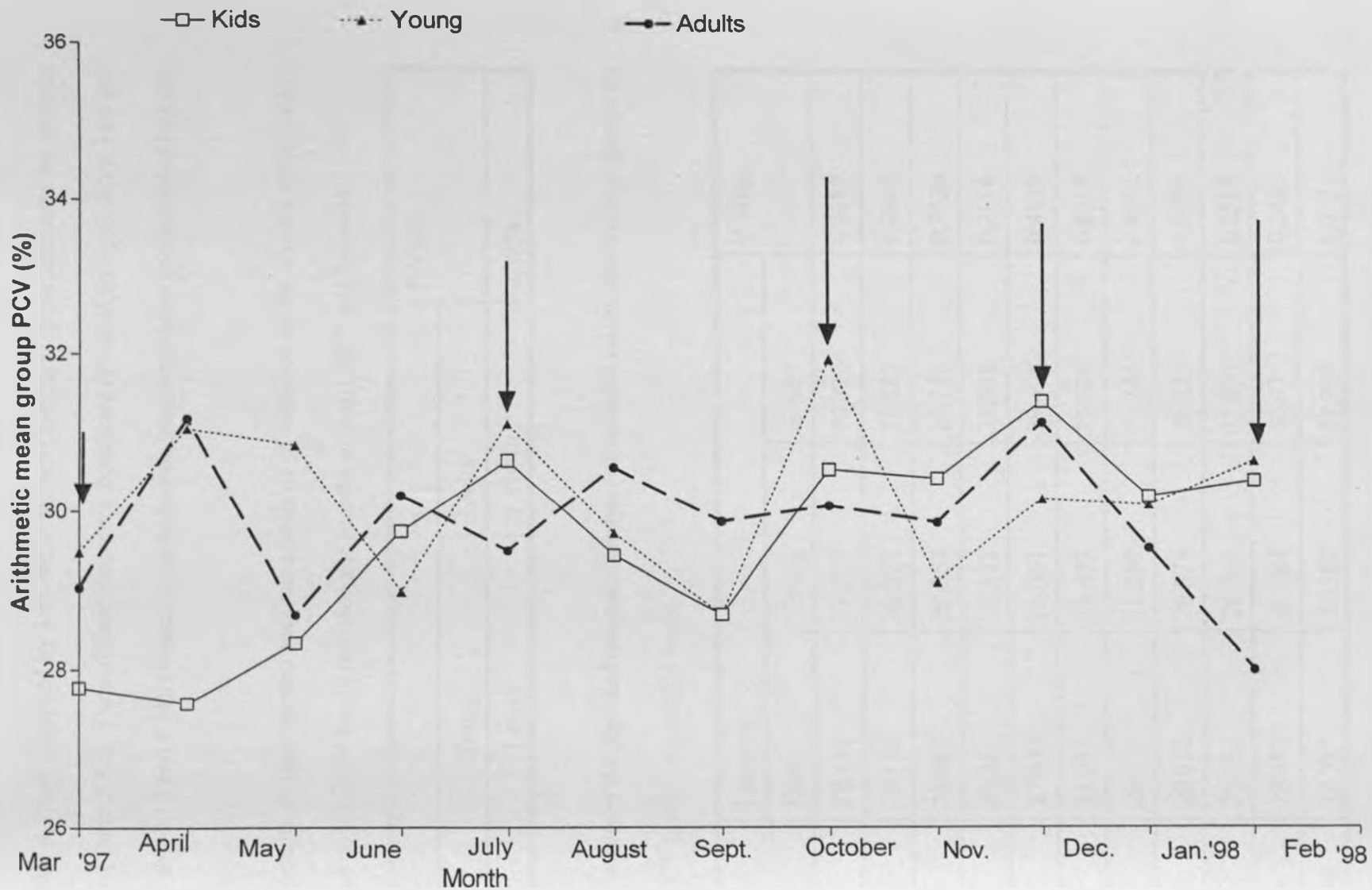


Figure 3.4: Arithmetic mean group Packed Cell Volume (PCVs) for the kids, young and adults between March 1997 and February 1998.

Arrows indicate flock anthelmintic treatments.

The PCV levels were significantly ( $P= 0.0028$ ) different between the farms (Table 3.12). The PCV levels in farm 1 dropped by 3.15 while those of the other farms increased by between 1.24 and 2.61 (Table 3.12). These differences were significant ( $P< 0.05$ ) in April, May, July and December (Table 3.13 and 3.14). Generally, anthelmintic treatments were accompanied by a rise in PCV values..

Table 3. 9: Results of the repeated measures analysis of variance on the overall effect of age groups on the PCV levels Between the months of April 1997 and February 1998.

| Lsmeans |         |          | P Value |
|---------|---------|----------|---------|
| Kids    | Young   | Adults   |         |
| 1.6408  | -1.0949 | -1.57970 | 0.0754  |

Table 3.10: Results of the repeated measures analysis of variance on the effect of age groups on the PCV levels on monthly basis.

| Month        | Lsmeans |        |        | P value |
|--------------|---------|--------|--------|---------|
|              | Kids    | Young  | Adults |         |
| April        | 27.144  | 31.377 | 30.323 | 0.0187  |
| May          | 29.152  | 30.277 | 28.885 | 0.2041  |
| June         | 26.943  | 29.557 | 29.748 | 0.3420  |
| July         | 29.316  | 31.511 | 30.058 | 0.2116  |
| August       | 27.979  | 30.001 | 29.400 | 0.4159  |
| September    | 28.577  | 29.475 | 29.924 | 0.8514  |
| October      | 29.019  | 31.209 | 31.244 | 0.4852  |
| November     | 29.783  | 29.574 | 30.425 | 0.6788  |
| December     | 28.954  | 29.767 | 31.077 | 0.2515  |
| January '98  | 28.498  | 30.264 | 30.67  | 0.3489  |
| February '98 | 28.785  | 30.282 | 31.908 | 0.0771  |

Table 3.11: Results of the repeated measures analysis of variance on the effect of age groups on the PCV levels during the long rains season (April, May and June), the dry season (July, August and September) and the short rains season ( October, November and December 1997, January and February 1998).

| Seasons     | Lsmeans |        |        | P value |
|-------------|---------|--------|--------|---------|
|             | Kids    | Young  | Adults |         |
| Long rains  | 27.746  | 30.437 | 29.654 | 0.0361  |
| Dry         | 28.624  | 30.329 | 27.794 | 0.4849  |
| Short rains | 29.026  | 30.219 | 31.065 | 0.1832  |

Table 3.12: Results of the repeated measures analysis of variance on the overall effect of the farms on the PCV levels between April 1997 and February 1998.

| Lsmeans  |        |        |        | P value |
|----------|--------|--------|--------|---------|
| Farm 1   | Farm 2 | Farm 3 | Farm 4 |         |
| - 3.1501 | 2.6147 | 1.2449 | 2.1246 | 0.0028  |

Table 3.13: Results of the repeated measures analysis of variance on the effect of farms on PCV levels on monthly basis.

| Month        | Lsmeans |        |        |        | P value |
|--------------|---------|--------|--------|--------|---------|
|              | Farm 1  | Farm 2 | Farm 3 | Farm 4 |         |
| April        | 32.269  | 28.816 | 29.849 | 27.532 | 0.0044  |
| May          | 30.782  | 31.202 | 27.478 | 28.423 | 0.0091  |
| June         | 29.967  | 28.385 | 29.984 | 26.862 | 0.2170  |
| July         | 30.140  | 28.208 | 32.062 | 30.771 | 0.0482  |
| August       | 29.649  | 27.393 | 30.368 | 29.096 | 0.1216  |
| September    | 29.882  | 28.595 | 29.195 | 29.629 | 0.8783  |
| October      | 28.645  | 29.322 | 33.102 | 30.894 | 0.0750  |
| November     | 30.321  | 28.780 | 31.046 | 29.679 | 0.3056  |
| December     | 29.667  | 28.028 | 32.260 | 29.775 | 0.0131  |
| January '98  | 29.527  | 28.644 | 31.681 | 29.391 | 0.1065  |
| February '98 | 29.119  | 31.431 | 31.093 | 29.657 | 0.1159  |

Table 3.14: Results of the repeated measures analysis of variance for the effect of farm on the PCV levels during the long rains season (April, May and June), dry season (July, August and September) and the short rains season (October, November and December 1997, January and February 1998).

| Season      | Lsmeans |        |        |        | P value |
|-------------|---------|--------|--------|--------|---------|
|             | Farm 1  | Farm 2 | Farm 3 | Farm 4 |         |
| Long rains  | 30.939  | 29.468 | 29.193 | 27.605 | 0.0109  |
| Dry         | 29.890  | 28.065 | 30.542 | 29.832 | 0.1916  |
| Short rains | 29.456  | 29.242 | 31.836 | 29.879 | 0.0463  |

The mean monthly plasma protein values for various age groups are shown on Figure 3.5. Overall there were no significant ( $P= 0.2734$ ) differences between the age groups (Table 3.15). The values in the groups decreased by 1.33 (kids), 3.76 (young) and 5.42 (adults). However the means were above the documented ranges of plasma proteins for goats (between 60 - 75g /l) (Radostits *et al.*, 1997). On monthly basis, the differences among the age groups were significant ( $P< 0.05$ ) in April, May, July and August sampling when the kids had relatively lower values compared to the young and the adults (Table 3. 16). Significant ( $P< 0.05$ ) differences were also found during the long rains and the dry season months (Table 3. 17). Kids had relatively lower values.

Table 3.15: Results of the repeated measures analysis of variance on the overall effect of age groups on the total plasma protein levels between April 1997 and February 1998.

| Lsmeans |         |         | P value |
|---------|---------|---------|---------|
| Kids    | Young   | Adult   |         |
| -1.3334 | -3.7604 | -5.4210 | 0.2734  |

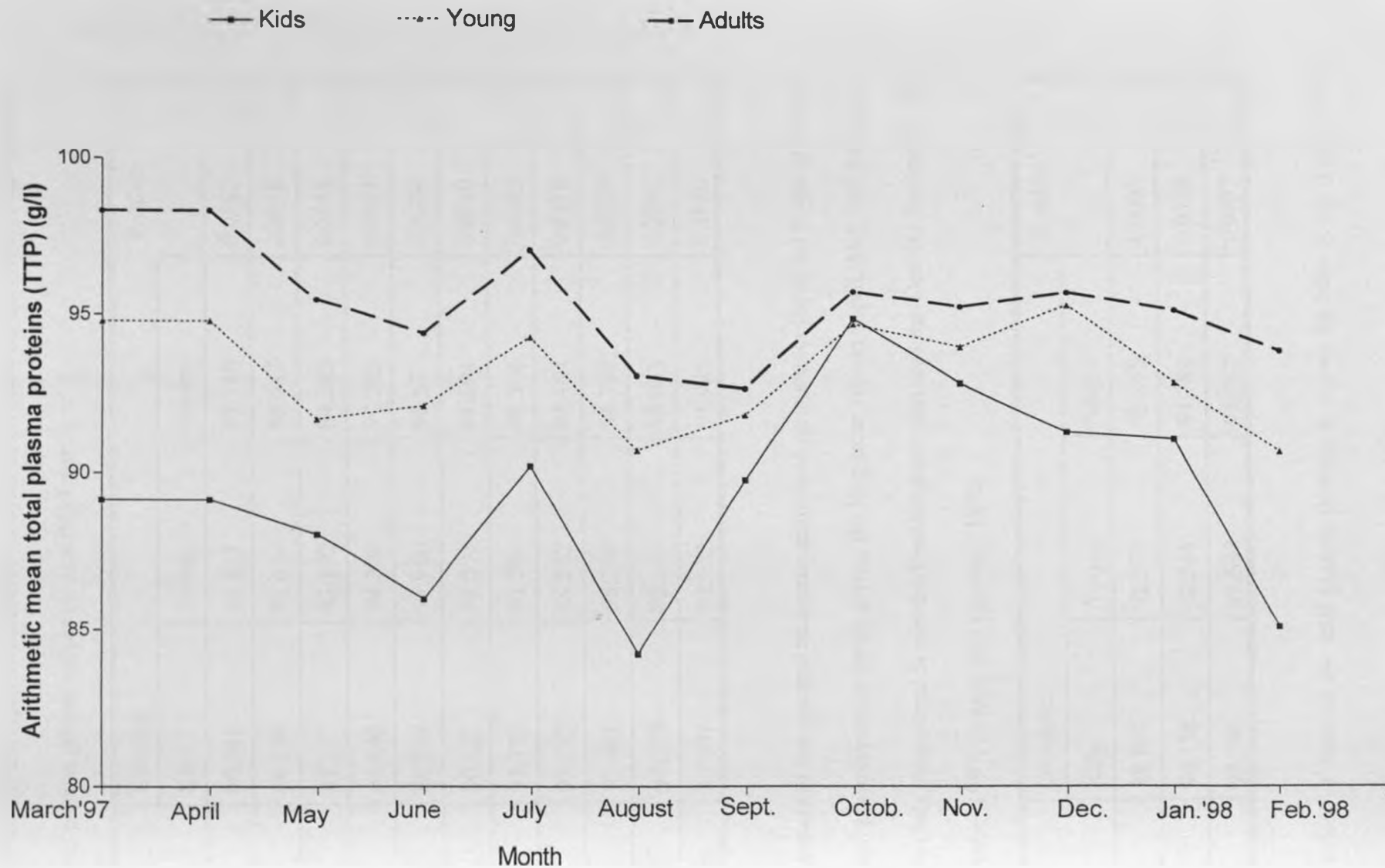


Figure 3.5: Arithmetic mean monthly group Total Plasma Proteins (TPP) for kids, young and adults during the period March 1997 and February 1998.

Table 3.16: Results of the repeated measures analysis of variance for the effect of age group on the total plasma protein values on monthly basis.

| Month        | Lsmeans |        |        | P value |
|--------------|---------|--------|--------|---------|
|              | Kids    | Young  | Adults |         |
| April        | 90.841  | 94.813 | 97.346 | 0.0187  |
| May          | 88.276  | 91.614 | 95.945 | 0.0028  |
| June         | 87.557  | 92.192 | 94.283 | 0.0554  |
| July         | 91.640  | 94.208 | 97.093 | 0.0461  |
| August       | 88.214  | 91.900 | 95.577 | 0.0024  |
| September    | 90.622  | 91.597 | 91.694 | 0.8830  |
| October      | 95.772  | 93.704 | 94.504 | 0.4143  |
| November     | 93.532  | 94.532 | 94.484 | 0.8378  |
| December     | 93.991  | 95.770 | 94.337 | 0.4259  |
| January '98  | 94.239  | 93.133 | 94.083 | 0.7046  |
| February '98 | 89.507  | 91.052 | 91.925 | 0.5926  |

Table 3.17: Results of the repeated measures analysis of variance for the effect of age groups on the total plasma protein levels during the long rains season (April, May and June), dry season (July, August and September) and the short rains seasons (October, November and December 1997, January and February 1998).

| Season      | Lsmeans |        |        | P value |
|-------------|---------|--------|--------|---------|
|             | Kids    | Young  | Adults |         |
| Long rains  | 88.891  | 92.873 | 95.858 | 0.0005  |
| Dry         | 90.158  | 92.569 | 94.788 | 0.0326  |
| Short rains | 93.390  | 93.638 | 93.867 | 0.9497  |

The effect of farm on the total plasma proteins is shown on table 3. 18. There were

significant ( $P=0.002$ ) differences among the farms. The mean levels in farm 1 rose by 2.05 units while those in farms 2, 3 and 4 fell by 3.56, 5.52 and 6.98 respectively. Significant ( $P<0.05$ ) differences were evident during the months of August, September and December 1997 and January and February 1998 (Table 3.19).

Table 3.18: Results of the repeated measures analysis of the overall effect of farm on the plasma protein levels between April 1997 and February 1998.

| Lsmeans |         |         |         | P value |
|---------|---------|---------|---------|---------|
| Farm 1  | Farm 2  | Farm 3  | Farm 4  |         |
| 2.0466  | -3.5621 | -5.5255 | -6.9789 | 0.002   |

Table 3.19: Results of the repeated measures analysis of variance on the effect of farm on the total plasma proteins on monthly basis.

| Month        | Lsmeans |         |        |         | P value |
|--------------|---------|---------|--------|---------|---------|
|              | Farm 1  | Farm 2  | Farm 3 | Farm 4  |         |
| April        | 96.731  | 95.283  | 92.156 | 93.163  | 0.1438  |
| May          | 91.665  | 92.628  | 91.077 | 92.410  | 0.8309  |
| June         | 93.7112 | 92.334  | 90.760 | 88.571  | 0.2254  |
| July         | 95.7149 | 95.823  | 93.198 | 92.520  | 0.2817  |
| August       | 95.1188 | 95.363  | 88.720 | 88.387  | 0.0002  |
| September    | 93.0321 | 93.664  | 88.060 | 90.461  | 0.0386  |
| October      | 94.464  | 96.7117 | 94.130 | 93.335  | 0.1369  |
| November     | 94.881  | 94.642  | 94.051 | 93.0367 | 0.7756  |
| December     | 99.349  | 95.455  | 92.406 | 91.587  | 0.0009  |
| January '98  | 99.639  | 94.707  | 90.502 | 90.426  | 0.0001  |
| February '98 | 98.777  | 91.721  | 86.630 | 86.184  | 0.0001  |

The effects of the farm on the total protein were significant ( $P<0.05$ ) during the dry

season months and the months of the short rains (Table 3. 20).

Table 3.20: Results of the repeated measures analysis of variance for the effect of farm on the total plasma protein levels during the long rains season (April, May and June), dry season (July, August and September) and the short rains season ( October, November and December 1997, January and February 1998).

| Season      | Lsmeans |        |          |         | P value |
|-------------|---------|--------|----------|---------|---------|
|             | Farm 1  | Farm 2 | Farm 3   | Farm 4  |         |
| Long rains  | 94.036  | 93.415 | 91.3309  | 91.3814 | 0.2239  |
| Dry         | 94.622  | 94.950 | 89.9922  | 90.4560 | 0.0034  |
| Short rains | 97.422  | 94.647 | 91. 5438 | 90.9137 | 0.0001  |

3.6. Discussion:

Similar variation in the seasonal prevalence and intensity of gastrointestinal nematode infections in goats observed in this study has been reported by other workers (Lutu 1983; Costa *et al.*, 1991; Rahman. 1992; Pandey *et al.*, 1994; Dorny *et al.*, 1995; Jacquiet *et al.*, 1995). In most of these reports, the prevalence and intensity measured by either faecal egg counts or total worm counts followed the rainfall patterns. They concluded that there is a direct relationship between rainfall and intensity of infection with gastrointestinal nematodes. Rainfall is the limiting environmental variable controlling translation of worm eggs to infective larvae in the tropics as temperatures are always favorable (Waller, 1997a). Jacquiet *et al.*, ( 1995), in a study on the seasonal evolution of helminth infection of sheep and goats in southern Mauritania found that season was the most important factor influencing the strongyle egg output, the rainy season being significantly the most favorable and dry season the least favorable. In the present study, high rainfall led to accelerated hatching of nematode eggs, improved survival of the infective larvae leading to heavy pasture infectivity, higher uptake of infective larvae leading to heavier

worm burdens and increased faecal egg counts. The duration and amount of rainfall during this study was higher than in the previous years. This may have led to maturation of hypobiotic larvae and also reduced or eliminated hypobiosis as reported by earlier workers (Pandey *et al.*, 1994; Gatongi, 1996; Gatongi *et al.*, 1998). These workers observed that the rate of hypobiosis is dependent on the amount and duration of the rainfall. They observed that the arrested larvae may co-exist with the adult egg laying worms during unfavorable periods. Therefore, heavy rainfall leads to maturation of the arrested larvae and may contribute to the increased faecal egg counts during the rainy season. Heavy rainfall leads to good vegetation cover which is critical for nematode transmission (Dinnik and Dinnik, 1961). This leads to improved larval survival and higher pickup leading to higher faecal worm egg counts.

Differences in grazing area and stocking density between farms may have an influence on the intake of infective larvae as observed by Dorny *et al.*, (1995). Goats grazing restricted areas will pick more larvae than those left to browse freely. The type of herbage may also have an effect on the faecal worm egg counts especially those with condensed tannins.

In the present study there were no significant differences in the intensity of infection with gastrointestinal helminths between the various age groups. This has been earlier reported in goats in Kenya on 4 farms where a point prevalence survey showed no differences in infection levels between the kids, immature and adult goats (Maingi *et al.*, 1993). Anene *et al.*, (1994), in Nigeria observed that adult goats ( more than 9 months) showed higher worm burdens than younger goats. In semi arid southern Mauritania, differences in faecal egg counts were only demonstrated during the dry season, with older animals excreting more eggs. Silva *et al.*, (1998), observed that old goats had higher parasite burdens than younger goats. Compared to sheep, goats have been shown to have a slow development of acquired immunity to gastrointestinal nematodes ( Dorny *et al.*, 1995; Vlassoff *et al.*, 1999). Le Jambre and Royal, (1976), suggested that goats as a

consequence of their browsing habit, have evolved a less efficient acquired resistance than sheep. When left freely to browse, goats have been thought to pick up fewer larvae than sheep. The slower build up of immunity with age may be the result of less contact with parasites (Dorny *et al.*, 1995). Unlike sheep where lambs accompany and are usually beside the ewes when feeding, in the tropics, goat kids practice “lying out” or “planting” behavior where kids are left in “camps” or penned around the homestead while does go out feeding (Peacock, 1996). This behavior delay contact with the infective larvae to the time the kids start accompanying the does which in some farms may be up to or after 3 months. This factor may also contribute to the lower level of prevalence and intensity of the gastrointestinal nematodes in the young and higher prevalence in the older goats. Vlassoff *et al.* (1999) observed that resistance to nematode establishment in goats develops up to 12 months and there is no marked increase in resistance beyond this age. This may explain the lack of significant differences in faecal egg counts in the present study.

The PCV values followed a similar pattern as the faecal egg counts with regard to the age groups and seasons. *Haemonchus contortus* which was the main nematode recovered is a blood sucker and had a significant effect on the PCV levels. The low levels during the dry season between July and September may be due to the poor quality feed during this period. Nutrition has been found to play a major role in buffering the pathogenic impact of parasites (Blackburn *et al.*, 1991).

The prevalence and intensity of gastrointestinal nematode parasites in goats in the marginal low potential areas of central Kenya varies with seasons. The intensity is highest during the rainy season and also varies with the amount and duration of rainfall. The intensity does not vary with age groups. All ages shed helminth eggs and age resistance seems not to play a major role. The intensity of infection also varies with individual farms. This may be due to a combination of factors such as management, nutrition, pasture types and levels of pasture

infection on each farm.

*Haemonchus contortus* was the main nematode infecting the goats in this area. The number of anthelmintic treatments given in this study which were highly influenced by the farmer, were on the higher side. This should be reduced in order to reduce cost to the farmer and also may delay the development and spread of anthelmintic resistant nematodes. This can be achieved by administering individual anthelmintic treatments instead of flock treatments. In this case only goats that have a certain level of faecal egg count per gram (EPG) should be drenched.

The second part of this study attempted this approach where anthelmintic treatments were only given to those goats that had an EPG of over 1500.

## SECTION 2:

### **3.8. Study on the epidemiology of gastrointestinal nematodes of goats when only individual anthelmintic treatments are given:**

#### **3.8.1. Introduction:**

Goats are kept worldwide in a range of different production systems from large numbers in extensive pastoral systems grazing common pastures in the arid and semi arid areas to intensively managed small flocks permanently housed as in rain forest / humid Java (Peacock, 1996). In the marginal agricultural areas, they serve as insurance to crop failure as they can be easily sold or exchanged for grain. They play a role in alleviating poverty of the poorest in many developing countries as the first step up and out of poverty. Many of farmers keep goats in small numbers on small farms.

It is estimated that over 80% of sheep and goats in Kenya are found in the arid and semi arid areas where they contribute an important source of dietary protein in form of milk and meat (Gatongi, 1996). However, their health and productivity depends on adequate disease control and husbandry. Gastrointestinal parasitism is one of the most prevalent problems and its chronic nature steadily eats away farmers profits . Most of the farmers in the semi arid areas can be classified as resource poor and are unable to afford the prices of anthelmintics. Any effort that may reduce the amount or reliance on anthelmintics in this areas is welcome. This study investigated the effect of individually treating goats with EPG counts of over 1500 instead of flock treatment and whether the numbers treated vary with age groups on two farms in a low potential area in Thika District of Central Kenya.

#### **3.8.2 Materials and Methods:**

##### **3.8.2.1. Study area and animals:**

The work was carried out in the coffee marginal low potential areas of Thika District

(Ecozone3). Coffee, horticultural and floricultural crops are grown under irrigation. During the dry season, goats may be grazed on roadside pastures and communal areas. The area receives an annual average rainfall of 1100mm which is bimodal in distribution. The long rains fall between March and May while the short rains usually occur between October and December but variation between years is substantial. The study was conducted between May (end of the long rains) and January (after the short rains) on two farms. Farm A (more than 50 ha) which had a flock of 90 goats composed of 29 adult does (over 2 years), 24 young (mainly females 1-2 years), 30 kids, 4 entire bucks and 3 castrates. Farm B (5 ha) had a flock of 68 goats composed of 20 adult does, 18 young, 20 kids, 4 entire bucks and 6 castrates.

Twenty and 32 goats of various ages were randomly selected from Farm A and Farm B respectively and ear tagged for identification. They were mainly of the Small East African breed. They were divided into 3 age groups (kids, young and adults) according to history and the number of permanent incisors. Majority in all the groups were females. The groups were maintained up to the end of the study with no transfers or replacements within the groups.

#### 3.8.2.2. Sampling and analysis:

The ear tagged goats were faecal sampled every two weeks and faecal egg counts determined by the modified McMaster technique with a lower limit of detection of 100 EPG of faeces (Anon., 1986; Hansen and Perry, 1994). Monthly Ethylenediaminetetra-acetic acid (EDTA) blood was collected by jugular venipuncture and packed cell volume (PCV) and total plasma protein (TP) determined using the microhaematocrit and refractometry methods, respectively. Farm pooled coprocultures were setup at monthly intervals and resultant larvae counted and identified (Hansen and Perry, 1994).

The animals were monitored and individual anthelmintic treatments given to those goats with EPG of over 1500 and/or PCV values lower than 20%. Such goats were then removed from the

study.

Rainfall data were recorded at Thika meteorological station.

### 3.8.2.3. Statistical analysis:

The faecal egg count data were logarithmically transformed and used for analysis of variance according to the expression  $Y = \log(x + 50)$  in order to stabilize the variances. The log-transformed faecal egg count data, PCV data and total plasma protein data were analyzed using repeated measures analysis of variance (ANOVA) in the GLM procedure of SAS (SAS, 1990).

### 3.8.3 Results:

The highest rainfall was received in May and was followed by a dry season up to October. The rainfall in the later period was very limited and the short rains which usually start in October were delayed until November (Figure 3.6). The total precipitation were below the long term average for the area during the short rains season.

There were no significant ( $P > 0.05$ ) difference in the faecal egg output (EPG) throughout the study during the various seasons (long rains - May and June, Dry season - July, August and September and the short rains - October, November and December). There was a general rise in faecal egg output on both farms in September and October although there were no significant rainfall received.

There was no significant differences in the faecal egg counts among the age groups although the kids had higher EPGs than the young and the adults throughout the study with peaks in June and December (Figure 3.7). They were followed by the adults while the young had generally lower EPG counts. Salvage treatment was given to 5 kids, 4 young goats and 7 adult goats from both farms which had over 1500 EPG. There were no significant ( $P > 0.05$ ) differences in the distribution of the number of animals given salvage treatment on both farms. There were no significant ( $P > 0.05$ ) differences among the age groups (Table 3.21). Peri-

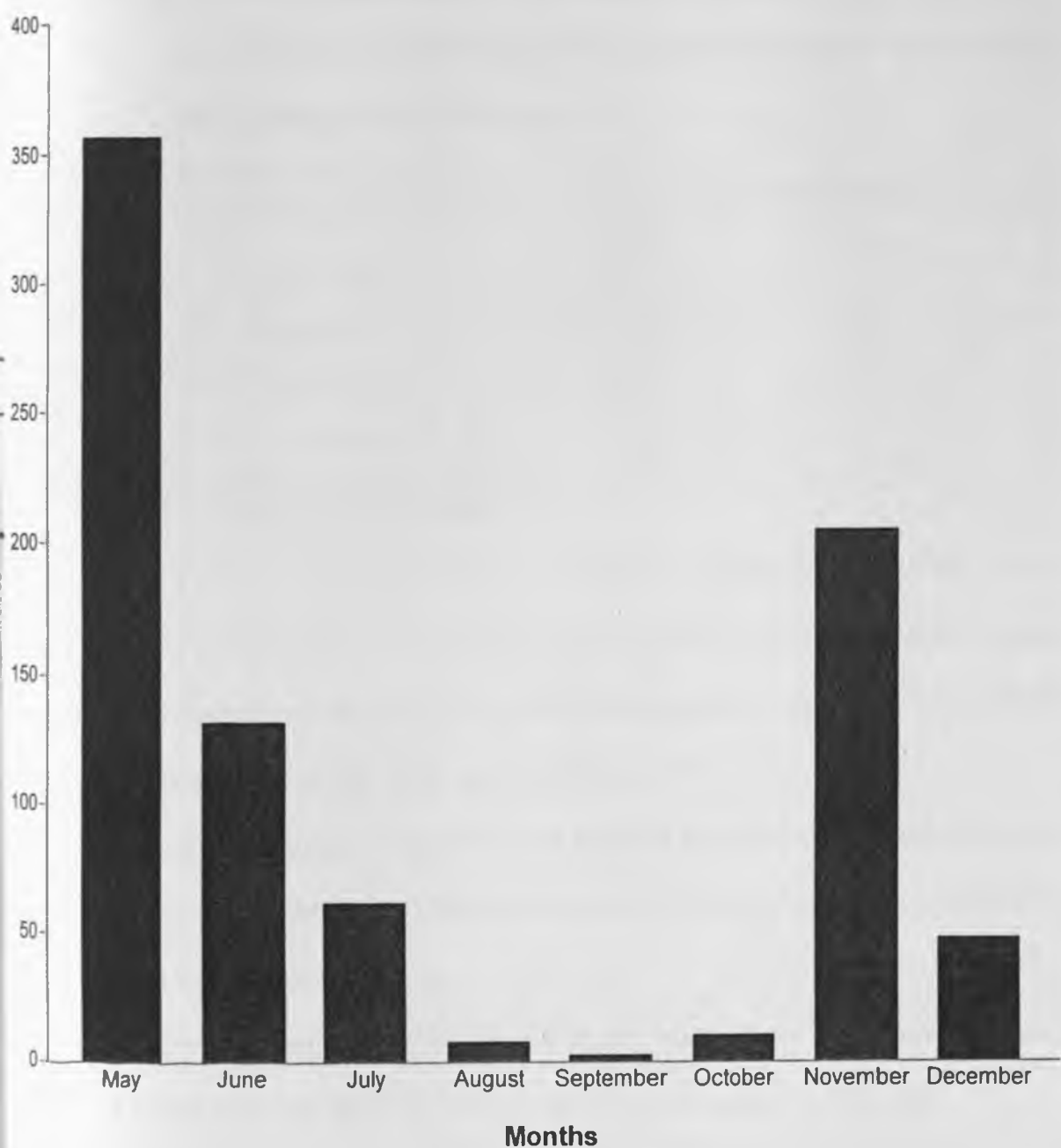


Figure 3.6: Monthly total rainfall recorded at Thika meteorological station 12 km from the study farms during the study period May - December 1998.

parturient rise in faecal output was seen in 4 out of 12 does that kidded during the study period. This had no statistical significance on the faecal egg counts between the age groups. In some, it was transient and did not require anthelmintic treatment while some had to be treated. The highest faecal egg count was 3600epg in an old doe which had given birth to twins. The twins died after 2 days due to malnutrition while the mother was given salvage treatment.

Table 3.21: Distribution of salvage treated goats among age groups during the rainy season (May, June, November and December) and the dry season (July, August, September and October) during the 8- month study.

| Age group | Rainy season - Number treated |             | Dry season Number treated |             |
|-----------|-------------------------------|-------------|---------------------------|-------------|
|           | Farm 1                        | Farm 2      | Farm 1                    | Farm 2      |
| Kids      | 2 (n = 7)*                    | 2 (n = 10)* | 1 (n = 7)*                | 1 (n = 10)* |
| Young     | 0 (n = 6)                     | 0 (n = 12)  | 3 (n = 6)                 | 1 (n = 12)  |
| Adults    | 0 ( n = 7)                    | 1 ( n = 10) | 2 ( n = 7)                | 3 ( n = 10) |
| Total     | 2 (n = 20)                    | 3 (n = 32)  | 6 (n = 20)                | 5 (n = 32)  |

(-)\* Total number for age group on the farm.

ThePCV values were depressed during the dry months of September and October. There was a difference in PCV values between various age groups in June, July and December (Figure 3.8). This was however not statistically significant although some kids and adult goats which were given salvage treatment had PCV values of less than 20% .

The mean total plasma protein (TP) values followed the same pattern as the PCVs and were different in June, September and December (Figure 3.9). These values were not statistically significant between the age groups.

*Haemonchus contortus* was recovered from the coprocultures throughout the study period. A few *Oesophagostomum* spp infective larvae were recovered in September.

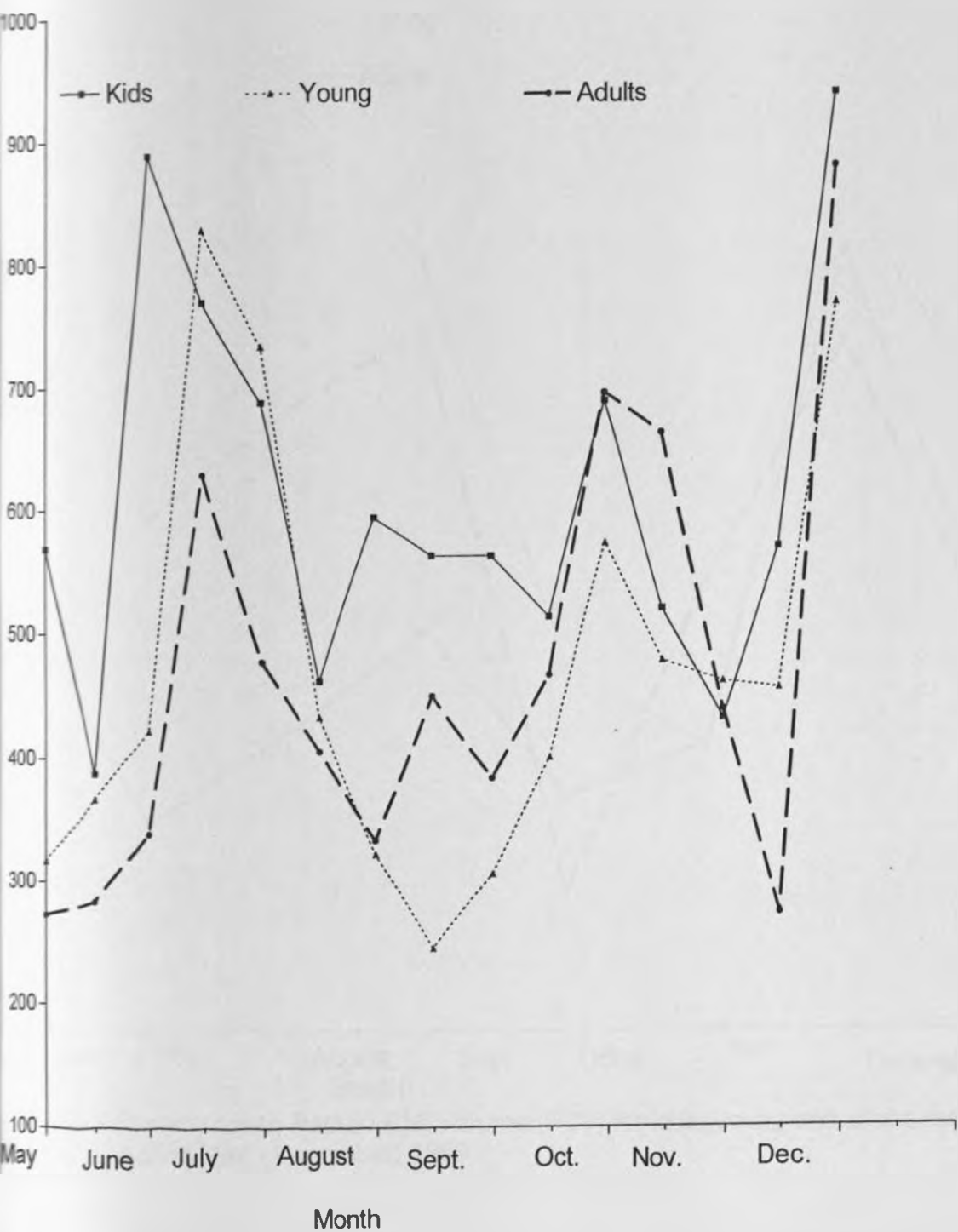


Figure 3.7: Arithmetic mean faecal counts for the kids, young and adults at each sampling during the study period May - December, 1998.

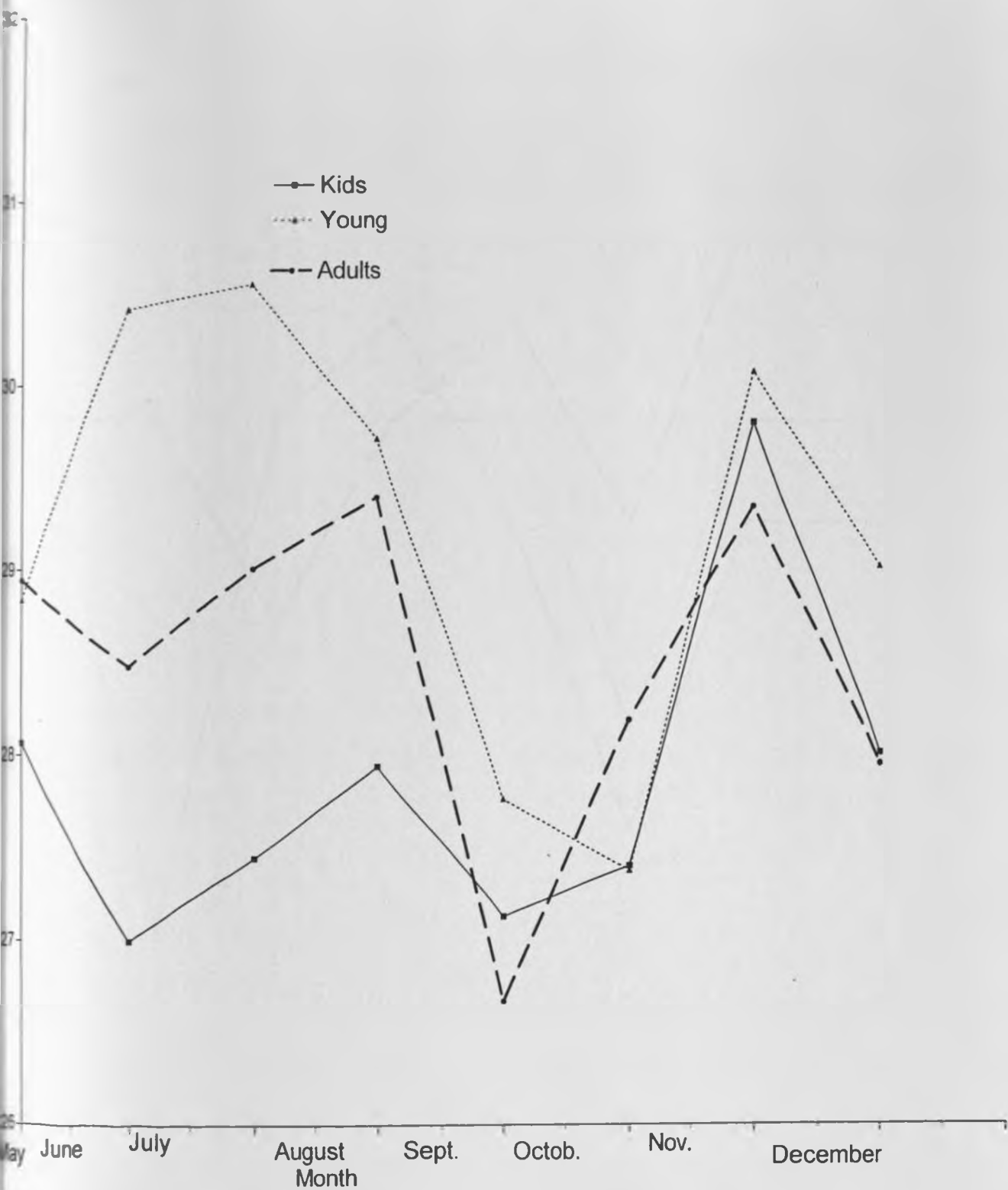


Figure 3.8: Arithmetic mean Packed Cell Volume (PCV) for kids, young and adults during the study period May - December, 1998.

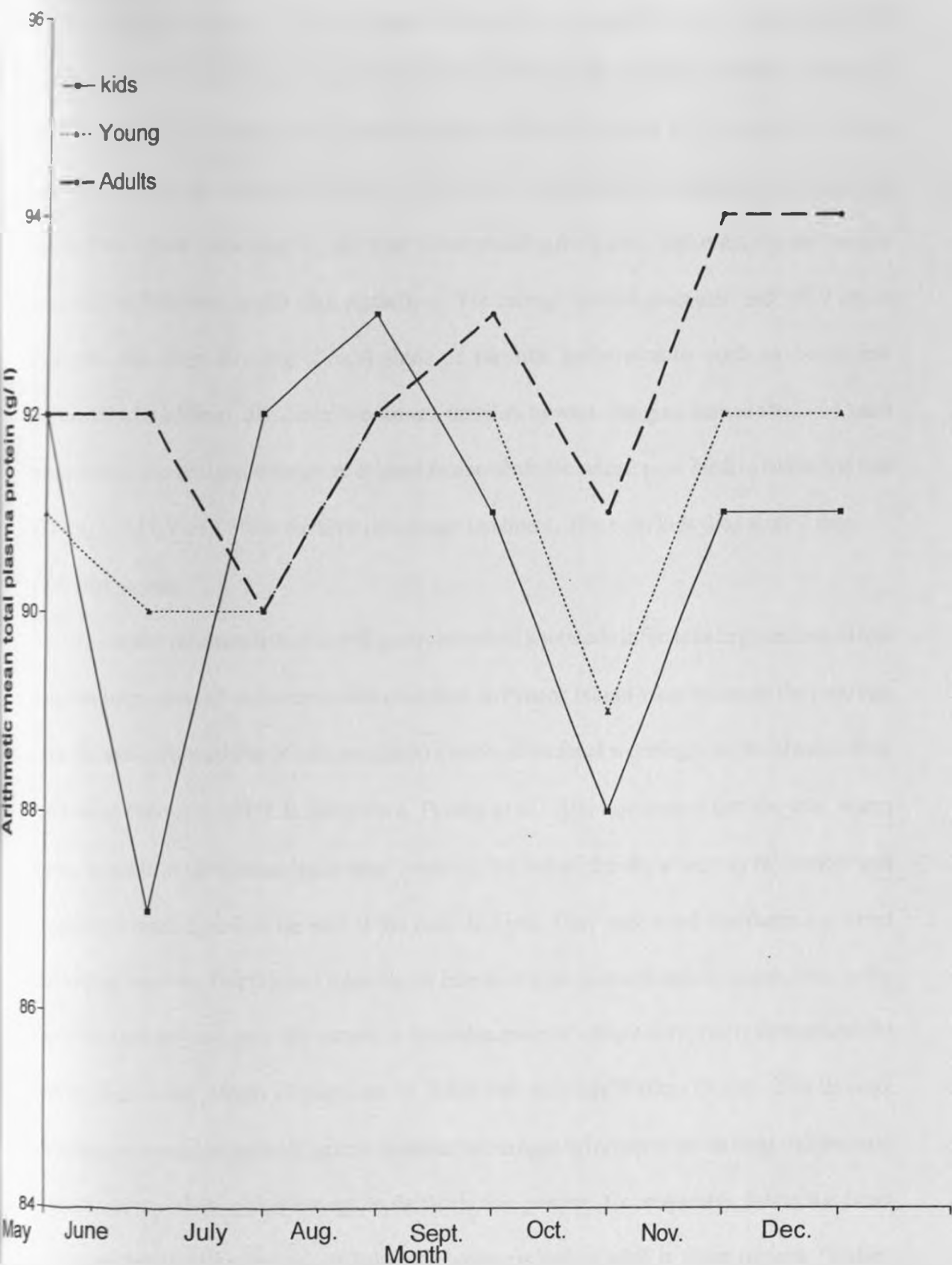


Figure 3.9: Arithmetic mean Total Plasma Protein (TPP) for the kids, young and adults during the study period May - December, 1998.

Four (4) kids out of 17 kids ear tagged were given salvage treatment in September and October when their faecal egg output exceeded 1500 and the PCV was less than 20% while 6 out of 18 ear tagged adult goats were given salvage treatment in October and November. Among these were 4 does that kidded during this period and showed peri parturient rise in faecal egg output. The highest faecal egg outputs were those resulting from peri - parturient rise and ranged from 1600 to 3600 one month after parturition. The salvage treated goats also had PCV lower than 20% and were showing clinical signs of parasitic gastroenteritis such as bottle jaw (submandibular edema), diarrhoea, weakness, inability to walk (lagging behind the flock) and in-appetence. These signs were more evident in one adult doe which gave birth to twins and had 3600epg and PCV of 17% at the time of salvage treatment. The twin kids died after 2 days.

#### **3.8.4. Discussion:**

Seasonal variation in the level of gastrointestinal nematode infections in goats seen in this study has been reported earlier in several countries. In Penang Island West Malaysia the fecal egg count patterns followed that of total rainfall in a study of the fecal worm egg counts of goats from two farms ( Rahman, 1992). In Zimbabwe, Pandey *et al.*, (1994), reported that the total worm burden in goats in communal lands were lowest at the end of the dry season in November and increased to reach a peak at the end of the rains in April. They concluded that there is a direct relationship between rainfall and intensity of infection with gastrointestinal nematodes. In the tropics and sub tropics, the environment is favorable more or - less continuously throughout the year for free living stages of parasites to hatch and develop(Waller, 1997a). The limiting environmental variable controlling translation of worm eggs to infective larvae is rainfall because temperatures are always high enough to facilitate this process. Consequently, there is a direct correlation between the severity of helminth problems and rainfall in these regions (Waller, 1997a). In a study on the effect of housing, pasture type and roughage supplements on nematode

parasitism in goats, Costa *et al.* (1991) found that the prevalence of infection was highest in the rainy season and in rainy years. Shavulimo and Semenye (1990), in a study of helminthosis in goats in some high, medium and low rainfall areas of Kenya found no correlation between rainfall and fecal egg counts (epg) in the medium and low potential areas. A correlation was however found in the high rainfall areas. In a study on internal parasitism in milk goats in Kenya, Lutu, (1983), reported fecal analysis being dominated by strongyle eggs which rose progressively throughout the study period in the high potential, high rainfall areas of Kenya. Jacquet *et al.*, (1995), in a study on the seasonal evolution of helminth infection of sheep and goats in southern Mauritania found that season was the most important factor influencing the strongyle egg output, the rainy season being significantly the most favorable and dry season the least.

The rise in faecal egg output before the rains seen in this study has been observed in the semi arid and arid areas where a sharp rise in fecal egg output occurs soon after the onset of the rains or even during the late dry season. This may be accompanied by acute parasitic gastroenteritis with high mortalities (Gatongi, 1996). This phenomenon cannot be accounted for by fresh infection in the pastures and may be due to hypobiosis. This is an adaptive characteristic that facilitates survival of nematodes especially *Haemonchus contortus* and subsequent transmission in the marginal and semi arid areas (Gatongi *et al.*, 1998). This allows the persistence of larval stages, mostly L4 in the host in a physiologically inert form that resume development at an opportune time when conditions become conducive for transmission. This phenomenon complicates interpretation of faecal egg counts as it only reflects the adult egg laying population and there may be many immature larval stages. These will resume development when conditions become conducive. The level of hypobiosis varies with seasons and may be as high as 80% in the long dry seasons and as low as 0% in the long rainy seasons (Gatongi *et al.*, 1998). This means that the hypobiotic larval population and the adult egg laying population

varies with seasons. A sharp rise in fecal egg output soon after the rains or during the late dry season is due to resumed development and maturation of the hypobiotic larvae to adults that start laying eggs. During the dry season most of the worm burden will be in the form of hypobiotic larvae and the egg output will thus be low. This is further complicated by the fact that hypobiosis is affected by not only the amount of rainfall but also the duration (Gatongi *et al.*, 1998). In the marginal areas hypobiotic larvae of *H. contortus* may not resume development during the short rains after the long dry season if the duration of rainfall is not long enough. Gatongi *et al.*, (1998) observed that there was no significant difference in the level of hypobiosis between the late dry period and the following short rain period. They observed that most of the worms survived the short rain season as hypobiotic larvae and the long rain season as adults. Similar results were observed by Pandey *et al.*, (1994) in goats raised in the high veld of Zimbabwe. Jacquet *et al.*, (1995) in mixed sheep and goat flocks in Mauritania observed that arrested development of larvae and long survival of adult worms constitute two complementary strategies for survival from one rainy season to the next in dry areas. Adult worms were able to survive the dry season..

In the present study, evidence of hypobiosis was seen in September and October when there was an increase in fecal egg counts but there was no significant rainfall for pasture transmission. The hypobiotic larvae may have resumed development in anticipation of the coming short rains for effective transmission. The sharp rise in December may have been due to pasture infection or enhanced development of the hypobiotic larvae.

Vegetation cover is critical for nematode transmission (Dinnik and Dinnik, 1961). The poor herbage that came up during the brief short rains during the study did not enhance parasite survival on pasture. Consequently the pickup was poor and possibly the parasites remained hypobiotic within the host. There were no significant differences in faecal egg output between the age groups in this study (as seen in section 1). This is unusual and may reflect that acquired

resistance to gastrointestinal helminth infections in goats does not develop with age as is the case in sheep and other livestock. All ages are susceptible. This phenomenon has been implicated in some earlier reports (Maingi *et al.*, 1993). Silva *et al.*, (1998), in a study of the evolution of natural gastrointestinal helminth infections in goats observed that old goats had higher parasite burdens than younger goats. In Nigeria, Anene *et al.*, (1994), observed that adult goats (more than 9 months) showed higher worm burdens than younger goats. In contrast, in the semi desert climate of Mauritania, Jacquiet *et al.*, (1995), reported that for goats the climate is a more important risk factor to gastrointestinal nematode infections than age. It was only during the dry season that differences related to age were demonstrated, the older animals excreting more eggs.

*Haemonchus contortus* was the most prevalent nematode recovered from the coprocultures. This nematode has been associated with hypobiosis during unfavorable conditions (Gatongi, 1996; Pandey *et al.*, 1994; Jacquiet *et al.*, 1995; Gatongi *et al.*, 1998). Costa *et al.*, (1991), suggested that *H. contortus* could be involved in hypobiosis in Brazil. Several workers have also reported that *H. contortus* is the most prevalent nematode of small ruminants in various parts of the world (Yadav and Uppal, 1992; Rahman, 1992; Maingi, 1991; Pandey *et al.*, 1994; Kochapakdee *et al.*, 1995; Mwamachi *et al.*, 1995; Jacquiet *et al.*, 1995; Gatongi *et al.*, 1998; Baker *et al.*, 1998 and Maingi *et al.*, 1998). It has also been reported as the most economically important nematode in Kenya (Waruiru *et al.*, 1991; Wanyangu *et al.*, 1996). Due to its pathogenicity, control failure is easily observed and has been associated with anthelmintic resistance in small ruminants in most tropical and sub tropical regions (Maingi *et al.*, 1991; Waruiru *et al.*, 1991; Yadav and Uppal, 1992; Kochapakdee *et al.*, 1995; Wanyangu *et al.*, 1996; Waller, 1997b and Maingi *et al.*, 1998).

Peri- parturient rise in fecal egg counts in goats has been documented in several tropical areas. It was observed in indigenous goats in Brazil (Costa, 1983), Angora goats in Australia

(Rahman and Collins, 1992) and in Kenya (Baker *et al.*, 1998). In the Kenyan study, peri-parturient rise was higher and more persistent in lactating Galla does compared to the Small East African does (Baker *et al.*, 1998). Peri-parturient rise in faecal egg counts was observed in this study in old lactating does which led to pre-weaning mortalities in kids whose mothers were heavily infected resulting in the reduction in milk yield.

The normal PCV threshold in goats has been given as 22% (Blackburn *et al.*, 1991). This level was surpassed by some goats that received salvage treatment. The threshold level set in this study (20%) was lower than the normal threshold and the impact on the animals may have been enormous. *H. contortus* which is a blood sucking nematode was the main nematode infecting the animals and probably contributed to the low PCV values. The PCV values were also low during the dry months probably due to poor quality feed. Blackburn *et al.*, (1991), observed that nutrition plays a major role in buffering the pathogenic impact of parasites. As nutrition improved PCV levels also increased. Intestinal helminths affect the nutritional status of the host by causing increased nutrient loss, in addition to decreased food intake and nutrient absorption. In the present study, the poor feed available during the dry months of September and October might not have buffered the pathogenic effects of the increased parasite levels. There was no significant differences in PCV between various age groups. This may be explained by lack of significant differences in nematode infections between the age groups, lack of acquired immunity and the nutritional stress affected all the age groups.

The total plasma proteins decreased during the dry period of September and October due to increased parasite levels and reduced feed quality. This has been observed by Blackburn, *et al.*, (1991), who concluded that as nutrition improved, total protein values were higher and as worm burden increased protein values were lower.

There was seasonal variation in the level of gastrointestinal nematode infection in goats

in the study area. The infection did not vary significantly with age indicating that age acquired resistance to gastrointestinal nematode infections did not develop in goats possibly due to their selective feeding habits. Clinical signs of parasitic gastroenteritis occur in the young goats and old lactating does. Peri parturient rise in faecal egg output occurs in some lactating does and though it may not have a significant effect on the group mean faecal egg counts, it led to pre-weaning mortalities in kids. There were no differences in the number of animals that required individual anthelmintic treatment between the age groups. Individual anthelmintic treatment reduces the amount of anthelmintic used and is therefore cheaper than the flock treatment. It does not have a significant effect on the epidemiology of gastrointestinal nematodes in goats. Where facilities are available, individual anthelmintic treatment is recommended. This is not only cheaper to the farmer but also delays or prevents the development of anthelmintic resistance.

*Haemonchus contortus* is the main nematode infecting goats on these farms. Hypobiosis may be occurring in goats in this area and may be playing a role in the epidemiology of *Haemonchus contortus*.

## CHAPTER 4:

### Studies on the impact of gastrointestinal helminths on production in young goats in the marginal low potential areas of Thika District of Central Kenya

#### 4.1. Introduction:

Helminth infections remain one of the major disease constraints to small ruminant production (FAO, 1992). Surveys indicate that up to 95% of sheep and goats are infected with helminths and that *Haemonchus* and *Trichostrongylus* are the main infecting species (Rey, 1991). The most striking effect of helminth infection in small ruminants is the death of the host. Mortality rates may exceed 40% while weight loss of 6 - 12 kg / year / animal may occur (IEMVT, 1980). Insidious production losses through reduced feed intake and decreased feed utilization efficiency, associated with subclinical or chronic conditions, are often the largest causes of economic losses (Holmes, 1993; Gatongi, 1996). These parasite nutrition interactions are exacerbated by the increasing pressure to cultivate marginal land, thereby further reducing available grazing for livestock. Under these conditions, the effects of poor nutrition and parasitism are frequently linked and additive ( Wanyangu and Bain, 1994). Infection with gastrointestinal nematodes in kids may occur as early as the first month of life in the semi arid areas during the rainy season (Silva *et al.*, 1998). Animals 1 - 2 months old should be housed in pens to avoid infection because the levels of infection at this age can cause high economic losses (Silva *et al.*, 1998). In a survey of goat diseases in Western Kenya, Shavulimo *et al.*, (1988), established that parasitic gastroenteritis was the most important health problem especially in exotic goat breeds. Parasitic gastroenteritis is a significant health problem in goats in all agroclimatic zones of Kenya (Lutu, 1983; Shavulimo, 1993; Gatongi, 1996; Gatongi *et al.*, 1998). They reduce productivity at clinical and subclinical levels. They cause production losses such as reduced weight gains, lower milk and meat production and mortalities. Though these losses have

been recognized in Kenya, there are no reports on the impact of natural gastrointestinal helminth infections in goats.

This study investigated the impact of natural gastrointestinal helminth infections on production and the species of helminths that infect goats in a low marginal area of central Kenya.

#### 4.2. Materials and Methods:

##### 4.2.1. Study area:

The study was conducted on a farm in the coffee marginal area of central Kenya (Ecozone3). The area lies at an altitude of 1500m. Coffee, floricultural and horticultural crops are grown under irrigation. There are several man made dams in the lower areas for harnessing rain water and the seasonal rivers. Most farms have mixed livestock species mainly crossbred Zebu cattle, Small East African goats and Dorper X Red Maasai sheep breeds. The animals graze and browse in areas with no coffee, which is the main enterprise. The vegetation consist of natural pasture which is dominated by *Themeda triandra*, *Cynodon dactylon* and *Pennisetum clandestinum*. There are also isolated *Lantana cammara* shrub and bushes with few acacia trees. The farmers have also modified the environment by planting several exotic trees. The soils are shallow, well drained and composed of clay with a lot of murram. Their water holding capacity is low and the vegetation dries up quickly during the dry season. The average annual rainfall in normal years is usually 1100mm. This varies from 750mm in dry years to over 1950mm in wet years. The rainfall is bimodal in distribution with the long rains falling between March and May and the short rains from October to December. The amounts and durations vary from year to year. The average temperature range is between 22°C - 30°C.

##### 4.2.2. Study design

A 4 -hectare pasture paddock which had been grazed by cattle, sheep and goats in the past year was fenced using wire mesh during the long rainy season in May 1998. The pasture was then

divided into 4 equal paddocks with even pasture cover.

Forty four weaned Small East African goat kids aged between 4 - 5 months were put into the fenced paddocks at the end of May 1998. They had free access to all the paddocks for 3 weeks. The kids were then separated into 4 groups of 11 with even weight, age and sex. They were treated with 10% albendazole at a dosage of 3.8mg per kg body weight and each group stocked in a paddock until end of December when the experiment was terminated and some of the animals slaughtered for total worm counts and identification (differential worm counts).

Two of the 4 groups of kids were given suppressive treatment with 10% albendazole every two weeks to prevent gastrointestinal nematode infection. The other two groups served as untreated controls. Animals in this group were only given individual salvage treatments when their EPG's exceeded 3000 and PCV lower than 18% to prevent mortalities. The treated animals were subsequently removed from the experiment.

Rectal faecal samples and EDTA blood by jugular venipuncture were obtained fortnightly. At the same time, all the kids were weighed and grass samples were collected from each paddock by hand following a W - shaped route across the paddock as described by Hansen and Perry, (1994). The eggs per gram (EPG) of faeces was determined by the modified McMaster technique with a lower limit of detection of 100 epg (Anon., 1986; Hansen and Perry, 1994). Blood Packed Cell Volume (PCV) and Total Plasma Protein (TPP) were determined using microhaematocrit and refractometry methods respectively. The herbage infective larvae were recovered using the method described by Hansen and Perry (1994). They were identified and counted according to the guidelines in Anon. (1986). The larvae recovered were expressed as counts per kilogram of dry herbage.

Two parasite naive tracer kids were introduced into each paddock at the end of the dry season (end of September) and at the end of the short rains season (end of December) for 3

weeks. They were then withdrawn and slaughtered for total and differential worm counts.

Six kids from each group were randomly selected and slaughtered for total and differential worm counts. The worms were recovered using the methods described in Anon.,(1986) and in Hansen and Perry, (1994). In this procedure, the abomasum, small intestines, cecum and colon were double ligated and separated immediately after slaughter. They were then opened into separate sieves, thoroughly washed and put into labeled containers. The clean contents containing the worms were preserved in 5% formalin until recovery and identification. The mucosa of the abomasum was scrapped off and digested in pepsin - HCL mixture as described in Hansen and Perry (1994). They were examined immediately after digestion for any inhibited larvae.

The contents of the abomasum and small intestines were examined under a dissecting microscope and all the recovered worms were counted and then differentiated into adults and immature larval stages. The large intestines were examined visually and the worms recovered. All the recovered worms were preserved in 10% alcohol before identification. The worms were identified to species level using morphological characteristics described in Anon. (1986) and in Hansen and Perry (1994).

Rainfall data were recorded at Thika meteorological station about 15 km from the farm.

#### 4.2.3. Statistical analysis:

Faecal egg counts (log - transformed), body weight, PCV, total protein and worm counts (log - transformed) were analyzed by repeated measures or non - repeated measures of analysis of variance ( ANOVA) using the following model (nested design) in the GLM procedure of SAS (SAS Institute Inc, 1989 - 1996):  $Y = TR + REP (TR)$ , where Y is the dependable variable, TR is the effect of treatment ( $\pm$ ) and REP(TR) represents the effect of the 2 replicates (REP) nested within treatments. Analysis was done for the first 11 sampling occasions (22 weeks after start of

the experiment), as from this point two animals in the control group died and two others from the same group were treated and removed from the experiment. This meant that the data could only be statistically comparable for the first 11 sampling (22 weeks after the start of the study). For ease of overview, the results of the replicate groups for each treatment were pooled in the graphs and figures.

### **4.3. RESULTS:**

#### **4.3.1 Rainfall data**

Rainfall data for the study period in 1997 and 1998 is shown on Figure 4.1. In 1998, the rainfall was heavier in May and June compared to 1997 where it was heavier in October, November and December. This was due to the El-nino phenomenon experienced between October 1997 and May 1998. Over the study period (May - December 1998), the highest rainfall was recorded in May and decreased through June and July to low levels in August, September and October. The amounts in June and July were above the average for the area. The short rains came in November and December. The rains were delayed as they are usually expected in October and the amount received over the period was below average. The duration of the rainy season was also short (approximately 2 months compared to 5 months in 1997)

#### **4.3.2. Faecal egg counts:**

The arithmetic mean strongyle EPG for the control and suppressively treated groups from May to December are shown in Figure 4.2. The mean EPG for the suppressively treated group were low throughout the study period reaching a maximum of 200 EPG in November and December. The control group had higher means than the suppressively treated group. The group mean in the control rose from 100 EPG in July to reach a level of 650 in August and September. The levels were lower for October and rose in November and December giving a peak of 800 EPG. This gave approximately 2 peaks: August and September, November and December.

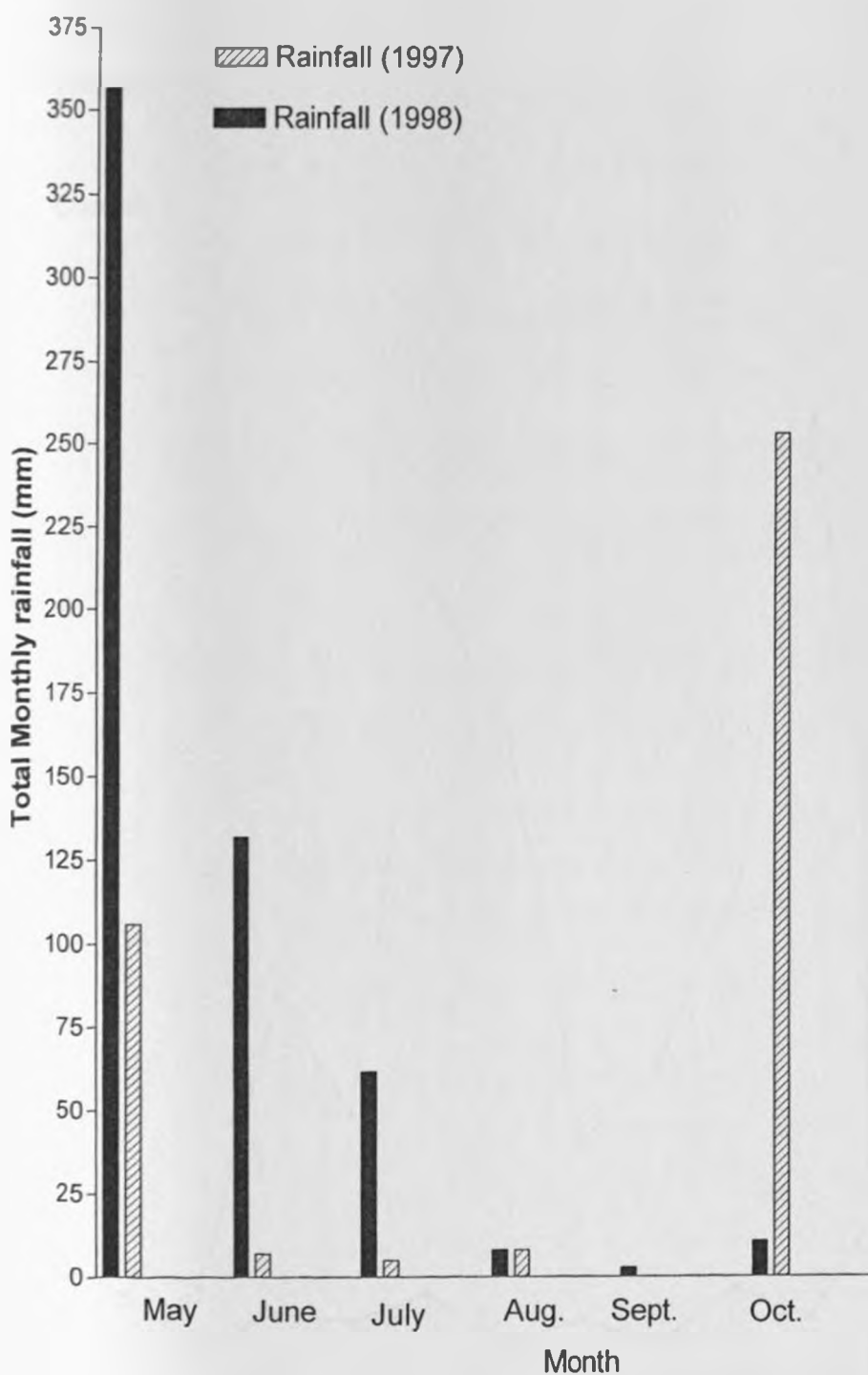


Figure 4.1: Total monthly rainfall recorded at Thika meteorological from experimental site for the months of May to December 1997 and 1998.

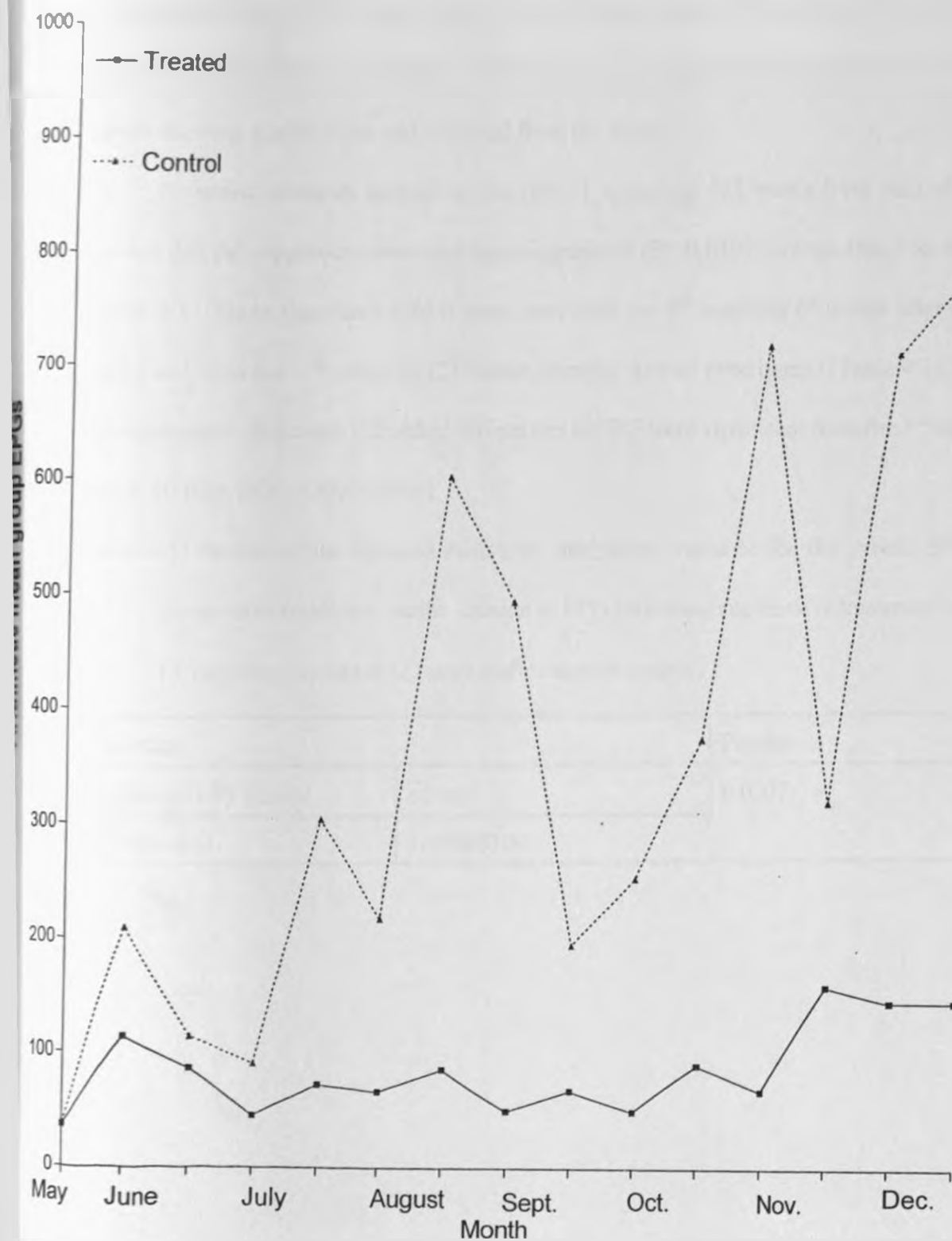


Figure 4.2: Arithmetic mean group strongyle faecal egg per gram (EPG) counts for the suppressively treated and control goats during the study period, May - December 1998.

Two goats in the control groups died in October with signs of parasitic gastroenteritis. They had bottle jaw, EPG counts of over 6500, PCV lower than 18% (one had 11%), weak and lagging behind the flock. Two others in the same group were given salvage treatments when they started showing similar signs and removed from the study.

Repeated measures analysis of the first 11 samplings (22 weeks from start of study) showed that the suppressive treatment had a significant ( $P=0.0307$ ) overall effect on the EPG (Table 4.1). These significant effects were seen from the 4<sup>th</sup> sampling (8 weeks after start of study) and up to the 11<sup>th</sup> sampling (22 weeks from the start of experiment) (Table 4.2). Within the suppressive treatments individual differences in EPG were significant from the 5<sup>th</sup> sampling (week 10 from start of experiment).

Table 4.1: Results of the repeated measures analysis of variance for the overall effects of suppressive treatment on the change in EPG following suppressive treatment over the 11 sampling occasions (22 weeks after start of study):

| Lsmeans               |            | P value |
|-----------------------|------------|---------|
| Suppressively treated | control    | 0.0307  |
| 0.37664861            | 1.30985300 |         |

Table 4.2: Results of the repeated measures analysis of variance on the effect of suppressive treatment at each of the 11 sampling occasions (weeks after start of experiment).

| Week | Lsmeans |         | P value         |                        |                   |
|------|---------|---------|-----------------|------------------------|-------------------|
|      | Treated | Control | treatment alone | treatment (with group) | Group (treatment) |
| 2    | 4.258   | 4.223   | 0.8496          | 0.8501                 | 0.4198            |
| 4    | 4.7219  | 5.1378  | 0.1339          | 0.1335                 | 0.3458            |
| 6    | 4.5104  | 4.6751  | 0.5253          | 0.5201                 | 0.2242            |
| 8    | 4.2848  | 4.755   | 0.0447          | 0.0462                 | 0.4767            |
| 10   | 4.3785  | 5.454   | 0.0004          | 0.0003                 | 0.1119            |
| 12   | 4.4437  | 5.1414  | 0.0121          | 0.0122                 | 0.3539            |
| 14   | 4.6243  | 5.7374  | 0.0006          | 0.0005                 | 0.1394            |
| 16   | 4.3731  | 5.8940  | 0.0001          | 0.0001                 | 0.0949            |
| 18   | 4.5542  | 5.4286  | 0.0014          | 0.0011                 | 0.1260            |
| 20   | 4.4613  | 5.7180  | 0.0001          | 0.0001                 | 0.7022            |
| 22   | 4.6347  | 5.5333  | 0.0161          | 0.0185                 | 0.8728            |

The group had no overall significant ( $P=0.1995$ ) effect during the 11 sampling occasions (Table 4. 3). However, the groups were different from the 5<sup>th</sup> up to the 10<sup>th</sup> sampling occasions (weeks 10 - 20). The EPG counts varied within each group in the treatments (Table 4.4).

Table 4.3: Results of the repeated measures analysis of variance for the overall effect of group on the change of EPG (DLEPG ) over the 11 sampling occasions.

| Lsmeans    |             |            |            | P value |
|------------|-------------|------------|------------|---------|
| Group 1    | Group 2     | Group 3    | Group 4    |         |
| 0.32321346 | 1.383889907 | 0.43008376 | 1.23580693 | 0.1995  |

Table 4. 4: Results of the repeated measures analysis of variance for the effect of group on LEPG

at each sampling for the 11 sampling occasions (weeks after start of study).

| Week | Lsmeans |         |         |         | P value |
|------|---------|---------|---------|---------|---------|
|      | Group 1 | Group 2 | Group 3 | Group 4 |         |
| 2    | 4.2116  | 4.0583  | 4.3045  | 4.3885  | 0.6166  |
| 4    | 4.7528  | 4.8559  | 4.6910  | 5.4197  | 0.2269  |
| 6    | 4.5911  | 4.3693  | 4.4296  | 4.9808  | 0.3312  |
| 8    | 4.4578  | 4.6576  | 4.1118  | 4.8527  | 0.1429  |
| 10   | 4.6987  | 5.1947  | 4.0583  | 5.7133  | 0.0008  |
| 12   | 4.5295  | 4.8809  | 4.3579  | 5.4019  | 0.0413  |
| 14   | 4.6910  | 5.3201  | 4.5577  | 6.1546  | 0.0015  |
| 16   | 4.5348  | 5.5554  | 4.2116  | 6.2326  | 0.0001  |
| 18   | 4.4578  | 5.07308 | 4.6506  | 5.7840  | 0.0028  |
| 20   | 4.6111  | 5.6739  | 4.3115  | 5.7622  | 0.0003  |
| 22   | 4.5348  | 5.4422  | 4.7346  | 5.6243  | 0.1151  |

The suppressive treatment did not completely eliminate the faecal egg out put. There was a gradual increase in the mean faecal egg counts (EPG) in the suppressively treated group up to the end of the study.

#### 4.3.3. Body weights (BWT):

The mean weight gains for the suppressively treated and control groups of kids are shown in Figure 4.3. The suppressively treated goats had a significantly higher weight gains from July up to November. The control group had lower weights and were more affected by the dry season in August, September and October. There was accelerated growth in December after the rains in November in both groups. Overall, the suppressively treated group gained a mean of  $3.1 \pm 0.3\text{kg}$  while the control group gained a mean  $1.1 \pm 0.2\text{kg}$  over the study period.

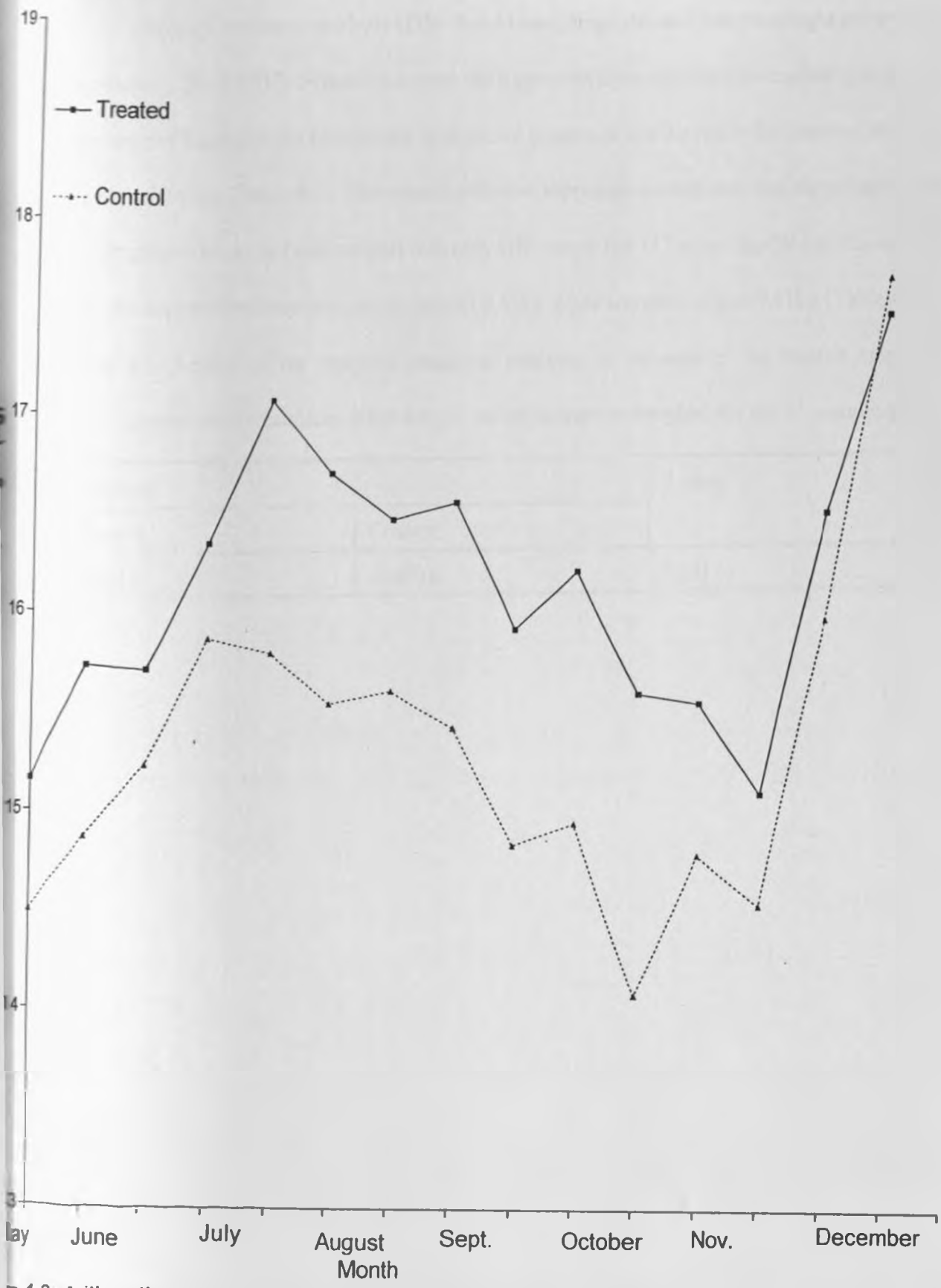


Figure 4.3: Arithmetic mean group weights for the suppressively treated and control goats during the study period, May - December 1998.

Repeated measures analysis of the first 11 samplings showed that the weight gains were significantly ( $P= 0.0317$ ) different between the suppressively treated and the control groups. At this time the suppressively treated kids had gained a mean of 0.45kg while the controls had lost a mean of 0.41kg (Table 4.5). The overall effect of suppressive treatment was significant. The weight gains (change in body weight) was only different at the 11<sup>th</sup> sampling (Week 22). At this time the suppressively treated groups gained 0.45kg while the control lost 0.41kg (Table 4.6).

Table 4.5: Results of the repeated measures analysis of variance of the overall effect of suppressive treatment on the weight gains( change in weights) for the 11 samplings.

| Lsmeans |         | P value |
|---------|---------|---------|
| Treated | Control |         |
| 0.4545  | -0.4091 | 0.0317  |

Table 4.6: Results of the repeated measure analysis of the effect of suppressive treatment and treatment nested in group on weight gain on each of the 11 sampling occasions ( change in body weight 1 minus change in body weight 11) (Dbwt 1 - Dbwt 11) (weeks after start of study).

| Weeks | Lsmeans |         | P value   |                  |
|-------|---------|---------|-----------|------------------|
|       | Treated | Control | Treatment | Treatment nested |
| 4     | 0.5682  | 0.3863  | 0.3474    | 0.9722           |
| 6     | 0.5909  | 0.7272  | 0.4274    | 0.4957           |
| 8     | 1.2045  | 1.3636  | 0.4893    | 0.0184           |
| 10    | 1.8181  | 1.2954  | 0.0499    | 0.2373           |
| 12    | 1.5682  | 1.0454  | 0.0504    | 0.1902           |
| 14    | 1.3182  | 1.1136  | 0.4927    | 0.3080           |
| 16    | 1.3863  | 0.9318  | 0.1556    | 0.5789           |
| 18    | 0.8182  | 0.3409  | 0.1311    | 0.5489           |
| 20    | 1.0454  | 0.4545  | 0.0959    | 0.0952           |
| 22    | 0.4545  | -0.4090 | 0.0212    | 0.0175           |

Repeated measures analysis of variance on the effect the group on the change in weight gains (Dbwt11) was significant ( $P= 0.0054$ ) (Table 4.7). There was a difference in weight gains between the two groups which were suppressively treated with one gaining 1.18kg while the other lost 0.27kg. The control groups lost 0.18kg and 0.64kg respectively (Table 4.7). The suppressive treatment had no significant effect on the body weights at each of the 11 sampling occasions ( $P> 0.05$ ) (Table 4.8).

Table 4.7: Results of the repeated measures analysis of variance on the overall effect of group on weight gain ( change in weight - Dbwt 11 ) for the 11 sampling occasions.

| Lsmeans |         |         |         | P value |
|---------|---------|---------|---------|---------|
| Group 1 | Group 2 | Group 3 | Group 4 |         |
| 1.1818  | -0.1818 | -0.2727 | -0.6364 | 0.0054  |

Table 4.8: Results of the repeated measures analysis of variance of the effect of suppressive treatment on the body weights for each of the 11 sampling occasions .

| Sampling | Lsmeans |         | P value |
|----------|---------|---------|---------|
|          | Treated | Control |         |
| 2        | 15.159  | 14.5    | 0.6057  |
| 4        | 15.727  | 14.886  | 0.5223  |
| 6        | 15.750  | 15.227  | 0.6920  |
| 8        | 16.364  | 15.227  | 0.7039  |
| 10       | 16.977  | 15.795  | 0.3561  |
| 12       | 16.727  | 15.545  | 0.3468  |
| 14       | 16.477  | 15.614  | 0.4867  |
| 16       | 16.545  | 15.432  | 0.3601  |
| 18       | 15.977  | 14.841  | 0.3341  |
| 20       | 16.204  | 14.954  | 0.2727  |
| 22       | 15.614  | 14.091  | 0.1839  |

The cumulative group weight gains for the 11 sampling occasions are shown in Figure 4. The weight gains during the long rainy season (May - June) were higher for the suppressively treated groups than the control. At the end of June (week 8) the suppressively treated kids had a mean weight gain of 1.81kg while the mean for the control kids was 1.3kg. The weight gain dropped during the dry period of July, August and September. The drop was more marked in the

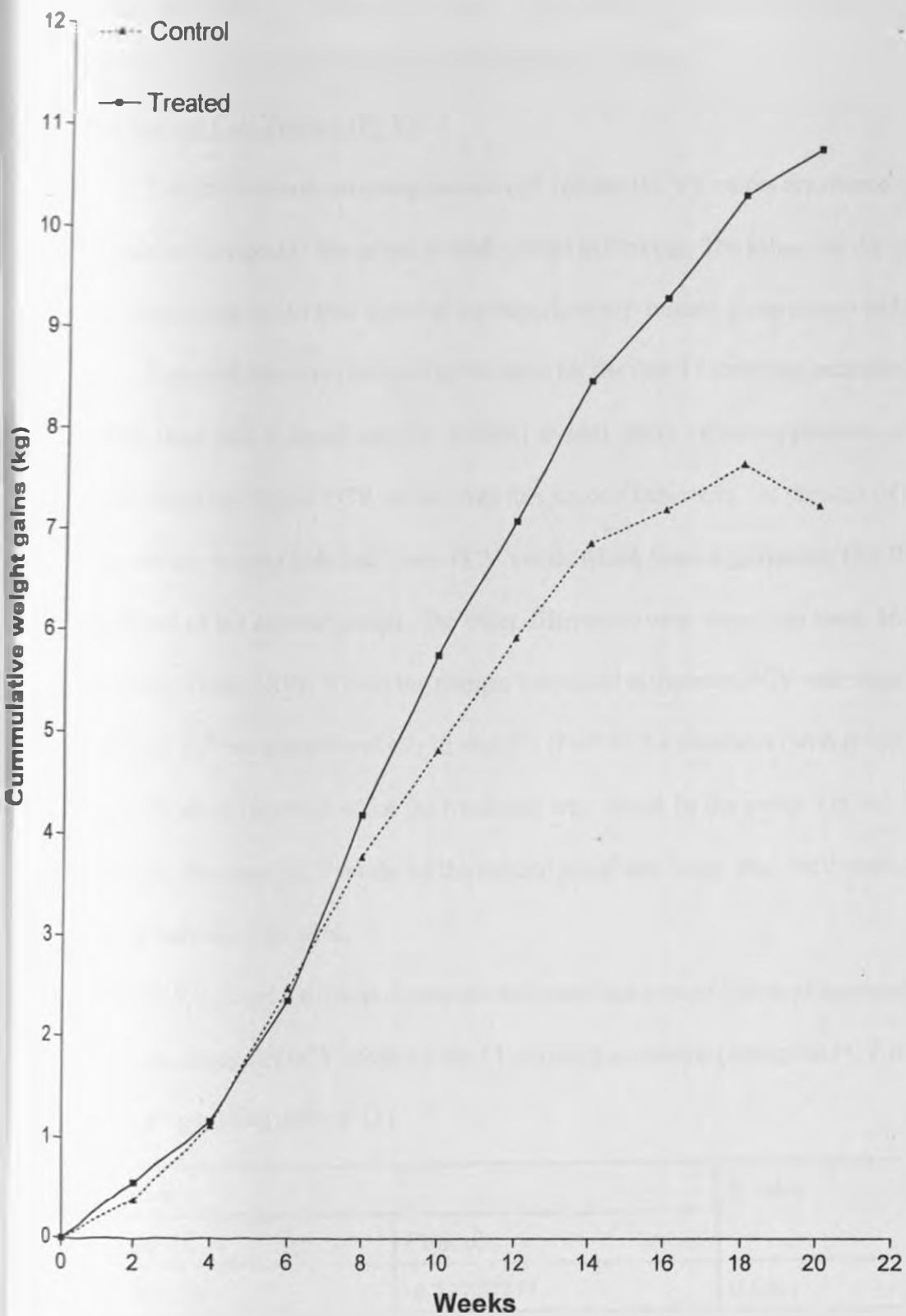


Figure 4.4: Accumulated weight gains for the suppressively treated and control goats for the first 11 sampling occasions.

control group where the kids had lost a mean of 0.41kg. The suppressively treated kids had a mean weight gain of 0.45kg at this time. The suppressive treatment enabled the animals to maintain a positive mean weight gain during the dry season.

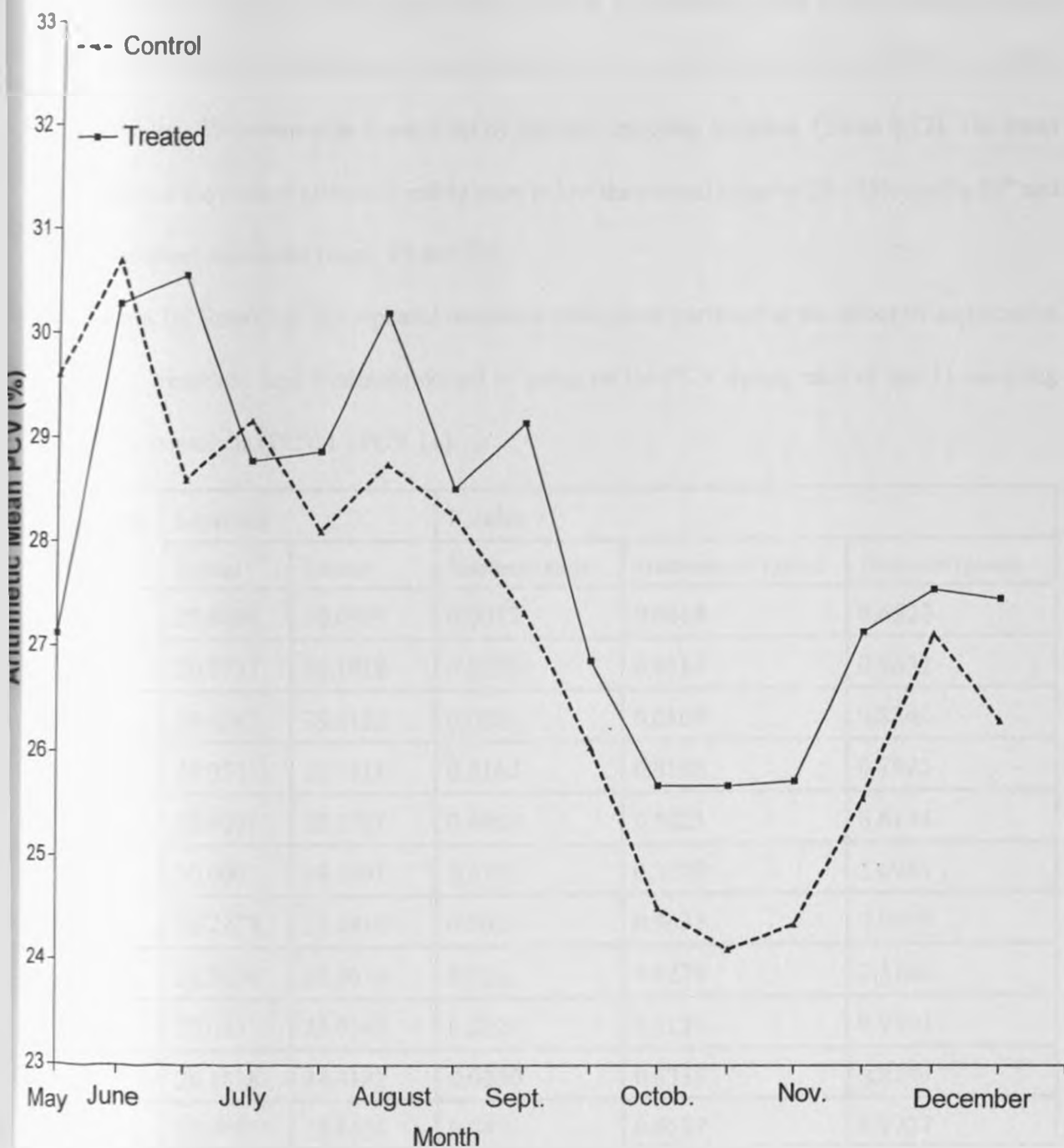
4.3.4. Packed Cell Volume (PCV):

The arithmetic mean group packed cell volume (PCV) values are shown on Figure 4.5. The values decreased to low levels in both groups in October. The values for the control groups were consistently lower than those of the suppressively treated group except in May and July.

Repeated measures analysis of variance for the first 11 sampling occasions (22 weeks), showed there was a significant ( $P= 0.0001$ ) overall effect of the suppressive treatment. The treated group had higher PCV values over this period (Table 4.9). At the start of the study, the suppressively treated kids had lower PCV values which were significantly ( $P< 0.05$ ) different from those of the control groups. The other differences were seen from week 16 (from the 8<sup>th</sup> sampling) (Table 4.10). Within the groups, individual difference, PCV were significant on the 1<sup>st</sup>, 8<sup>th</sup> and 10<sup>th</sup> sampling (weeks 2, 16 and 20) ( $P< 0.05$  for treatment (with group)). There was no significant differences when the treatment was nested in the group. On the 11<sup>th</sup> sampling (week 22), the mean PCV levels for the control group was lower than the documented range in goats of between 25 - 45%.

Table 4.9: Results of the repeated measure analysis of the overall effects of suppressive treatment on change of PCV levels for the 11 sampling occasions ( change in PCV levels from the 1<sup>st</sup> sampling -DPCV 11).

| Lsmeans    |             | P value |
|------------|-------------|---------|
| Treated    | Control     |         |
| 0.86363636 | -6.22727273 | 0.0001  |



4.5: Arithmetic mean group Packed Cell Volume ( PCV) for the suppressively treated and control goats during the study period, May - December 1998.

During the 11 samplings, the groups were significantly ( $P=0.0002$ ) different in the PCV values (Table 4.11). The mean PCV levels in group 1 (suppressively treated) fell by 1.82 while in group 3 (suppressively treated), it rose by 0.09. In the control groups 2 and 4, the PCV levels fell by 5.64 and 6.82 respectively over the 11 samplings. The levels were significant ( $P<0.05$ ) on the first sampling when the suppressively treated group had lower PCV values than the control. They were able to catch up by the next sampling occasion (Table 4.12). The mean values for the control groups (2 and 4) were below the normal range of 25 - 45% on the 10<sup>th</sup> and 11 sampling occasions (week 20 and 22).

Table 4.10: Results of the repeated measures analysis of variance of the effect of suppressive treatment and treatment nested in group on the PCV during each of the 11 sampling occasions (PCV 1 - PCV 11).

| Week | Lsmeans |         | P value         |                      |                   |
|------|---------|---------|-----------------|----------------------|-------------------|
|      | Treated | Control | Treatment alone | Treatment with group | Treatment (group) |
| 2    | 26.8636 | 30.0909 | 0.0012          | 0.0014               | 0.6527            |
| 4    | 30.2727 | 30.1818 | 0.9298          | 0.9314               | 0.9632            |
| 6    | 30.4545 | 28.8182 | 0.0801          | 0.0869               | 0.8840            |
| 8    | 28.9545 | 29.1818 | 0.8162          | 0.8195               | 0.7825            |
| 10   | 28.9091 | 28.2727 | 0.4968          | 0.5023               | 0.6184            |
| 12   | 30.000  | 28.9091 | 0.3195          | 0.3272               | 0.6984            |
| 14   | 28.2273 | 28.1818 | 0.9624          | 0.9633               | 0.9479            |
| 16   | 28.8636 | 26.8636 | 0.026           | 0.0274               | 0.5140            |
| 18   | 27.045  | 25.9545 | 0.2024          | 0.2128               | 0.9303            |
| 20   | 26.1818 | 24.3182 | 0.0300          | 0.0335               | 0.8297            |
| 22   | 26.0000 | 23.8636 | 0.0493          | 0.0537               | 0.7727            |

Table 4.11: Results of the repeated measures analysis of variance on the overall effect of group on the change in PCV levels over the 11 sampling occasions (DPCV 11).

| Lsmeans  |         |         |         | P value |
|----------|---------|---------|---------|---------|
| Group 1  | Group 2 | Group 3 | Group 4 |         |
| - 1.8182 | -5.6364 | 0.0909  | -6.8182 | 0.0002  |

Table 4. 12: Results of the repeated measures analysis of variance on the effect of each group on the PCV levels at each of the 11 sampling occasions.

| Week | Lsmeans |         |         |         | P value |
|------|---------|---------|---------|---------|---------|
|      | Group 1 | Group 2 | Group 3 | Group 4 |         |
| 2    | 27.454  | 29.909  | 26.273  | 30.373  | 0.0112  |
| 4    | 30.091  | 30.373  | 30.454  | 30.091  | 0.9937  |
| 6    | 30.636  | 28.545  | 30.273  | 29,091  | 0.3566  |
| 8    | 28.545  | 28.909  | 29.364  | 29.454  | 0.9079  |
| 10   | 28.364  | 27.909  | 29.454  | 28.636  | 0.700   |
| 12   | 30.182  | 28.273  | 29.818  | 29.545  | 0.6384  |
| 14   | 29.091  | 28.364  | 28.364  | 28.000  | 0.9905  |
| 16   | 28.636  | 27.545  | 29.091  | 26.182  | 0.1032  |
| 18   | 27.273  | 25.909  | 26.818  | 26.000  | 0.630   |
| 20   | 26.000  | 24.636  | 26.364  | 24.000  | 0.1739  |
| 22   | 25.636  | 24.373  | 26.364  | 23.454  | 0.2317  |

4.3.5. Total Plasma Proteins (TPP):

The arithmetic mean group total plasma protein values are shown in Figure 4.6. There were no significant differences between the groups. Both groups had peaks in August - September with a sharp decline and depression in October and November.

There was no significant (P= 0.9102) differences on the 11 sampling occasions. The mean

total plasma protein values dropped by 3.36 and 3.54g/l for the suppressively treated and the control groups respectively (Table 4. 13). The levels were not different ( $P > 0.05$ ) at any sampling occasion (Table 4.14). The group effect was also not significant ( $P = 0.6035$ )(Table 4. 15). There was an overlap of the plasma protein values between the suppressively treated and the control (Table 4. 16). All the values were above the normal documented ranges for goats (between 60 - 75g/l) (Radostits *et al.*, 1997).

Table 4. 13: Results of the repeated measures analysis of variance of the overall effect of suppressive treatment on the change in total plasma protein levels over the 11 sampling occasions (DTPP11).

| Lsmeans |         | P value |
|---------|---------|---------|
| Treated | Control |         |
| -3.364  | -3.545  | 0.9102  |

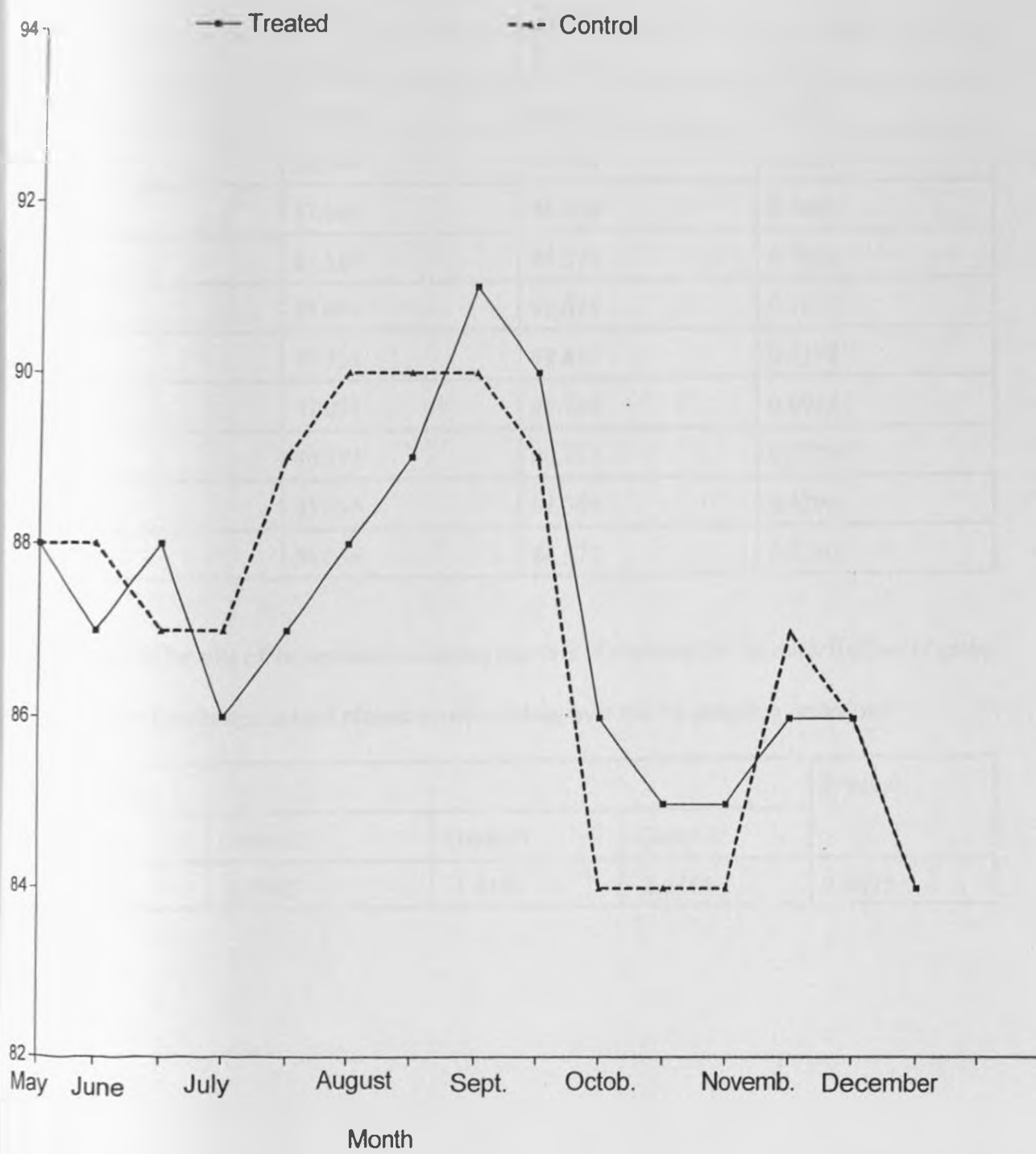


Figure 4.6: Arithmetic mean group total plasma protein for the suppressively treated and control goats during the study period, May - December 1998.

Table 4.14: Results of the repeated measures analysis of variance on the effect of suppressive treatment on total plasma protein at each of the 11 sampling occasions (TP 1 - TP 11).

| Week | Lsmeans |         | P value |
|------|---------|---------|---------|
|      | Treated | Control |         |
| 2    | 88.000  | 87.818  | 0.914   |
| 4    | 86.818  | 88.364  | 0.4575  |
| 6    | 88.364  | 87.364  | 0.6521  |
| 8    | 87.000  | 86.909  | 0.9664  |
| 10   | 87.636  | 88.373  | 0.7554  |
| 12   | 88.091  | 90.636  | 0.1666  |
| 14   | 89.364  | 89.818  | 0.8108  |
| 16   | 91.091  | 90.364  | 0.6954  |
| 18   | 90.373  | 88.727  | 0.3726  |
| 20   | 85.636  | 84.364  | 0.4299  |
| 22   | 84.636  | 84.373  | 0.8280  |

Table 4.15: Results of the repeated measures analysis of variance for the overall effect of group on the change in total plasma protein values over the 11 sampling occasions.

| Lsmeans |         |         |         | P value |
|---------|---------|---------|---------|---------|
| Group 1 | Group 2 | Group 3 | Group 4 |         |
| -4.9091 | -3.4545 | -1.8182 | -3.6364 | 0.6035  |

Table 4. 16: Results of the repeated measures analysis of variance for the effect of group on the total plasma protein values at each of the 11 sampling occasions.

| Week | Lsmeans |         |         |         | P value |
|------|---------|---------|---------|---------|---------|
|      | Group 1 | Group 2 | Group 3 | Group 4 |         |
| 2    | 88.000  | 86.545  | 88.000  | 89.0909 | 0.7672  |
| 4    | 85.818  | 84.182  | 87.818  | 92.545  | 0.0191  |
| 6    | 88.545  | 83.091  | 88.182  | 91.636  | 0.0418  |
| 8    | 86.545  | 82.909  | 87.454  | 90.909  | 0.0599  |
| 10   | 85.818  | 83.818  | 89.454  | 92.727  | 0.0053  |
| 12   | 86.545  | 88.727  | 89.636  | 92.545  | 0.1334  |
| 14   | 87.454  | 87.636  | 91.273  | 92.000  | 0.1779  |
| 16   | 90.545  | 87.636  | 91.636  | 90.909  | 0.1469  |
| 18   | 88.909  | 86.545  | 91.636  | 90.909  | 0.1469  |
| 20   | 84.000  | 82.727  | 87.273  | 86.000  | 0.1813  |
| 22   | 83.091  | 83.081  | 86.182  | 85.454  | 0.4275  |

4.3.6. Herbage Larval counts:

These are shown in Figure 4.7. The levels were higher in the paddocks grazed by the control goats with significant differences in the months of August, September, December and January. The recovered larvae were identified as *Haemonchus contortus*. No infective larvae were recovered in the paddocks grazed by the suppressively treated groups in September, December and January.

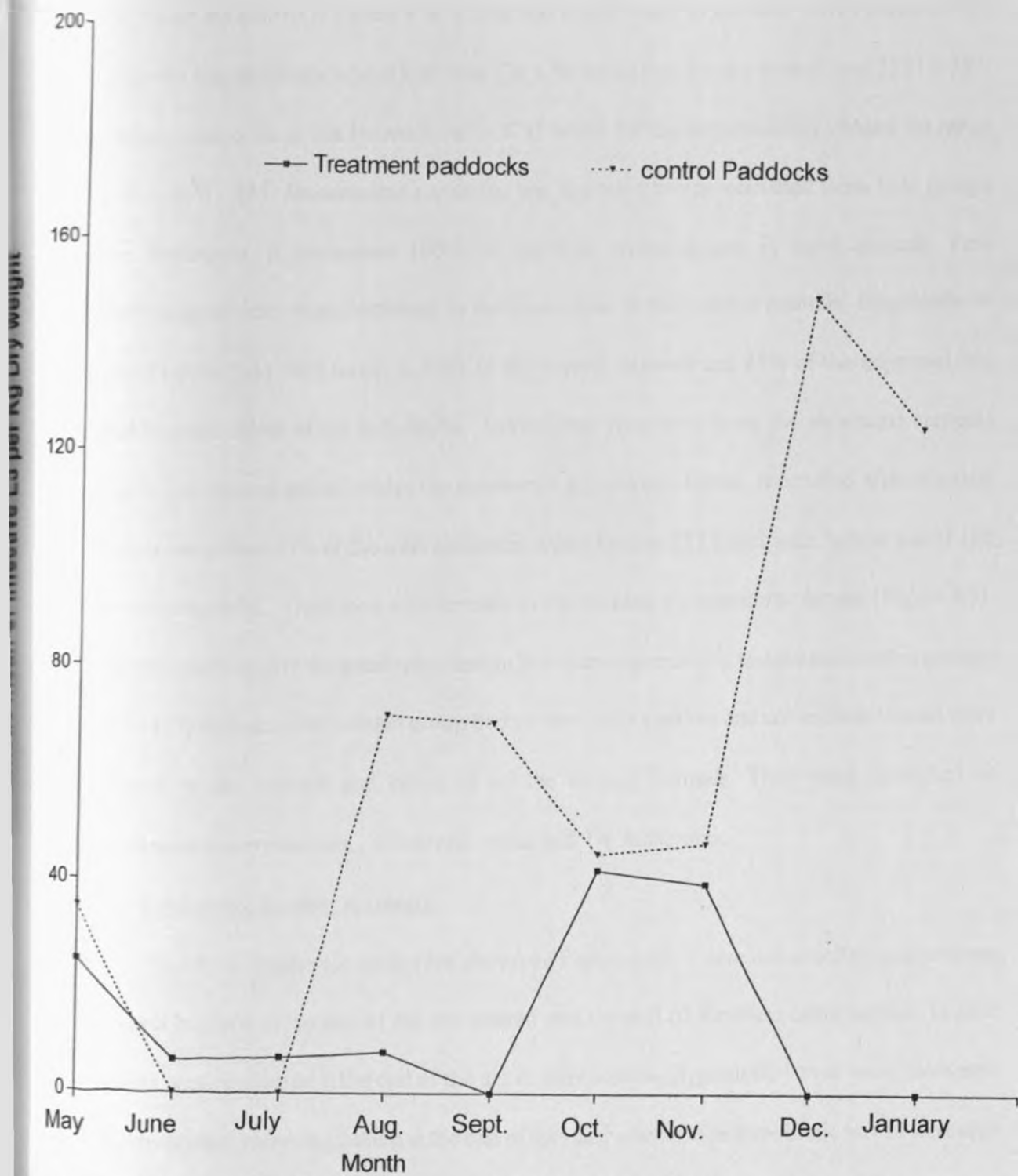


Fig. 4.7: Arithmetic mean of infective larvae (L3) per kg dry herbage for the paddocks grazed by the suppressively treated and control goats during the study period, May - December 1998 and January 1999.

#### 4.3.7. Total worm burdens in experimental goats:

These are shown in Figure 4. 8. There was a difference in the total worm burdens. The mean for the suppressively treated kids was  $124 \pm 56$  while that for the control was  $1133 \pm 387$ . The range in the control was between 161 - 3741 while for the suppressively treated the range was between 0 - 485. *Haemonchus contortus* was the main worm recovered from both groups in the abomasum. It comprised 100% of the total worm counts in most animals. Few *Trichostrongylus axei* were recovered in the abomasum in two control animals. Hypobiotic or inhibited larvae (L4) were found in 88% of the control animals and 25% of the suppressively treated animals. Most of the hypobiotic larvae were recovered from the abomasal contents except in one control animal where the number of hypobiotic larvae recovered after mucosal digestion comprised 17% of the total abomasal worm burden (27 hypobiotic larvae out of 150 total worm burden). There was a difference in the number of hypobiotic larvae (Figure 4.9). There were no worms in the small intestines in both the suppressively treated and control groups. Only two (2) animals in the treated group had worms in the caecum and colon. Few worms were recovered in the caecum and colon of all the control animals. They were identified as *Oesophagostomum radiatum* , *Chabertia ovina* and *Trichuris ovis*.

#### 4.3.8. Total worm burdens in tracers:

The worm burdens in tracers are shown on Figure 4.10. There was a difference between the worm burdens at the end of the dry season and the end of the short rainy season. Higher numbers were recovered at the end of the short rainy season. Hypobiotic larvae were recovered in the tracers that were slaughtered at the end of the rainy season. The hypobiotic larvae were also recovered in the study animals slaughtered at this time. The differences showed that the pasture infection was higher at the end of the short rainy season. *Haemonchus contortus* was the main nematode

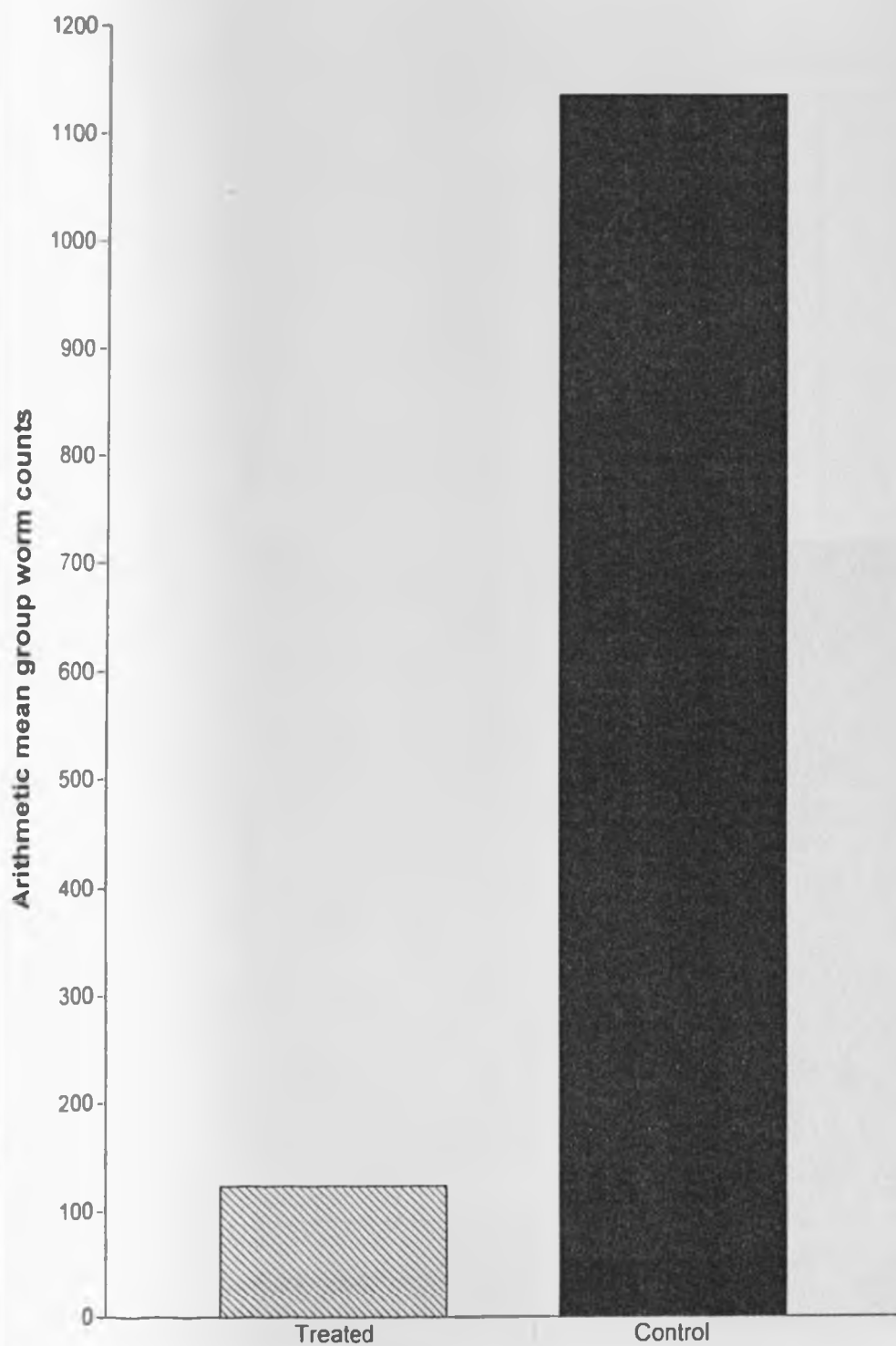
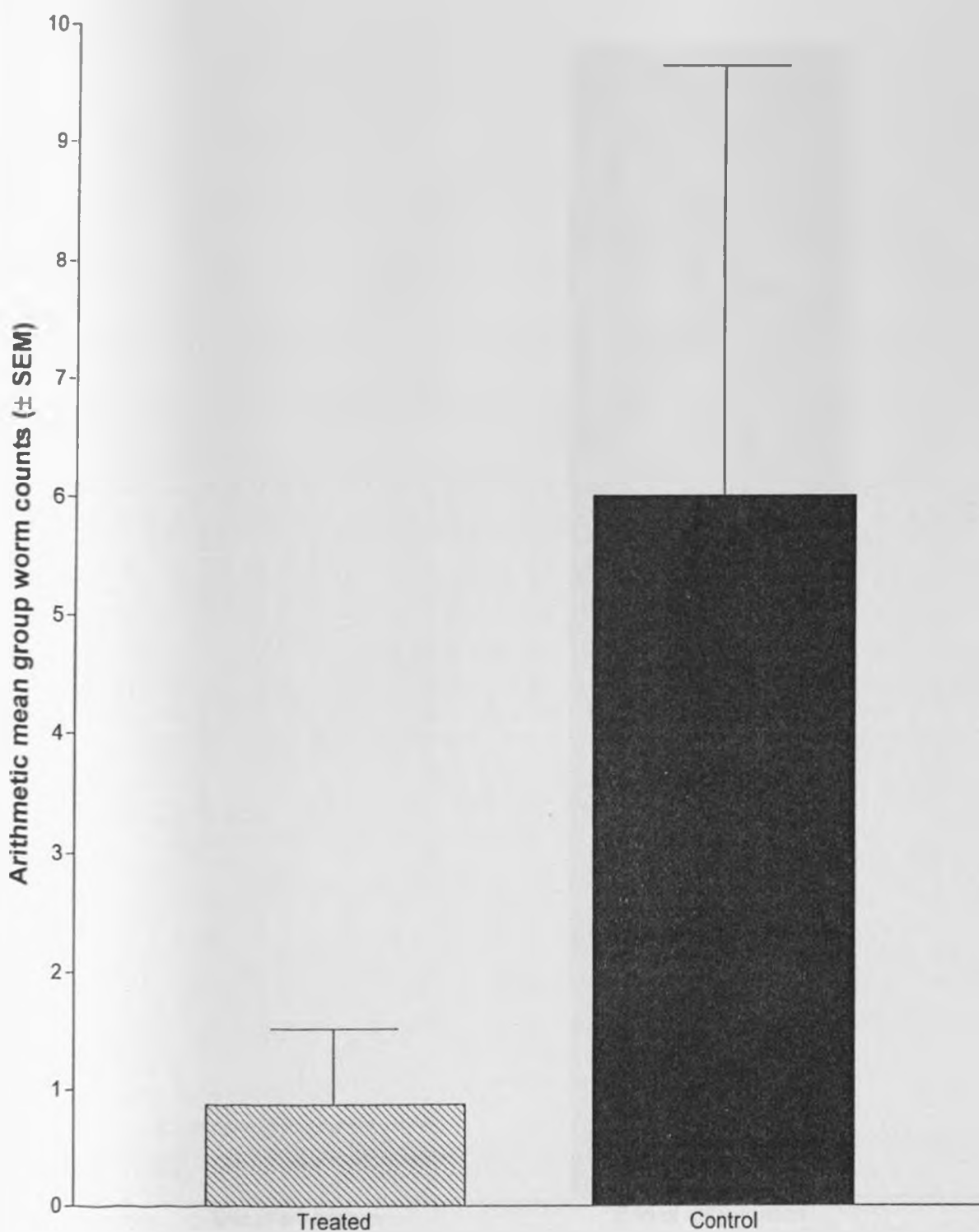
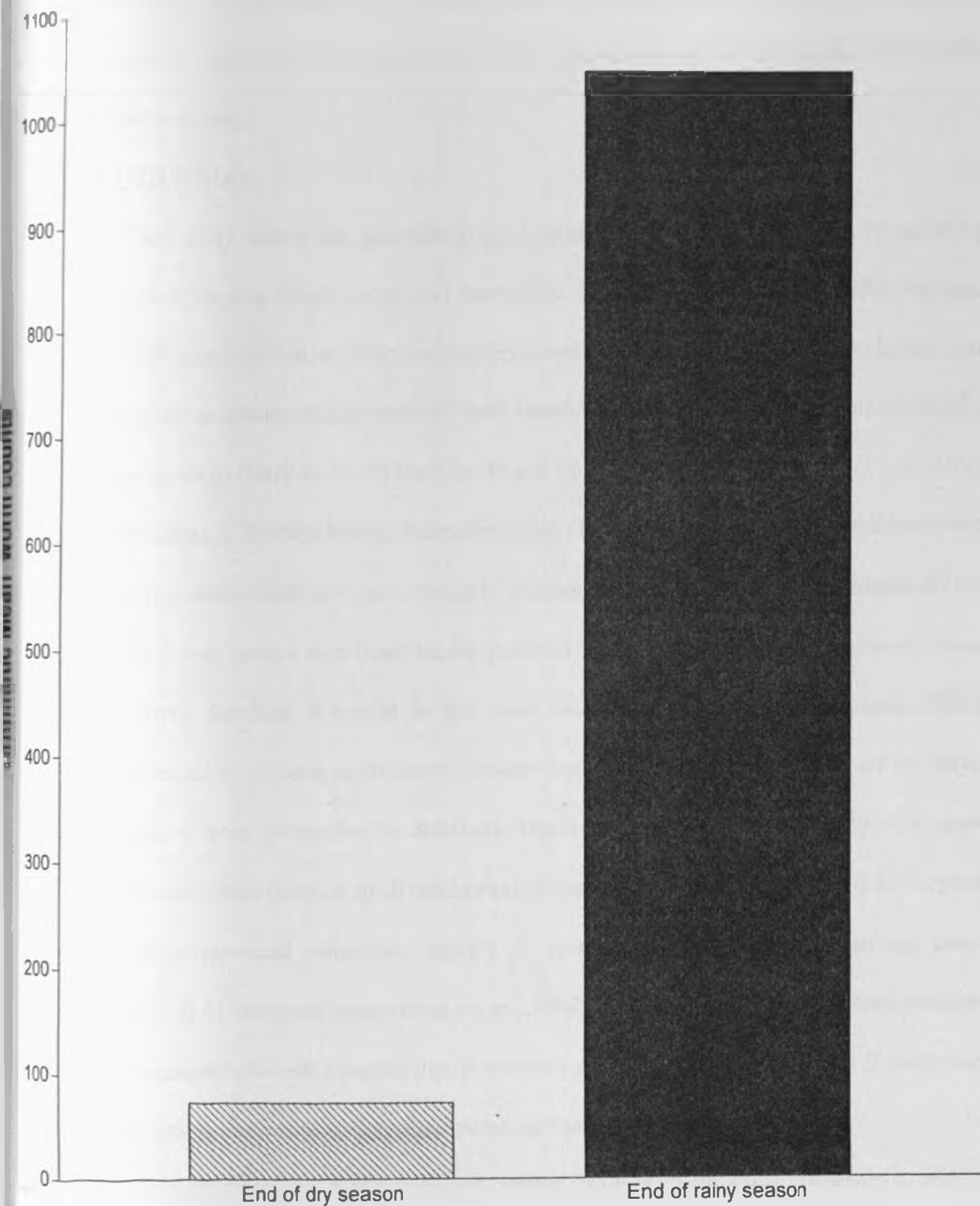


Figure 4.8: Arithmetic mean group total worm burdens for the suppressively treated and control goats at slaughter



4.9: Arithmetic mean ( $\pm$  SEM) group abomasal immature worm counts for the suppressively treated and control goats at slaughter.



4.10: Arithmetic mean total worm counts for the tracers introduced into the paddocks at slaughter at the end of the dry and short rainy seasons.

recovered in both seasons. *Moniezia expansa* tapeworms were recovered in the small intestines of the tracers in both seasons. Few worms were found in the large intestines of the tracers slaughtered at the end of the dry season. None was recovered from those slaughtered at the end of the rainy season.

#### 4.4. DISCUSSION:

This study shows that gastrointestinal helminths affect goat production by reducing weight gains, causing weight losses and mortalities. This has been reported by other workers. Joshi, (1994) reported that parasitic gastroenteritis was one of the major causes of production loss in goats under sedentary management in Nepal. Gastrointestinal nematodes particularly limit the raising of goats in Brazilian North East (Costa and Vieira, 1983; Costa *et al.*, 1987). In a survey of goat diseases in Western Kenya, Shavulimo *et al.*, (1988), established that gastroenteritis was the most important health problem especially in exotic goat breeds. Parasitic gastroenteritis has also been shown to be a significant health problem in sheep and goats in agroclimatic zones ranging from the high potential to the semi arid zones of Kenya (Shavulimo, 1993). Gastrointestinal nematodes, particularly *Haemonchus contortus*, are common and are the major constraints to goat production in Southern Thailand (Saithanoo, 1996). They also cause significant economic losses in small ruminants in Bangladesh (Mostofa *et al.*, 1996). In Haryana, India, gastrointestinal nematodes, mainly *H. contortus* and *Trichostrongylus* spp cause gastroenteritis in sheep and goats (Gupta *et al.*, 1987). In a survey of gastrointestinal parasites in small ruminants in south Eastern Nigeria over two seasons, strongyliasis (due to *H. contortus*) was the most prevalent gastrointestinal parasitism in goats (Anene *et al.*, 1994).

Though goats in Spain are not treated with anthelmintics, parasitism due to trichostrongyles appeared to be of some importance as the goats had high egg and worm counts, and showed signs of parasitic gastroenteritis (Terazona *et al.*, 1982 ). They were also very

sensitive to Ostertagiasis type II. Subclinical gastrointestinal parasitism leads to loss in body condition and reduced milk yield in dairy goats (Hoste and Chartier, 1993).

The clinical signs observed in the present study have previously been observed in dairy goats (Lutu, 1983) and goats under sedentary management (Joshi, 1994). Deaths after such signs in dairy goats were sudden in some very acute infections but usually occurred after several days of clinical disease (Lutu, 1983). In the present study, deaths occurred after five days of clinical disease. The highest EPGs recorded by Lutu, (1983), in the dairy goats for the dying animals (12,200 - 50,000) were not recorded in the present study in goats that died.

There were two EPG peaks observed during this study, the August and September peak was due to the larvae picked in June and July which were relatively wet and possibly some maturation of hypobiotic larvae while the November and December peak may have been due to larvae picked during the short rainy season.

The sudden rise in egg counts in October and the appearance of the clinical disease was possibly due to resumed development and maturation of hypobiotic larvae. Several authors have reported on hypobiosis in goats (Pandey *et al.*, 1994; Gatongi *et al.*, 1998) involving mostly *H. contortus* which was the main nematode recovered in this study. Such instances have been reported in the semi arid areas of Kenya where acute haemonchosis occurs during the dry season just before the onset of the rains (Gatongi *et al.*, 1998). In the present study, the short rains were expected in October and it is possible that the hypobiotic larvae resumed development in anticipation of these rains hence no hypobiotic larvae were recovered from the tracers introduced at the end of the dry season. Variations in the proportions of hypobiotic larvae of *H. contortus* with season as seen in this study have been reported by Pandey *et al.*, (1994), in the high veld of Zimbabwe. In this veld the hypobiotic larvae of *H. contortus* comprised 46.1% of the total worm burdens in the middle of the dry season in August. For the rest of the year, hypobiotic larvae

accounted for between 0 - 6.8% of the total worm burden.

In a study carried out in Naivasha in the semi arid area of Kenya, Gatongi, *et al.*, (1998), indicated that hypobiotic larvae of *H. contortus* and adult population co - existed in proportions that varied with the seasons. The hypobiotic larvae increased during the dry season and then declined during the wet season while the adult worms decreased during the dry season and increased during the wet season. Similar situation has been reported in Saudi Arabia where *H. contortus* survived the dry season as adults and larvae (El -Azazy, 1995). This may explain the occurrence of the reduced eggs observed in the dry season and the increased eggs at the onset of the rainy season without a corresponding increase in pasture infectivity. A dry season rise in helminthosis was shown to occur in gastrointestinal helminths of small farm ruminants in Sierra Leone (Asanji and Williams, 1987). In the present study, tracers introduced at the end of the dry season did not have any hypobiotic larvae while those introduced at the end of the short rains had hypobiotic larvae. The short rains are usually followed by a long dry season and it is possible that the larvae picked towards the end of the short rains are prone to undergo hypobiosis. The hypobiotic larvae resume development at the end of the dry season. This may be a mechanism inherent in the worms for survival which responds to weather changes in a pre - programmed pattern (Blitz and Gibbs, 1971; Connan, 1978; Williams *et al.*, 1983; Gatongi *et al.*, 1998).

Pasture infectivity occurs even during the dry season as seen in the present study where the tracers picked infective larvae at the end of the dry season. The infective larvae may have been picked in the early morning when there was dew or in some areas of the paddocks where there was sufficient vegetation cover that retained enough moisture for the hatching and survival of the infective larvae. Areas around the watering troughs could also be sources of infection for these tracers during the dry season. However, it was more pronounced during the rainy season as observed by other workers (Pandey *et al.*, 1994; Jacquet *et al.*, 1995; Silva *et al.*, 1998).

Suppressive treatment decreased the pasture infectivity due to the reduced egg shedding by the treated animals. However, there were still some pasture larval contamination as shown by the tracers grazed in the paddocks previously grazed by the treated animals. Such treatment where egg output is suppressed until the initial population of infective larvae on pasture has declined to safe levels has been classified as 'preventive' (Michel, 1985; Barger, 1997). The treatment may have also selected for resistant worms. Michel, (1985), indicated that selection pressure on worms to anthelmintic resistance is not only proportional to the frequency of treatment but to the success of treatment. Selection for resistance is more closely related to the degree of success of the control strategy than the frequency of treatment it entails (Barger, 1995). The selection pressure is measured by the contribution that resistant survivors of anthelmintic treatment make to subsequent generation of worms. This is large when animals are treated frequently or when given a single treatment and moved to a clean pasture. In the present study, the frequency of treatment was high and may have exerted the selection pressure even if the treatment was successful. Barnes *et al.*, (1995), advised the use of highly effective anthelmintics or combinations which select less strongly as they leave few survivors. Prichard, (1990), reported that prolonged use of an anthelmintic selects effectively for resistance to the anthelmintic being used plus others with similar mode of action.

*Haemonchus contortus* was the main nematode recovered. It has been reported by several workers that this is the main nematode that affects sheep and goats in the tropics and subtropical areas (Charles, 1989; Yadav and Uppal, 1992; Rahman, 1992; Chaudhri *et al.*, 1993; Anene *et al.*, 1994; Pandey *et al.*, 1994; Dorny *et al.*, 1994; 1995; Kochapakdee *et al.*, 1995; Mwamachi *et al.*, 1995; Jacquet *et al.* 1995; Saithanoo, 1996; Beriajaya and Copeman, 1997; Baker *et al.*, 1998). Due to its pathogenicity, control failure is easily noticeable and has been associated with anthelmintic resistance in small ruminants in the tropics and sub tropics (Dorny *et al.*, 1994;

Yadav and Uppal, 1992; Waller, 1997b; Maingi *et al.*, 1998; Chandrawathani *et al.*, 1999).

The mean PCV levels in control goats in this study showed a sharp decline from August to October. This is also the time when two goats died in this control group and two others had to be given salvage treatment when their PCV values were below 18%. The depression in October in the suppressively treated group may have been due to the low quality of the feed. Both groups, the suppressively treated and control showed improved PCV levels after the rains in November. Suppressive treatment prevented clinical parasitic gastroenteritis especially in October when there was a significant difference in the PCV levels between the treated and control groups.

This study has shown that gastrointestinal helminths reduce the productivity of goats by causing reduced weight gains, weight losses, mortalities and parasitic gastroenteritis. The effects are more profound during the periods of feed scarcity like the dry season. Frequent suppressive anthelmintic treatment prevent these, reduce pasture contamination but may also select for anthelmintic resistant nematodes.

Infective larvae are able to hatch, survive and become infective during the dry season possibly from moist areas in the paddocks and areas around the watering points.

*Haemonchus contortus* was the main nematode and contributed up to 100% of the total worm burdens. This species undergoes hypobiosis and maturation of these larvae possibly occurs at the beginning of the rainy season.

### 5.0. Studies to determine the effectiveness of grazing management in controlling gastrointestinal nematodes in weaner lambs on pasture in Denmark.

#### 5.1. Introduction:

Based on the epidemiology of nematodes, integrated or strategic control measures have been developed. The dose and move (Weybridge) system, where calves were treated and moved to a “clean” (safe) pasture was one of the first to utilize this knowledge (Stromberg and Averbeck, 1999). In sheep, the principle of “dose and move” combines a move of ewes with lambs or weaner lambs to “clean” (safe) pasture in July with an anthelmintic treatment (Michel, 1976). This method has been advocated as an option for practical parasite control in the United Kingdom (Coles and Roush, 1992). Michel, (1985) classified such a strategy as “evasive”. These rely on anthelmintic treatment and movement of livestock to clean pasture just before the larvae resulting from eggs shed in the original pasture appear in significantly dangerous levels. This was originally devised by Michel, (1969) for the control of gastrointestinal nematodes in calves and Thomas and Boag for ewes and lambs (Thomas, 1982). In cattle, the move is done in early July to a clean pasture and the animals are moved monthly and the last move should not be more than a month before housing (Eysker *et al.*, 1998 b). In sheep, ewes and lambs are moved at the beginning of July. Lambs are weaned and share the pasture with ewes and lambs are then moved again with or without treatment before housing or slaughter (Coles and Roush, 1992). The anthelmintic removes the worms that may contaminate the clean pasture. The move enables young animals to escape exposure to mid-summer larval peak resulting from the hatching of eggs passed earlier in the season. Variations of the dose and move method have been extended to control lung worms (Jorgenson, 1981). However, there is a risk of build up of infections in late season and secondly, the dose and move has been associated with the development of

anthelmintic resistance because any nematode capable of surviving the anthelmintic treatment will be the founder nematode population in the new clean pasture (Cawthorne and Cheong, 1984). This strategy is also not compatible with non-chemical pest management control methods of organic farming systems (Thamsborg *et al.*, 1999).

Michel, (1976) showed that moving cattle to graze the aftermath of a harvested crop such as wheat or corn offered relatively safe pastures. These fields had been tilled, planted and harvested since they were last grazed. The eggs/larvae were moved into the soil and given time to die.

Nematophagous fungi have in plot studies (Waller and Faedo, 1993; Mendoza -de Gives *et al.*, 1992; 1994) and in a single field study ( Githigia *et al.*, 1997) shown promising results in the control of ovine nematode infections in set-stocked lambs on contaminated pasture. However, the period of fungi feeding should preferably be shortened ( at present 2 - 3 months). This may be accomplished if the animals are removed from the contaminated paddocks at weaning to a clean paddock and fungi dosed for an initial period of six weeks.

The present study compared the dose and move , move and fungi feeding for a short period and the move only strategies at weaning on the performance and build up of gastrointestinal nematode infections in weaner lambs in Denmark.

## 5.2. Materials and Methods.

### 5.2.1. Experimental design:

Twenty four Suffolk cross ewes (sired by a Texel ram) with twin lambs (2 - 3 weeks old) were grazed on a contaminated 1.2 ha pasture on 4<sup>th</sup> May 1999. The pasture had been used for sheep grazing for 9 consecutive years and was known to be infected with *Ostertagia*, *Trichostrongylus* and *Nematodirus spp* ( Githigia *et al.*, 1997). The ewes were supplemented with a commercial concentrate at the rate of 1 kg per ewe per day as group feeding.

On 29<sup>th</sup> June the lambs were weaned and allocated to 6 groups of 8 lambs according to body weight, sex and faecal egg counts. Two of the groups were treated with fenbendazole (Panacur®) at a dosage rate of 7.5 mg kg<sup>-1</sup> - Dose and Move (DM1 + 2) while four groups were not treated - Move and Fungi (MF1 + 2) and Move only (M1 + 2) groups. All the lambs were moved to a clean pasture subdivided into 6 equal sized paddocks of 0.25 ha. The pasture had not earlier been grazed by sheep but was grazed by cattle and horses during the previous year's grazing season. The pasture was cut for silage making at the beginning of June. Each group was randomly allocated to one paddock and set stocked until 27<sup>th</sup> September when they were all slaughtered for worm counts and identification. The move and fungi group was supplemented daily with 100 g commercial concentrate per lamb per day mixed with freeze dried *Duddingtonia flagrans* chlamydospores for six weeks (1<sup>st</sup> July to 5<sup>th</sup> August). The fungi dosage was between 375,000 to 400,000 chlamydospores per kg body weight of the lambs at the beginning and was given as group feeding on an open feeding trough. This dosage was not increased over the six weeks and the dosage at the end of the fungi feeding period was approximately 250,000 chlamydospores per kg body weight of the lambs. The other groups were supplemented daily with a similar amount of concentrate without the fungi up to the end of the study. The lambs were observed daily while feeding to ensure that all lambs consumed the offered material. The pasture was cut in mid July at 8 - 10 cm and the seed heads removed.

#### 5.2.2 Meteorological data:

Total monthly temperature and rainfall data were obtained from a meteorological unit within 1 km of the experimental paddocks.

#### 5.2.3 Sampling and analysis:

Rectal faecal samples and blood samples by jugular venipuncture for serum were obtained every fortnight. All lambs were weighed and grass samples (200 - 400g) per paddock

were collected by hand following a W- shaped route across the paddock. The lambs were observed daily with a more detailed examination on sampling days. Faecal nematode egg counts were determined using the modified McMaster procedure (Henriksen and Aagaard, 1976). *Nematodirus* eggs were identified to the level of species based on morphology (Barth and Visser, 1991) and eggs other than *Nematodirus* were categorized as other strongyles. Group faecal cultures in duplicate were made by modifying the method of Henriksen and Korsholm, (1983), using 1 g of faeces from each animal in the group (= total 8 g) mixed with 10 ml water and 4 g vermiculite. The cultures were incubated for 2 weeks in a climatic chamber at 20°C and 95% R.H. The resulting larvae (L<sub>3</sub>) were enumerated and identified to genus or species level based on size and morphology (Anon., 1986). Serum was analyzed for albumin using the bromocresol-green method ( Nisbeth, *et al.*, 1973).

The herbage samples were soaked overnight and infective larvae were isolated and enumerated according to the method of Mwegoha and Jorgensen (1977). The method was modified by omission of ox bile in the agar gel.

Total worm counts were performed on all lambs in the groups DM1 + 2 and M1 + 2. The abomasa and small intestines were ligated and separated immediately after slaughter. The organs were opened into individual containers and rinsed with warm saline (approximately 38°C). Two 10% subsamples were drawn and processed for nematode recovery and enumeration by the agar gel method (Van Wyk and Gerber, 1978; Slotved, *et al.*, 1996) which was previously found comparable to the method of sieving traditionally used in the laboratory (Thamsborg, *et al.*, 1996) but was more labour saving. Digestion of the abomasal mucosa was performed according to Anon. (1986) and the resultant larvae (mainly *Ostertagia*) counted. For the large intestines 10% sub samples were observed directly after sieving. Nematodes recovered from the sub samples were identified to level of species based on morphology of adults (Anon., 1986; Barth and Visser,

1991).

#### 5.2.4. Statistical analysis:

Faecal egg counts (log- transformed), body weight, serum albumin and worm counts (log- transformed) for the groups DM1 + 2 and M1 + 2 were analyzed by repeated measures or non - repeated measures of analysis of variance (ANOVA) using the following model  $Y = TR + REP(TR)$ , (nested design) in the GLM procedure of SAS (SAS Institute Inc, 1989 - 1996): Y is the dependable variable, TR is the effect of treatment ( $\pm$  Dose at moving) and REP(TR) represents the effect of the 2 replicates (REP) nested within treatments. The MF1 + 2 groups were left out of the analysis due to failure of the fungi to reduce the number of infective larvae in coprocultures.

### 5.3. RESULTS:

#### 5.3.1. Rainfall and temperature

The monthly precipitation was 53.6, 99, 16, 114 and 31mm for May - September compared with a long term average (1960 - 1990) of 44, 53, 67. 62 and 59mm for the same months. The months of May, June and August were wetter while July was dry and warm. Heavy rainfall was recorded in August (Figure 5.1).

#### 5.3.2. Feeding, clinical observations and weight gains

In the MF1 + 2 groups problems were identified regarding the feeding of fungal chlamydospores.. Some fungal spores were found in the feeding troughs after feeding which may indicate that the lambs were selecting the concentrate and leaving the spores. The dry spores were not adhering to the commercial concentrate hence they were easily blown by the lambs when feeding. This was confirmed during faecal egg count analysis where very few chlamydospores were encountered. Very few mycelia were also encountered when the coprocultures from these groups were harvested and there was no differences in the percent reduction of the numbers of

infective larvae between groups which showed that the fungi had no effect. A sample from this batch was found to work and reduce infective larvae under laboratory conditions when the lambs were bucket fed individually indoors and monitored to ensure they consumed all the material. From this it was concluded that there was a problem with the delivery system of the fungal spores to the lambs and the MF1 + 2 groups were excluded from the study at this point.

For the other groups, no clinical disease related to gastrointestinal helminthosis was observed. One lamb died during the study from strangulation and one from suspected *Clostridium perfringens* (pulpy kidney) which was not confirmed at postmortem. There was also no breech soiling in any of the lambs. Some lambs in the move only (M1 + 2) groups had loose semi formed faeces for 4 weeks after moving to the clean pasture. Later other lambs from both groups had loose faeces but this was not consistent.

Mean weights were comparable for the two groups throughout the study period (Figure 5.2). There was a rapid weight gain immediately after the lambs were moved to the clean pastures with a high availability of good quality herbage. The grass heights in the 4 paddocks were comparable and decreased from 10cm after the cutting to 4cm at the end of the study. The accumulated weight gains of groups dose and move (DM1 + 2) were  $12.3 \pm 1.6\text{kg}$  and move only (M1 + 2) were  $13.1 \pm 0.2\text{kg}$  per lamb for the season (Figure 5.2). Repeated measures analysis of variance showed no significant ( $P > 0.05$ ) differences in weight gain between the dose and move and move only groups of lambs over the study period and at no point during the study (Table 5.1 and 5.2).

Table 5.1: Results of the repeated measures analysis of variance for the effect of anthelmintic treatment before moving to clean pasture (dose and move) on weight gains of the weaner lambs over the study period

| Lsmeans                 |                    | P value |
|-------------------------|--------------------|---------|
| Dose and Move (DM 1 +2) | Move only (M1 + 2) |         |
| 14.9643                 | 15.4375            | 0.5667  |

Table 5.2: Results of the repeated measures analysis of variance for the effect of anthelmintic treatment before moving to clean pasture on accumulated weight gains over the study period.

| Weeks | Lsmeans  |         | P value |
|-------|----------|---------|---------|
|       | DM 1 + 2 | M 1 + 2 |         |
| 2     | 6.3218   | 5.6562  | 0.1364  |
| 4     | 9.5000   | 8.8437  | 0.1763  |
| 6     | 10.8571  | 10.7187 | 0.8051  |
| 8     | 11.1428  | 10.8437 | 0.6209  |
| 10    | 12.5357  | 12.6562 | 0.8574  |
| 12    | 14.9642  | 15.4375 | 0.5667  |

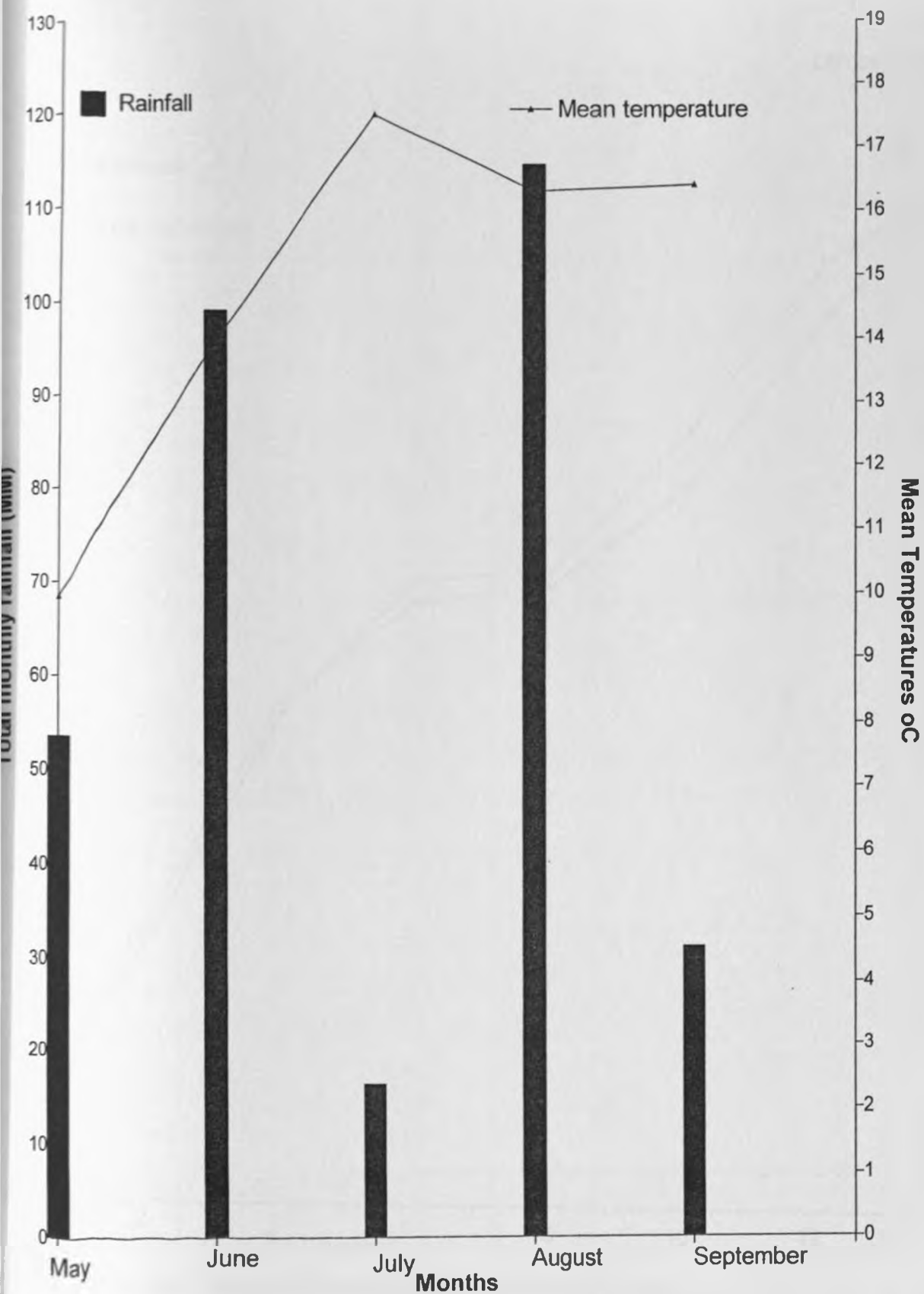
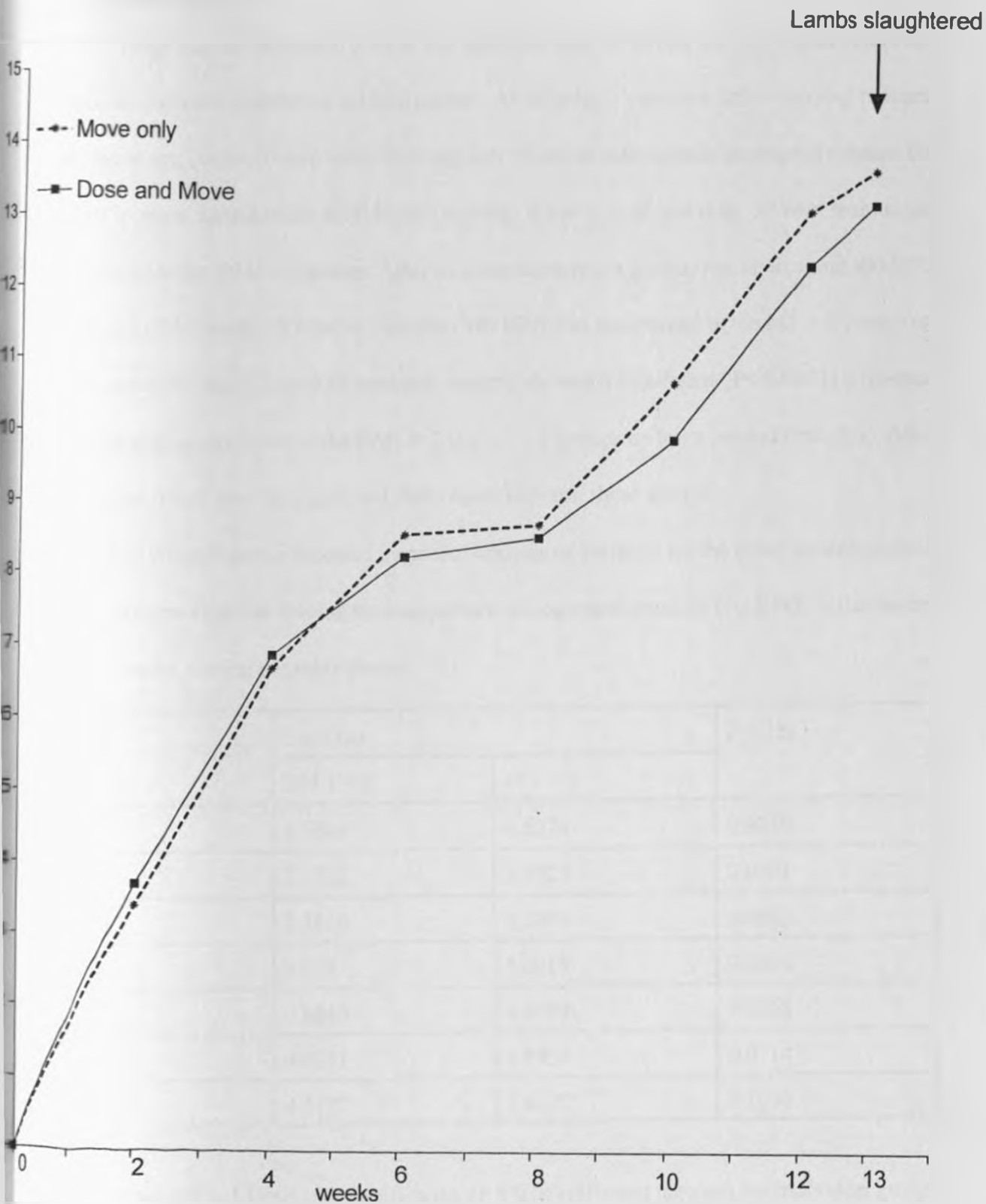


Figure 5.1: Monthly rainfall and mean temperatures obtained from a meteorological station within 1 km from the experimental paddocks



5.2 Accumulated mean group weight gains from moving to clean pasture to slaughter

5.3.3. Faecal egg counts:

There was no difference ( $P>0.05$ ) in the faecal egg counts for the two weeks before the lambs were weaned and moved to clean pasture. Anthelmintic treatment before moving reduced the faecal egg counts to zero while moving only (without anthelmintic treatment) reduced the counts to about a third of the level before moving. A mean level less than 50 was maintained for 6 weeks in the DM1 + 2 groups. After this time there was a gradual rise up to about 400 EPG at the end of the study. A level of less than 400 EPG was maintained in the M1 + 2 groups up to the end of the study. Repeated measures analysis showed a significant ( $P<0.0001$ ) difference in faecal egg counts between the DM1 + 2 and M1 + 2 groups up to six weeks (Table 5.3). After this period, there was no significant differences between these groups.

Table 5.3: Results for the repeated measures analysis of variance for the effect of anthelmintic treatment before moving to clean pasture on Log transformed EPG (LEPG) of the weaner lambs during the study period.

| Week | Lsmeans  |         | P value |
|------|----------|---------|---------|
|      | DM 1 + 2 | M 1 + 2 |         |
| 0    | 6.5606   | 6.5278  | 0.9030  |
| 2    | 2.3025   | 5.7627  | 0.0001  |
| 4    | 2.3810   | 4.5890  | 0.0001  |
| 6    | 3.118    | 5.0210  | 0.0001  |
| 8    | 3.8645   | 4.4587  | 0.1263  |
| 10   | 4.4231   | 4.8956  | 0.0714  |
| 12   | 4.5127   | 5.0120  | 0.1098  |

The EPG (LEPG) was significantly ( $P < 0.05$ ) different between the individual groups across the treatments by repeated measures analysis of variance (Table 5.4).

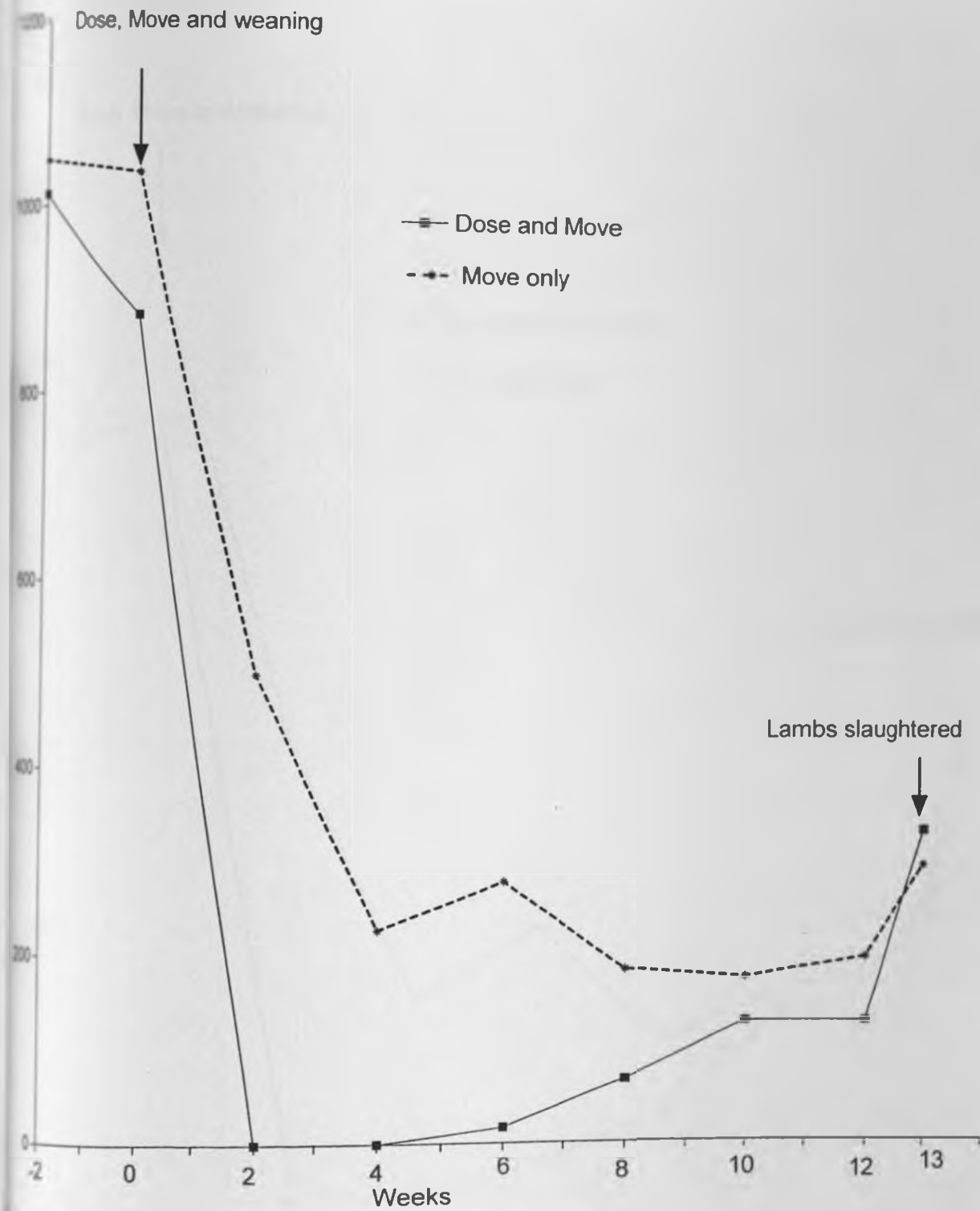
Table 5.4: Results of the repeated measures analysis of variance for the effect of the different groups on the log transformed EPG of the weaner lambs during the study period.

| Week | Lsmeans |       |       |       | P value |
|------|---------|-------|-------|-------|---------|
|      | DM 1    | M 1   | DM 2  | M 2   |         |
| 0    | 6.598   | 6.609 | 6.523 | 6.445 | 0.9115  |
| 2    | 2.302   | 6.08  | 2.302 | 5.445 | 0.0001  |
| 4    | 2.302   | 4.419 | 2.459 | 4.759 | 0.0001  |
| 6    | 3.16    | 5.18  | 3.076 | 4.861 | 0.0001  |
| 8    | 4.383   | 4.029 | 3.345 | 4.887 | 0.0043  |
| 10   | 5.186   | 4.521 | 3.659 | 5.268 | 0.0091  |
| 12   | 5.171   | 5.186 | 3.853 | 4.837 | 0.0053  |

Overall, there was a significant ( $P < 0.0001$ ) difference with the treatment by repeated measures analysis.

The highest mean level reached in the treated groups was 250 eggs per gram (EPG) of faeces. The moving to clean pasture sharply reduced the total faecal egg counts in the moved only group. The total EPG were reduced to less than half the level before moving to clean pasture (1100 to 500 EPG) (Figure 5.3). This level was maintained below 400epg up to the end of the grazing season.

The counts for the strongyles followed the pattern of the total faecal egg counts in both groups. These were responsible for the gradual rise in total faecal egg counts in the dose and move groups after one month and up to the end of the grazing season (Figure 5.4).The *Nematodirus* faecal egg counts only contributed significantly to the total faecal egg counts before moving to the clean pasture. Moving reduced the *Nematodirus* egg counts rapidly to levels less than 100epg. There was a gradual decrease up to the end of the grazing season. The level did not



5.3: Arithmetic mean group faecal egg counts (EPG) over the study period from 2 weeks before moving to clean pasture until slaughter date

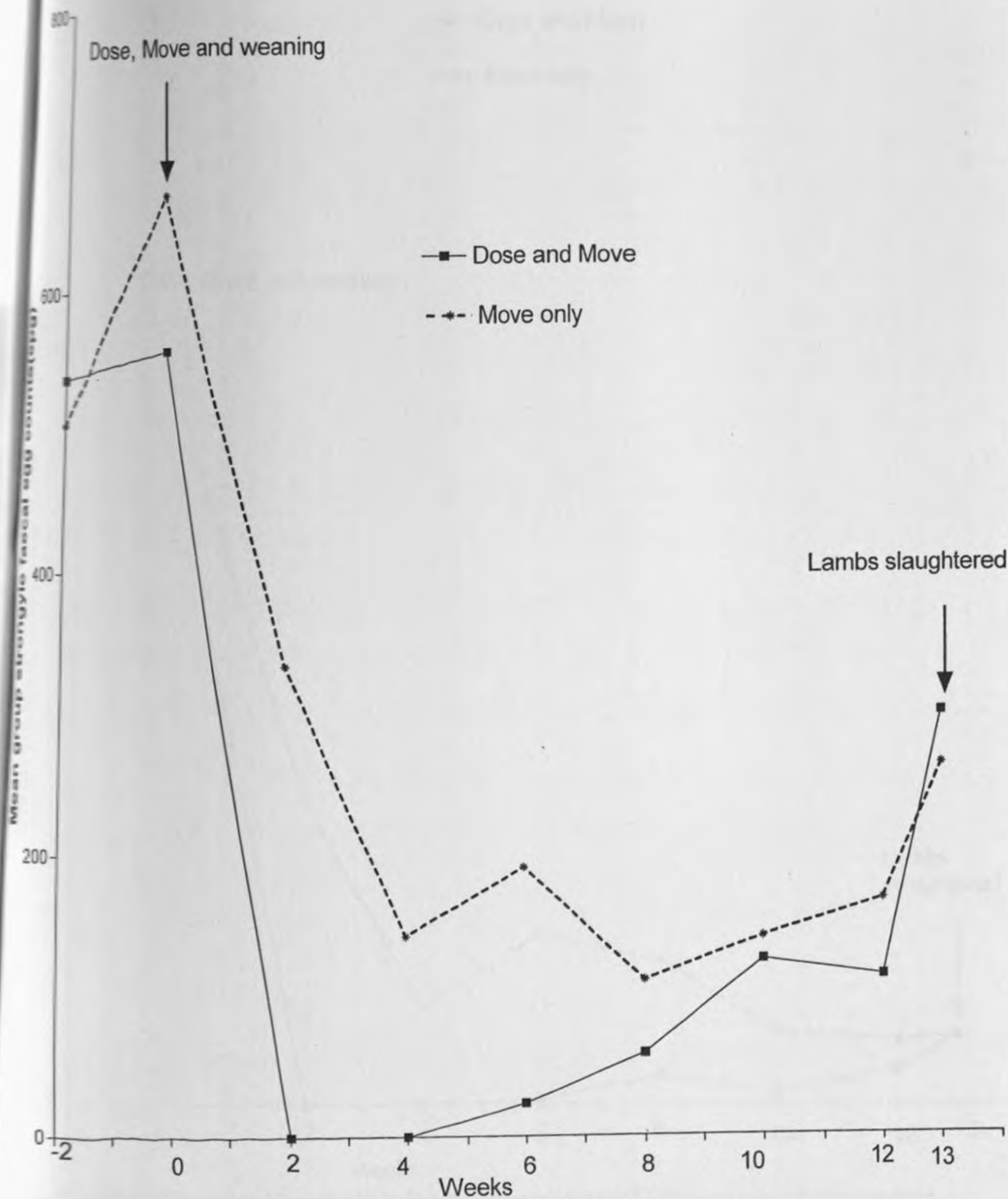


Fig 5.4: Arithmetic mean group strongyle faecal egg per gram (EPGs) counts from two weeks before moving to clean pasture up to slaughter.

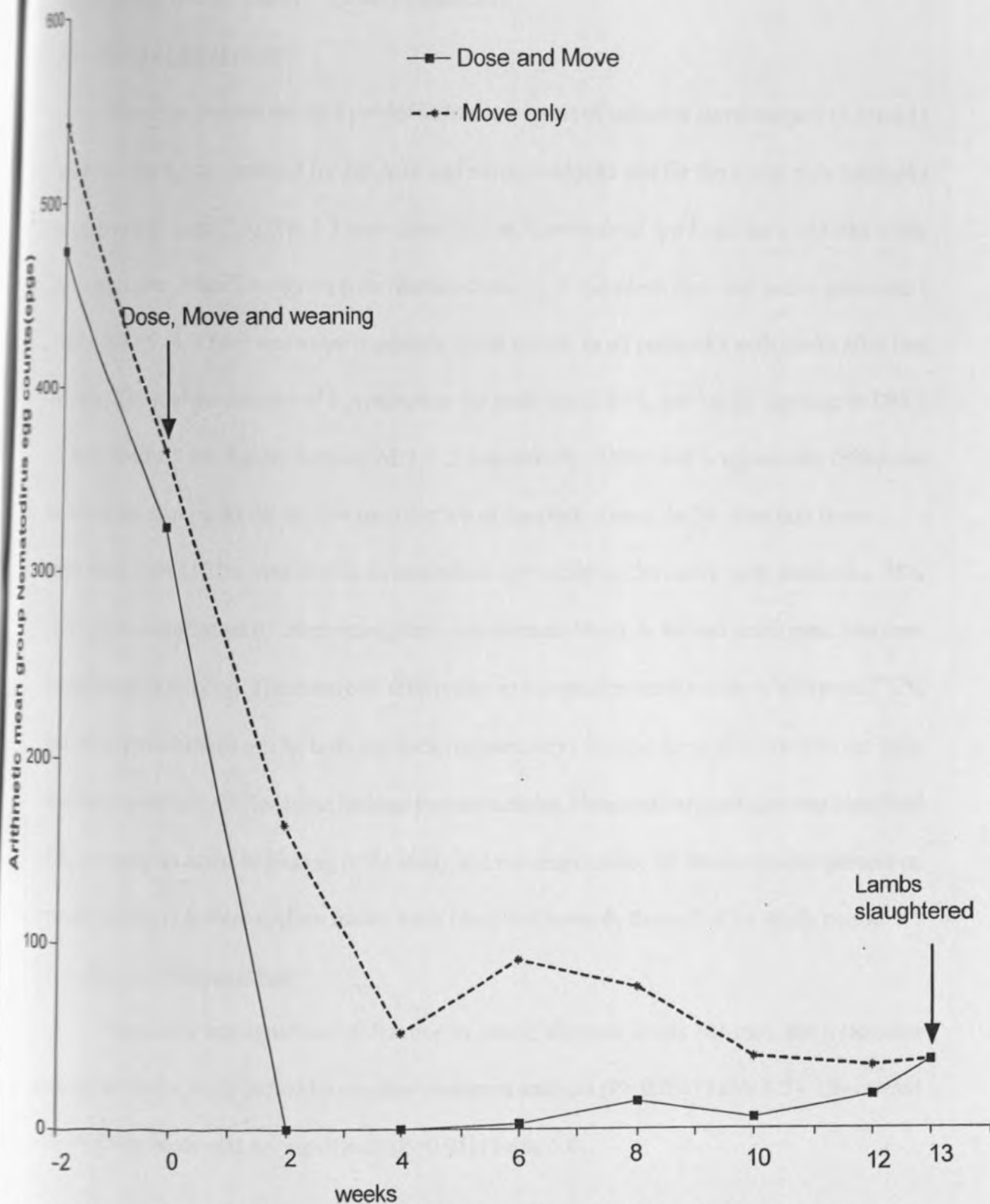


Figure 5.5: Arithmetic mean *Nematodirus* faecal egg per gram (EPGs) counts for the weaner lambs from two weeks before moving to clean pasture until slaughter

the beyond 40 EPG in the dose and move group. The main species identified by egg morphology was *N. fillicolis* with a few *N. battus* (Figure 5.5).

#### 5.3.4. Pasture Larval counts:

The clean pasture for the 4 paddocks had low levels of infective larval stage 3 (L3) i.e. 33 and 81 L3 per kg dry herbage for the dose and move paddocks and for the move only paddocks respectively (Figure 5.6). The L3 were identified as *Nematodirus* spp in all the paddocks while there were few other Strongyles (non *Nematodirus*) L<sub>3</sub> in the clean dose and move paddocks (Figure 5.7 - 5.8). There was a rise in pasture larval counts in all paddocks with peaks after two months. The highest number of L<sub>3</sub> reached at the peak was 1354 L<sub>3</sub> per kg dry herbage in DM 1 +2 and 2045 L3 per kg dry herbage M 1 + 2 respectively. There was a significant difference between the groups on the species contribution of the peak counts. In the dose and move paddocks, 90% (1216) was due to *Nematodirus* spp while in the move only paddocks, 75% (1544) was contributed by other strongyles ( non *Nematodirus*). A second small peak was seen 3 months after moving. There were no differences in the species contribution of this peak ( 72% and 74% *Nematodirus* spp for both paddocks respectively). *Nematodirus fillicolis* was the most abundant species identified in the herbage pasture samples. *Nematodirus spathiger* was identified in a few samples at the beginning of the study and was responsible for the low counts present on moving while few *Nematodirus battus* were identified towards the end of the study period.

#### 5.3.5. Serum albumin levels

There was no significant difference in serum albumin levels between the treatments during the entire study period by repeated measures analysis ( $P > 0.05$ ) (Table 5.5). The overall effect of treatment was not significant ( $P > 0.05$ ) (Table 5.6).

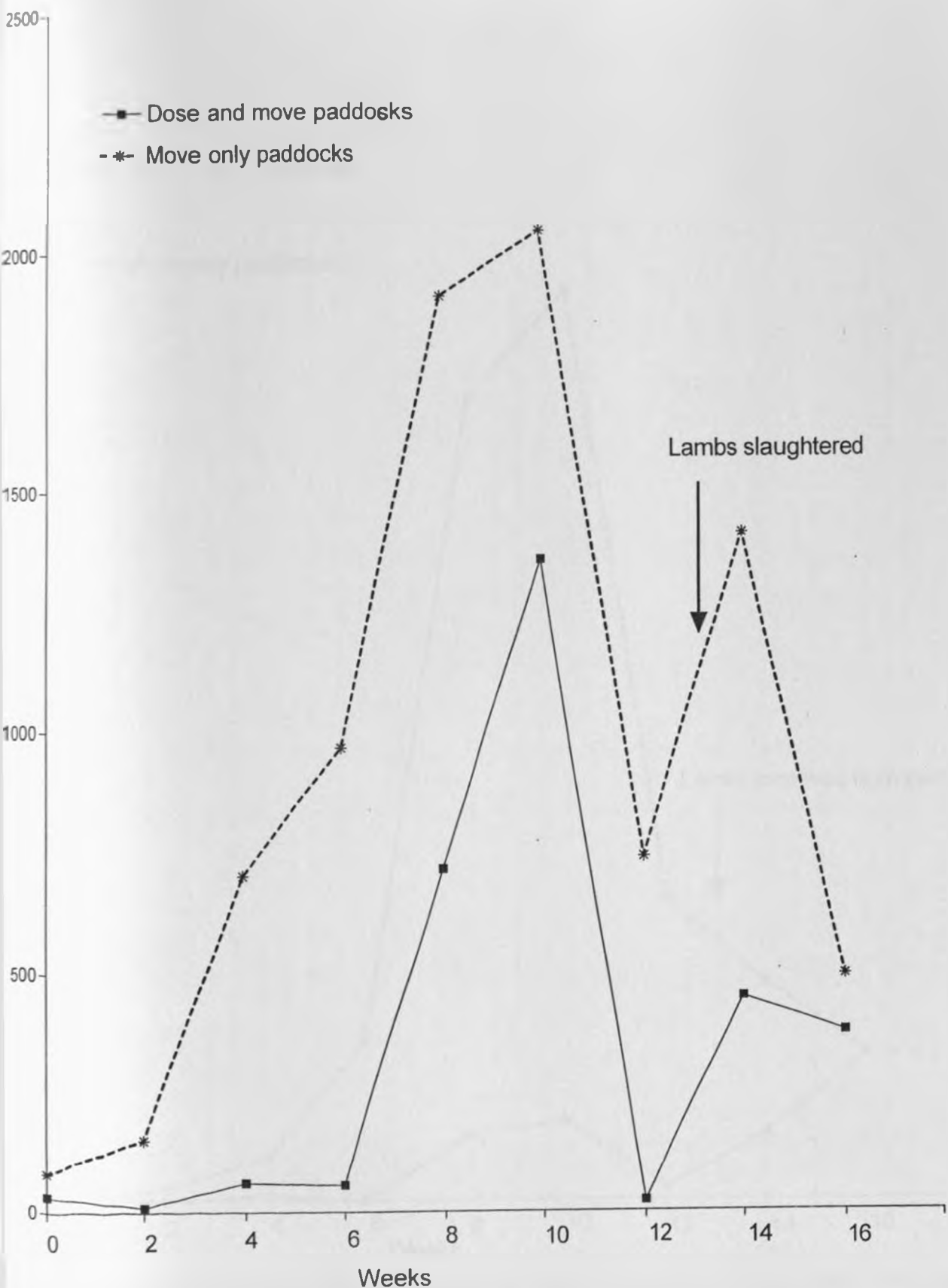


Figure 5.6: Arithmetic mean infective larvae (L3) per kg dry herbage in the paddocks grazed by the groups from move to clean pasture to 3 weeks after the slaughter of the lambs.

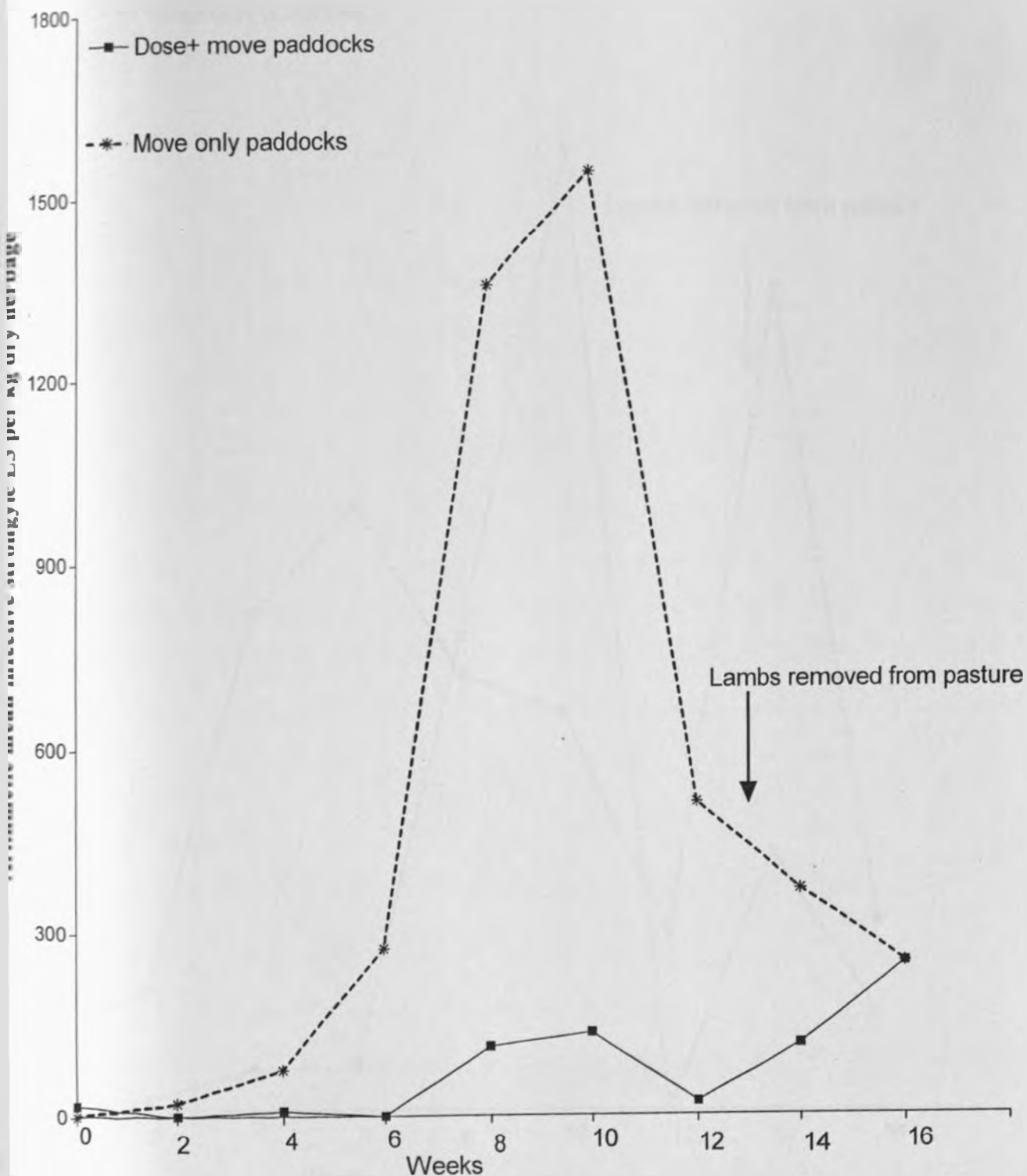
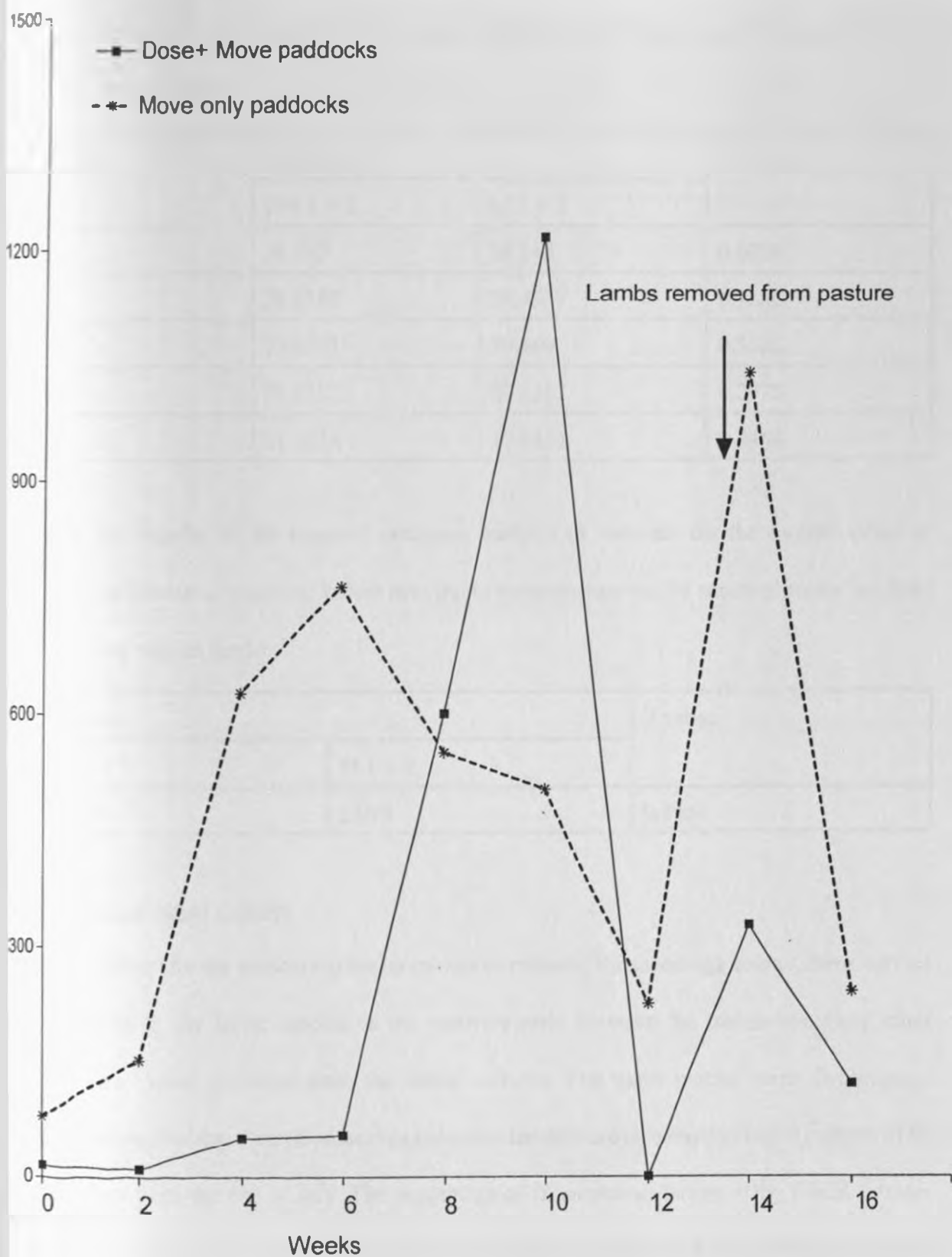


Figure 5.7: Arithmetic mean strongyle infective L3 per kg dry herbage in the paddocks from date of moving to 3 weeks after slaughter of lambs.



5.8: Arithmetic mean infective *Nematodirus* L3 per kg dry herbage in the paddocks on moving and up to 3 weeks after slaughter of the lambs.

Table 5.5: Results of the repeated measures analysis of variance for the effect of treatment before moving to clean pasture on the serum albumin levels of the weaner lambs during the study period.

| Week | Lsmeans  |         | P value |
|------|----------|---------|---------|
|      | DM 1 + 2 | M 1 + 2 |         |
| 2    | 28.707   | 28.143  | 0.0709  |
| 4    | 28.0785  | 28.4937 | 0.7686  |
| 6    | 29.6571  | 29.400  | 0.3022  |
| 8    | 29.7271  | 29.3562 | 0.2975  |
| 10   | 31.2214  | 30.8812 | 0.3364  |

Table 5.6: Results of the repeated measures analysis of variance for the overall effect of anthelmintic treatment before moving to clean pasture on the serum albumin levels of the weaner lambs.

| Lsmeans  |         | P value |
|----------|---------|---------|
| DM 1 + 2 | M 1 + 2 |         |
| 3.0500   | 2.075   | 0.3364  |

5.3.6. Faecal larval cultures

Except for the numbers of the larvae which reflected the faecal egg counts, there were no differences in the larval species or the recovery rates between the treatments. Only other strongyles were recovered from the faecal cultures. The main species were *Ostertagia* / *Trichostrongylus spp.* Few *Haemonchus contortus* larvae were recovered in faecal cultures of all the groups from the end of July. The percentage of *H. contortus* larvae in the faecal cultures increased from 2.1% to 2.8% in the cultures from DM 1 +2 and from 4% to 24% in those from M 1 +2 from 4 weeks up to the end of the study period. Few *Cooperia spp* were recovered in the

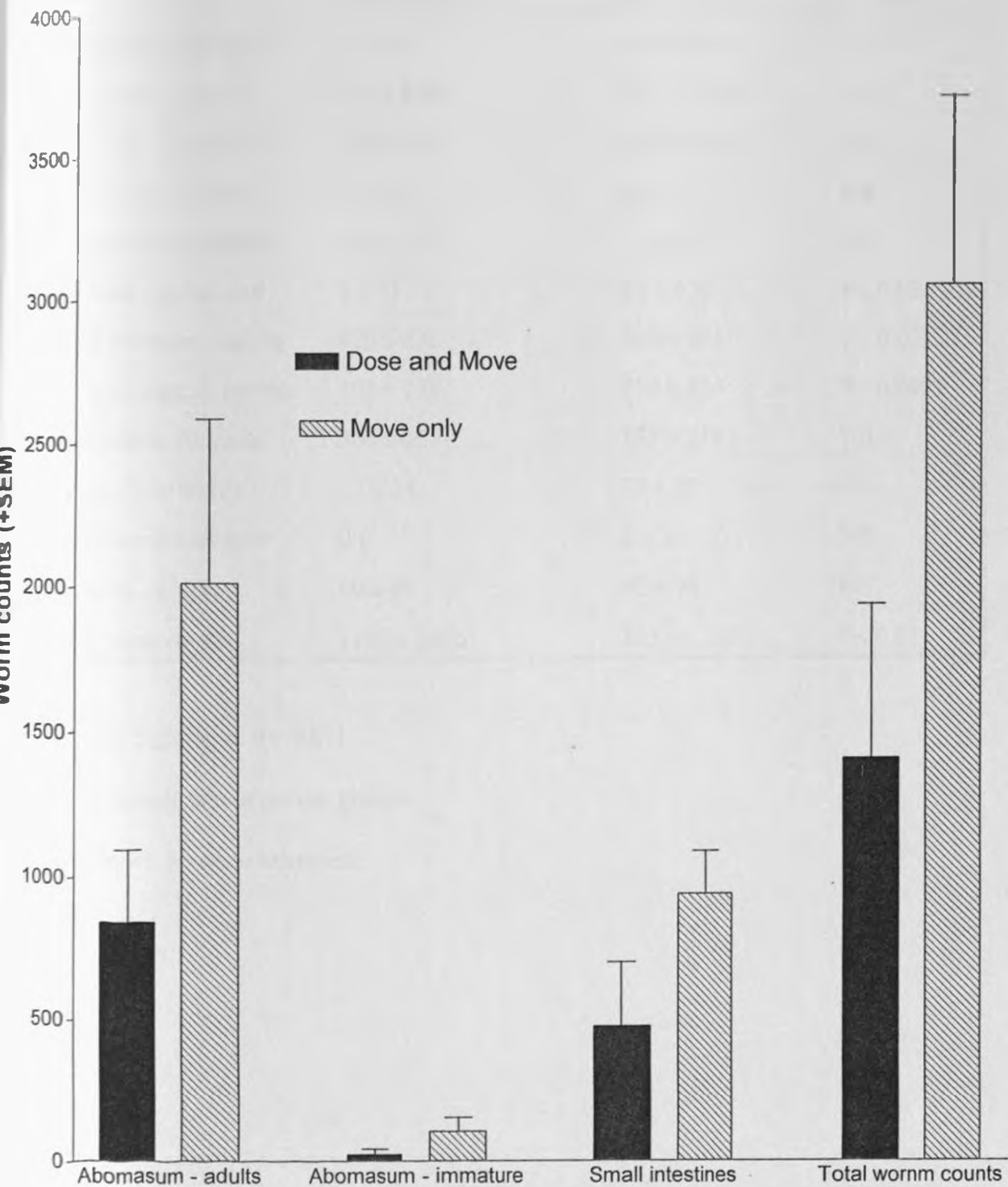
faecal cultures from both groups at irregular intervals. The recovery rates (percentage of faecal eggs hatching to larvae) in all the groups dropped to below 50% at the end of the study compared to the beginning when it ranged between 75 - 91%.

#### 5.3.7. Worm counts

The worm counts are shown on Table 5.7 and Figure 5. 9. Treating lambs before moving reduced the total worm burdens to a half of those lambs that were only moved without treatment.

The abomasal worm counts in the dose and move lambs ranged from 120 to 4000 while those for the move only ranged from 0 to 7400. The small intestinal worm counts ranged from 0 to 2400 in the dose and move lambs and between 20 and 2020 in the move only lambs.

Immature stages were found in only 2 lambs in the DM 1 +2 while 10 out of 16 lambs in the M1 +2 had immature stages in the abomasum. The counts for immature stages in the abomasum and the small intestinal worm counts were significantly different while the abomasal adult worm counts were not significantly different by non repeated measures analysis. The total worm counts (abomasal counts (adults + immature) and small intestinal counts) were statistically significant between the groups ( $P < 0.05$ ) ( Table 5.8). The M 1 + 2 group had higher total counts.



5.9: Arithmetic mean group worm counts (+ SEM) for the abomasum (adults and immature), small intestines and the total worm counts of the lambs at slaughter

Table 5.7: Arithmetic mean total worm counts ( $\pm$  SD) and the mean ( $\pm$  SD) for species identified

|                                  | Dose and Move            | Move only       | P -value <sup>b</sup> |
|----------------------------------|--------------------------|-----------------|-----------------------|
| Abomasum- Immature               | 23 $\pm$ 75              | 104 $\pm$ 190   | P< 0.05               |
| Abomasum- Adults                 | 840 $\pm$ 959            | 2014 $\pm$ 2304 | NS                    |
| <i>Ostertagia circumcinta</i>    | 734 $\pm$ 907            | 1764 $\pm$ 2081 | NS                    |
| <i>Ostertagia trifurcata</i>     | 84 $\pm$ 58              | 94 $\pm$ 132    | NS                    |
| <i>Haemonchus contortus</i>      | 14 $\pm$ 19 <sup>a</sup> | 61 $\pm$ 191    | NS                    |
| <i>Trichostrongylus axei</i>     | 8 $\pm$ 21               | 94 $\pm$ 130    | P< 0.05               |
| Small Intestine - Adults         | 473 $\pm$ 836            | 939 $\pm$ 601   | P< 0.001              |
| <i>Trichostrongylus vitrinus</i> | 108 $\pm$ 178            | 710 $\pm$ 414   | P< 0.0001             |
| <i>Nematodirus fillicolis</i>    | 304 $\pm$ 658            | 157 $\pm$ 219   | NS                    |
| <i>Nematodirus battus</i>        | 22 $\pm$ 24              | 23 $\pm$ 32     | NS                    |
| <i>Nematodirus spathiger</i>     | 0.0                      | 5 $\pm$ 20      | NS                    |
| <i>Cooperia curticei</i>         | 40 $\pm$ 66              | 41 $\pm$ 79     | NS                    |
| Total worm counts                | 1407 $\pm$ 1986          | 3057 $\pm$ 2691 | P< 0.05               |

NS = Not Significant (P> 0.05)

<sup>a</sup> Found in only one of the sub groups.

<sup>b</sup>Differences between treatments

**Table 5.8:** Results of the analysis of variance for the effect of anthelmintic treatment before moving to clean pasture on the log transformed immature abomasal worm counts, adult abomasal counts, small intestinal worm counts and the total counts of the weaner lambs.

|                      | Lsmeans  |         | P value |
|----------------------|----------|---------|---------|
|                      | DM 1 + 2 | M 1 + 2 |         |
| Abomasum<br>Immature | 2.0551   | 3.4323  | 0.0207  |
| Abomasum adults      | 6.3594   | 6.6821  | 0.5563  |
| Small Intestines     | 4.8913   | 6.5341  | 0.0044  |
| Total Worm counts    | 6.7133   | 7.599   | 0.0285  |

*Ostertagia circumcinta* was the main species in the abomasum. The other species recovered were *Ostertagia trifurcata*, *Haemonchus contortus* and *Trichostrongylus axei*. There were no significant differences in the total count of these species between the treatments by ANOVA analysis ( $P > 0.05$ ) except for *Trichostrongylus axei* which though significant was recovered in very low numbers (Table 5.9). *Haemonchus contortus* was only recovered from one of the sub groups of the DM 1 + 2. In the small intestines, *Trichostrongylus vitrinus* was the main species. The other species were *Nematodirus fillicolis*, *Nematodirus battus* and *Cooperia curticei*. Only *T. vitrinus* was significantly different between the two groups ( $P < 0.0001$ )( Table 5.9).

Table 5.9: Results of the analysis of variance for the effect of anthelmintic treatment before moving on the log transformed counts of various species of worms recovered from the weaner lambs.

| Species               | Lsmeans  |         | P value |
|-----------------------|----------|---------|---------|
|                       | DM 1 + 2 | M 1 + 2 |         |
| <i>O. circumcinta</i> | 6.1911   | 6.5346  | 0.5418  |
| <i>O. trifurcata</i>  | 3.9942   | 3.2111  | 0.2235  |
| <i>H. contortus</i>   | 2.4573*  | 2.4952  | 0.9332  |
| <i>T. axei</i>        | 2.0397   | 3.4080  | 0.0144  |
| <i>T. vitrinus</i>    | 3.8249   | 6.2943  | 0.0001  |
| <i>N. fillicolis</i>  | 2.9170   | 3.7983  | 0.2847  |
| <i>N. battus</i>      | 2.7947   | 2.7355  | 0.8636  |
| <i>C. curticei</i>    | 2.8339   | 2.9562  | 0.8089  |

\* Only in one of the subgroups

A few *Trichuris ovis* and *Oesophagostomum spp* were recovered in the large intestines of M 1 +2 lambs.

### 5.4. Discussion:

The present study has shown the benefits of the dose and move and move only strategies in controlling gastrointestinal tract nematodes in sheep on pasture. It has shown almost similar production parameters in both systems. It has shown that weaning lambs at the beginning of July and moving them alone from an infected pasture to a clean pasture will prevent parasitic gastroenteritis and achieve a good production whether the move is accompanied by anthelmintic treatment or not. The anthelmintic treatment has an effect of removing the nematodes in the body of the lambs and lowering the pasture infectivity on the clean pasture and thereby reducing the worm burden of the lambs grazing such pastures. There was a period of approximately 4 - 6

weeks when the lambs were ingesting none or few larvae with herbage while the resident worm numbers were decreasing due to natural mortality.

The study has also shown that contamination of clean pastures may occur even when the animals are treated before moving to the clean pasture. The source of contamination may be eggs which are shed in faeces on the first day the animals are on the clean pasture immediately after treatment or resistant worms which are not eliminated by the anthelmintic. Some animals may harbor immature stages which are not affected by the anthelmintic; these mature and become a source of contamination in the clean pasture. Eggs of some nematodes such as *Nematodirus* can survive for long periods in pasture and hatch when the conditions are conducive. *Nematodirus* spp were identified in the herbage samples of the clean pasture immediately the lambs were introduced. The infective larvae of sheep parasites were found to survive for 6 months or more in Australia (Southcott *et al.* 1976). The free living stages of all species studied by Helle, (1981) in Norway were found to survive over the winter. Stromberg *et al.*, (1991) reported that larvae survive on pasture throughout winter for periods of 6 - 12 months. In a study in a winter rainfall region in Australia, Basier and Dunsmore, (1993), showed that maximum recoveries of larvae of *H. contortus* from eggs deposited on pasture plots at intervals over a 3 year period occurred in late autumn and late spring when adequate moisture coincided with warm temperatures. Larval development was low and sporadic over the hot and dry summer period, and depressed during winter, although most egg depositions in winter yielded L3 at some time. In the present study, some of the infective larvae may have survived for the one year that the pasture was rested, through winter and infected the lambs.

Treatment reduced the numbers of *Ostertagia* / *Trichostrongylus* faecal egg contamination in the clean pastures. The numbers of *Nematodirus* were also reduced but their role was diminished as the lambs acquire resistance to these especially *Nematodirus battus*. The total

worm burdens were reduced by treatment before moving. The worm burdens acquired in the clean paddocks were half those of the groups that were only moved both in the abomasum and small intestines. The higher number of immature stages in the abomasum of M1 + 2 lambs may indicate inhibition but also increased uptake of larvae. This may make a second move to clean pasture before housing necessary especially in this group as these immature stages may mature to adults and cause parasitic gastroenteritis.

Barger, (1999), reported that dosing of the young animals and then moving to clean pasture is a common practice in commercial small ruminant production. The clean pasture is usually provided more for nutritional than parasitological reasons. This may lead to accumulation of nematode infections in the clean pastures if the animals are kept for prolonged periods as long as there is enough pasture. In cattle, the concept of dosing first year grazing heifers in mid- July and moving them to clean pasture has been proved to be efficacious in the northern temperate conditions ( Eysker *et al.*, 1998a). A similar beneficial effect has been seen when cattle have been moved to clean pasture without any anthelmintic treatment (Foldager *et al.*, 1981; Nansen *et al.*, 1988b). However, repeated moves are necessary and the animals should not stay in one paddock for more than one month from mid summer when the pasture infectivity is highest. The last move should be less than one month before housing ( Eysker *et al.*, 1998b). This simplified the system developed by Oostendrop and Harmsen (1968) ,which required moves every 2 - 3 weeks throughout the grazing season, to only 3 monthly moves from July when the calves are housed in October. The limited number of moves makes it applicable to dairy farmers. Stromberg *et al.*, (1991) observed that there is a general increase in the number of larvae on pasture throughout the grazing season as observed by total worm recoveries from tracer calves on pasture for 1 month periods. The only decrease observed during the grazing season was for the month of July when cattle were moved to a clean pasture. Stromberg and Averbeck, (1999), suggested that an

anthelmintic treatment of cattle before moving to clean pasture will reduce larvae contamination of the clean pasture. When used with active pasture management, the parasite levels can be kept at low level.

In the present study the lambs were only moved once at the beginning of July and were set stocked up to the end of the grazing season. No parasitic gastroenteritis was observed and their weight gains were comparable to those treated before being moved. Coles and Roush, (1992), recommended a dose and move strategy for ewes with lambs. The ewes were dosed at weaning. Often the weaners had to be moved to a clean pasture a second time with or without anthelmintic treatment. In the present study, only lambs were moved to the clean pasture. This greatly reduced the pasture contamination from ewes hence there was no need of a second move. This may show some benefit of only moving the weaned lambs. In ewes with lambs, a single move at the beginning of July without anthelmintic treatment limited *Ostertagia* / *Trichostrongylus* infections on pasture but had no effect on *Nematodirus* spp in an earlier study in Denmark, (Boa and Thamsborg - In press).

A non- chemotherapeutic approach consisting of repeated move for goats has been developed in a wet tropical environment (Barger *et al.*, 1994). A grazing system was developed with 10 paddocks each grazed in a sequence of 3.5 days then spelled for 31.5 days. Though anthelmintic treatments were given to pregnant and lactating does, there were indications that this was not necessary in the repeated moved goats (Barger. 1997). In this environment, larvae (predominantly *Haemonchus*) survived for less than 2 weeks and peak larval concentrations occurred one week post contamination. In temperate conditions this may not be successful especially with *Nematodirus* spp whose eggs survive for long periods. An interesting finding in the present study was the presence of *Haemonchus contortus*, which is not commonly reported in sheep in Denmark, in the move group. In this particular year, it was reported to cause

mortalities in some organic farms during the same grazing season (Thamsborg - personal communication). The warm and wet summer may have facilitated the hatching of *Haemonchus* eggs (possibly present in the clean pastures) in large numbers and their survival.

This study has shown that it is possible to achieve sufficient nematode control and good production levels in weaned lambs without the use of anthelmintics when the lambs are weaned at the beginning of July and moved to a clean pasture for up to 3 months. This is only possible in the temperate countries where the animals are removed from pasture and either housed or slaughtered at the end of the grazing season. As indicated by the number of immature stages in the abomasum of the lambs that were only moved and the increased pasture infectivity at the end of the study, the possibility of an outbreak of parasitic gastroenteritis could not be ruled out if the lambs were to stay any longer in the paddocks. Another move to a clean pasture or an anthelmintic treatment would have been necessary. Such a system will not be applicable in the humid tropics where the animals are on pasture throughout the year. As the temperatures are always conducive, larvae hatching occurs through out the year. In the humid tropics, provision of safe pasture is only possible through rapid rotational grazing if there is enough available land for paddocking or tethering. This system is readily adaptable to more traditional husbandry methods such as tethering and shepherding which are commonly used by the small holders in developing countries. This may be the only viable alternative grazing method with potential for integrated nematode parasite control in the humid tropics. Other methods may include the use of medicated molasses blocks as shown in Malaysia (Chandrawathani *et al.*, 1997). Exploring the potential of biological control using nematophagous fungi is also another alternative. This has been explored in Malaysia with promising results (Chandrawathani, 1997; Chandrawathani *et al.*, 1998). This was the subject of a field study in Denmark reported in chapter 6.

Dose and move and move only systems will not be possible in the humid tropics where

*Haemonchus contortus* is the main nematode species in small ruminants. This species has a short generation interval in the tropics of 4 - 7 days and is a prolific egg layer and a voracious blood sucker. Pasture contamination builds up fast and mortalities may occur before the animals are moved. *H. contortus* is also very pathogenic and a blood sucker. This greatly affect production and reports have shown that it is the most economically important species in the tropics.

## Chapter 6:

### 6.0. Studies on the preventive effect of the fungus *Duddingtonia flagrans* on trichostrongyle infections of weaner lambs on pasture in Denmark

#### 6.1. Introduction

Worldwide reports on increasing levels of anthelmintic resistance in sheep covering all the major anthelmintic classes, as evidenced lately in South-America (Waller *et al.*, 1996), call for intensified research into development of alternative strategies for worm control, including the use of nematode-destroying micro fungi.

Nematophagous fungi have been isolated from faecal pellets of grazing sheep (Juniper, 1957; Jancke and Schmecktal, 1990; Larsen *et al.*, 1994). With regard to the potential of such fungi as biological control agents against ovine parasitic nematodes, efforts so far have concentrated on laboratory work. *In vitro* tests on agar media or in faecal cultures have shown that these fungi are able to significantly reduce the number of free-living larval stages (Nansen *et al.*, 1988a; Waller and Faedo, 1993; Mendoza-de Gives and Vazquez-Prats, 1994; Mendoza de-Gives, *et al.*, 1992, 1994). Furthermore, it has been demonstrated that several species of fungi have the ability to survive passage through the gastrointestinal tract of sheep and subsequently reduce the number of parasitic nematode larvae in faecal cultures (Grüner *et al.*, 1985; Peloille, 1991; Larsen *et al.*, 1994; Waller and Faedo, 1993; Waller *et al.*, 1994). By incorporating fungal treatment as a parameter in their model Barnes and coworkers (1995) estimated that a nematode-destroying fungus, which potentially could reduce the number of infective larvae on pasture by 90% over a 3 months period would be able to be as efficient as a vaccine or anthelmintic treatment with regard to survival of lambs exposed to gastrointestinal nematodes. Whether the effect demonstrated in the laboratory and in *in vivo* passage studies can be expected under field conditions still needs testing although there has been some limited reports indicating or

postulating such an effect (Roubaud and Deschiens, 1941; Hashmi and Connan, 1989). However, at present no study has been reported on the effect of fungal treatment of sheep on naturally infected pasture during an entire grazing season.

The net-trapping predacious fungus *Duddingtonia flagrans* has demonstrated good potential as a biological control agent against free-living stages of bovine parasitic nematodes in several field studies (Grønvold *et al.*, 1993; Larsen *et al.*, 1995; Nansen *et al.*, 1995) and was considered a likely candidate in sheep as well. The present experiment investigated the effect of *D. flagrans* on acquisition of trichostrongyle infections by weaned lambs grazing a naturally infected pasture in Denmark

## 6.2. Materials and methods

### 6.2.1 Experimental Design

Thirty-two parasite naive Dorset-crossbred lambs, 3-4 months old and recently weaned, were allocated to four groups of eight according to body weight, sex and age. On 2 May 1995, all lambs were turned out on a 1.2 ha permanent pasture (15 km west of Copenhagen, Zealand), previously grazed for several years by sheep and known to be infected with *Ostertagia*, *Trichostrongylus* and *Nematodirus* spp. During the initial period, lambs were accustomed to a daily feeding with barley in a sectioned trough. After three weeks, the pasture was divided into four equal sized paddocks (0.3 ha) and the above groups were randomly allocated to the paddocks. From 23 May to 5 September, two groups ( $F_1$  and  $F_2$ ) received *D. flagrans* ( $10^6$  chlamydospores per kg body weight per lamb per day) mixed in 20 g of wheat bran and 100 g of barley per lamb. The lambs of the groups  $C_1$  and  $C_2$  received a similar amount of wheat bran and barley without the fungi. The lambs were observed daily while feeding to ensure that all lambs consumed the offered material. Fungi-feeding was discontinued between 27 June and 11 July following an anthelmintic treatment of all groups (see below). All groups remained set-stocked until slaughter

for worm counts on 10 October. The pasture was cut in late June at 8-10 cm to remove stems and seed heads which were left in the paddock.

Sixteen Texel-crossbred lambs (6-7 months old) were kept free of nematodes on pasture by monthly anthelmintic treatments (fenbendazole, 7.5 mg/kg) from May until introduced as tracer lambs. On 3 October, the lambs were allocated to four groups; TC<sub>1</sub>, TC<sub>2</sub>, TF<sub>1</sub> and TF<sub>2</sub>, and grazed in the paddocks of groups C<sub>1</sub>, C<sub>2</sub>, F<sub>1</sub> and F<sub>2</sub>, respectively. The tracers grazed until 23 October and were slaughtered for worm counts after 3 weeks of housing.

#### 6.2.2. Fungal material

*A. D. flagrans* isolate, previously selected by Larsen and coworkers (1991), was cultivated on barley grains as described by Grønvold *et al.* (1993). The spores were washed off, added to a small volume of wheat bran and kept refrigerated. Before feeding, the daily dose per lamb was mixed with 100 g of whole barley. The dose was adjusted according to increases in average body weight of the lambs during the study. In August, the ration in both fungi fed and untreated groups was subjected to standard chemical analysis for dry matter, crude protein and net energy calculated according to the Scandinavian feed unit system (1 F.U. equivalent to the net energy content of 1 kg barley).

#### 6.2.3. Meteorological data

Total monthly rainfall and temperature data was obtained from a meteorological unit within 1 km of the experimental plot.

#### 6.2.4. Sampling and analysis

Rectal faecal samples and blood samples by jugular venipunctures for serum were obtained every fortnight. At the same time, all lambs were weighed and two grass samples (300 - 800 g) per paddock were collected by hand following a W-shaped route across the paddock. The lambs were observed daily with a more detailed examination on sampling days. Tracer lambs

were sampled when introduced to the paddocks and during the housing period.

Faecal nematode egg counts were determined using a modified McMaster procedure (Henriksen and Aagaard, 1976). *Nematodirus* eggs were identified to the level of species based on morphology (Barth and Visser, 1991) and eggs other than *Nematodirus* were categorized as other strongyles. Group faecal cultures in triplicate were made by modifying the method of Henriksen and Korsholm (1983), using 1 g of faeces from each animal in the group (= total of 8 g) mixed with 8 ml water and 4 g vermiculite. The cultures were incubated for two weeks in a climatic chamber at 25°C and 95% RH. The resulting larvae (L3) were enumerated and identified to genus or species level based on size and morphology (Anon., 1986). The yield of L3 in faecal cultures (*Ostertagia*/ *Trichostrongylus* or *Nematodirus* spp.), expressed as a percentage of faecal egg counts (other strongyles or *Nematodirus* spp.), was used to evaluate the *in vitro* nematode destroying effect of *D. flagrans*. Serum was analyzed for albumin using the bromocresolgreen method (Nisbeth *et al.*, 1973). Serum of tracer lambs were further analyzed for pepsinogen using the procedure described by Ross *et al.* (1967). The herbage samples were soaked overnight in water, washed in a concrete mixer and sieved through a cloth of 31 µm. The debris content was embedded in agar on a cloth, submerged in water overnight at room temperature and infective larvae were isolated and enumerated according to method of Mwegoha and Jørgensen (1977). This method has been reported to show a higher recovery of L3 of *Ostertagia* / *Trichostrongylus* and *Nematodirus* as compared to the Baermann technique (Boa *et al.*, 1998). The samples processed by this method are clean and hence the larvae are easily detected (Stephan, 1993). The method was modified by omission of ox bile in the agar-gel. The recovered larvae were stained with iodine and counted under a microscope. The genera of the parasitic larvae were determined on morphometric details. Double determinations were averaged.

Total worm counts were performed on all flock and tracer lambs. The abomasa and small

intestines were ligated and separated immediately after slaughter. The organs were opened into individual containers and rinsed with warm saline (approx. 38°C). Two 10% sub-samples were drawn and processed for nematode recovery and enumeration by the agar gel method (Van Wyk and Gerber, 1978; Slotved *et al.*, 1996) which was previously found comparable to the method of sieving traditionally used in the laboratory (Thamsborg *et al.*, 1996) but was more labour-saving. Digestion of abomasal mucosa was performed according to Anon. (1986). Nematodes recovered from the sub-samples of all tracer lambs and two flock animals per group were identified to level of species based on morphology of adults (Anon., 1986; Barth and Visser, 1991).

#### 6.2.5. Statistical analysis

Faecal egg counts (log-transformed), body weight, serum albumin and worm counts (log-transformed) were analysed by repeated measures or non repeated measures analysis of variance (ANOVA) using the following model (nested design) in the GLM procedure of SAS (SAS, 1990):  $Y = TR + REP(TR)$  Y is the dependent variable, TR is the effect of treatment (+/- fungi feeding) and REP(TR) represents the effect of the two replicates (REP) nested within treatments. For the ease of overview, the results of replicate groups ( $F_1$  and  $F_2$ ;  $C_1$  and  $C_2$ , respectively) were pooled in figures and tables as they were not found statistically different by the above analysis of variance. Data from faecal cultures were analysed by a one-way ANOVA with the factor +/- fungi feeding.

### 6.3. RESULTS

#### 6.3.1. Rainfall and Temperature:

The monthly precipitation was 65, 49, 19, 30 and 88 mm for May-September compared to a long term average (1960-1990) of 44, 53, 67, 62 and 59 mm for the same months. The months of July and August were unusually dry and warm whereas heavy rainfall and low

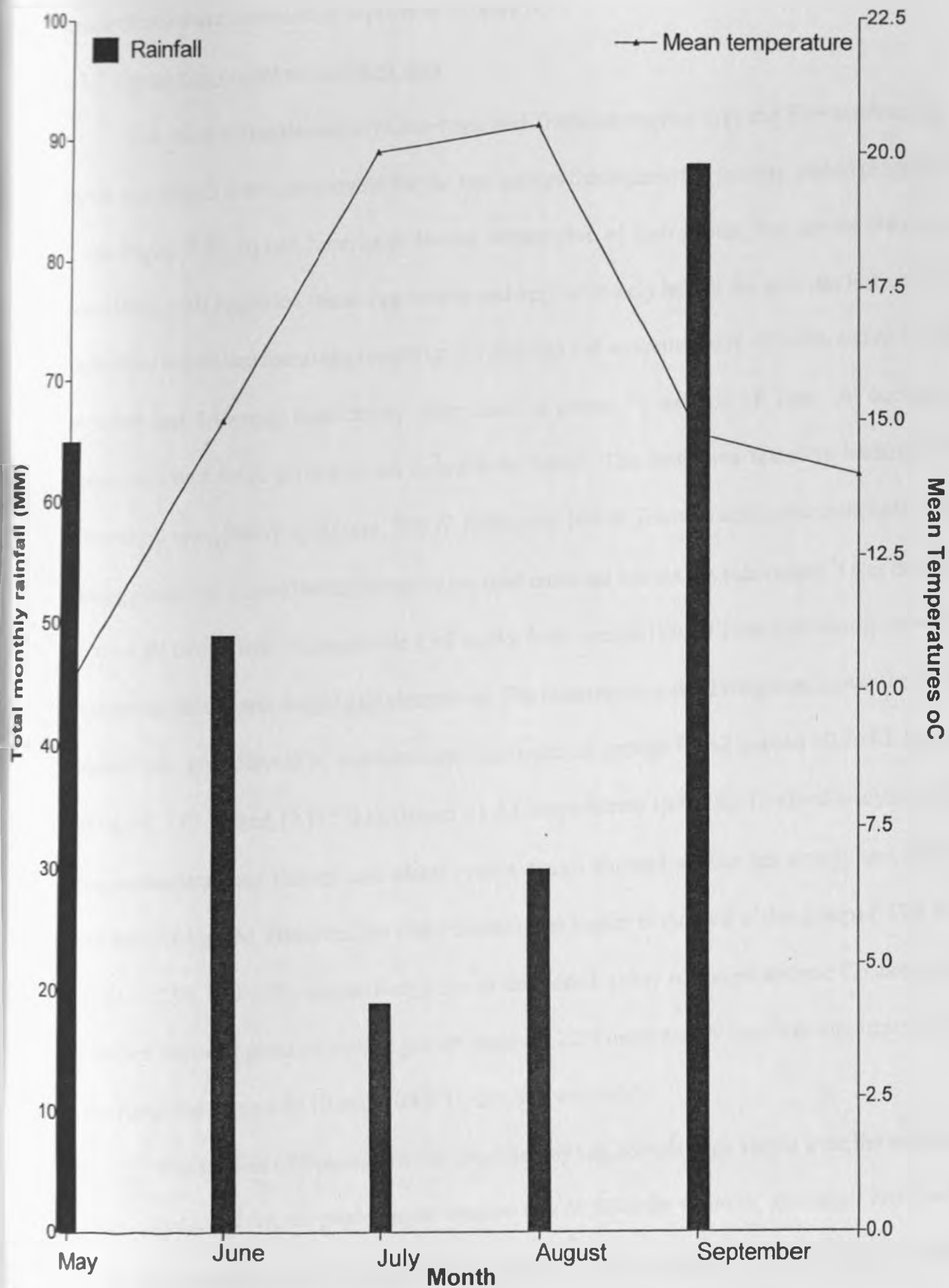


Figure 6.1: Monthly rainfall and mean temperatures obtained from a meteorological station within 1 km from the experimental paddocks for the year 1995

temperatures were recorded in September (Figure 6.1).

### 6.3.2 Faecal egg counts and clinical data

The other strongyle (mainly *Ostertagia* and *Trichostrongylus* spp) and *Nematodirus* spp. faecal egg counts were comparable for the two groups throughout the grazing period (Figure 6.2 and Figure 6.3). In late June, most lambs, irrespective of their group, had severe diarrhoea coinciding with increased faecal egg counts and approximately half of the animals lost weight. Individual lambs had faecal egg counts up to 7,500 epg and excretion of *N. filicollis* and *N. battus* at 1,800 and 140 epg, respectively. One lamb in group F1 died on 18 June. At necropsy, abomasitis and focal peritonitis on colon were found. The lamb was found to harbour 670 *Ostertagia* spp., 900 *N. spathiger*, 500 *N. battus* and 100 *N. filicollis* and it was concluded that strongylosis was a contributing factor to the fatal outcome but not the sole cause. It was decided to treat all lambs with fenbendazole (5.0 mg/kg body weight) on 29 June and shortly after the symptoms ceased and weight gains improved. The mean accumulated weight gain over the entire season was not affected by experimental treatments as groups F 1+2 gained  $10.7 \pm 7.1$  kg and groups C 1+2 gained  $13.1 \pm 5.0$  kg (mean  $\pm$  s.d.), respectively ( $p=0.28$ ). The feed analysis of the supplementary feed (barley and wheat bran  $\pm$  fungi) showed similar net energy and protein content per kg DM. However, the water content was higher in the feed of the groups F 1+2 than C 1+2 (27% and 11%, respectively) due to the added slurry of fungal spores. Consequently, besides intake of grass the control groups received 22% more energy from the supplements than the fungi-fed groups (0.10 and 0.08 F.U./day, respectively).

The species of *Nematodirus* as identified by egg morphology varied over the season. In May, June and July, the predominant species was *N. filicollis* while *N. spathiger* increased in July and dominated up to the end of the experiment. The levels of *N. battus* remained constant at a low level (Figure 6.4). The mean *Nematodirus* spp. faecal egg count had a course over the

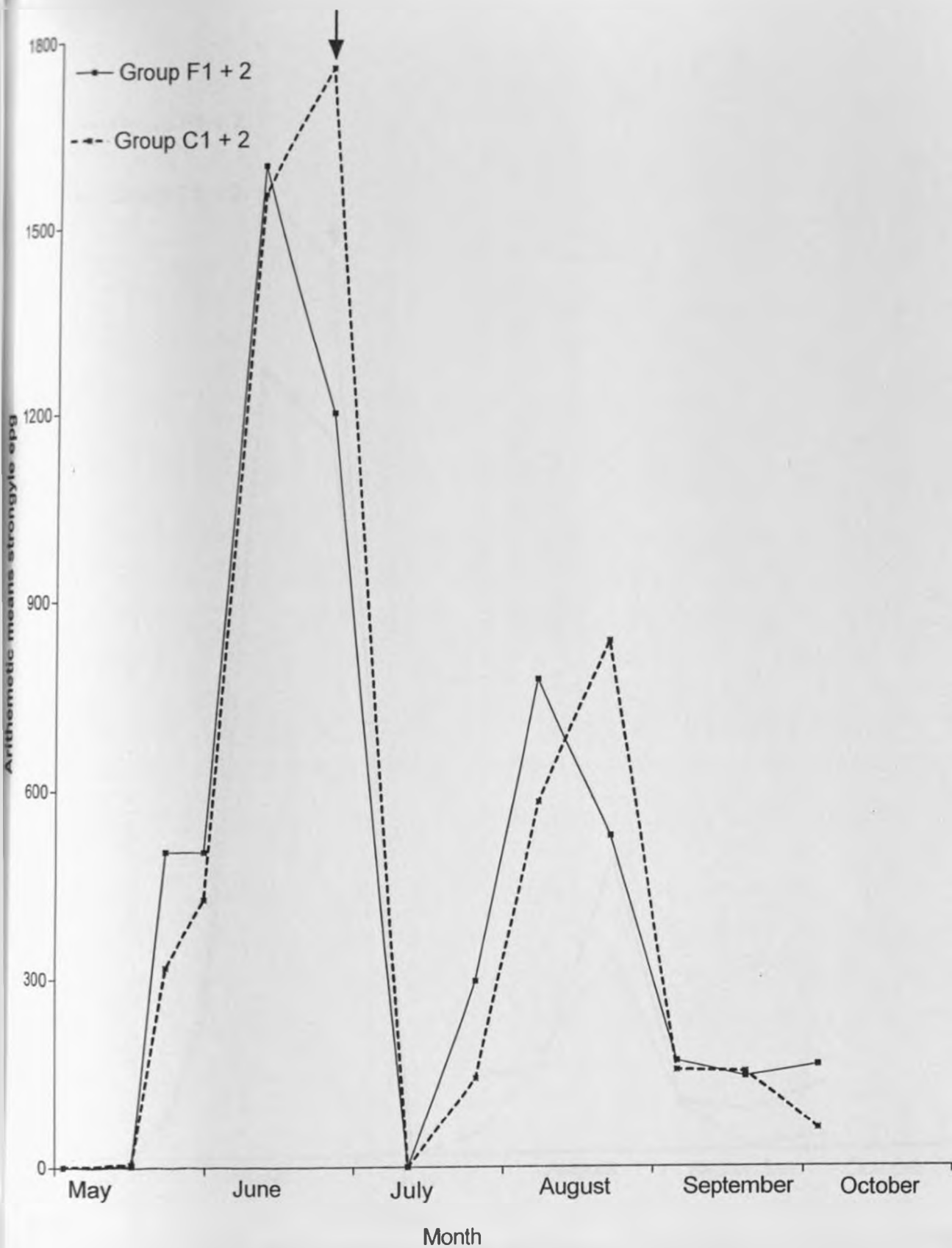


Figure 6.2: Arithmetic means Strongyle (mainly *Ostertagia* and *Trichostrongylus* species) faecal egg count of lambs dosed with fungi (groups F1 + 2) and untreated lambs (groups C1 + 2). Arrow indicate anthelmintic treatment.

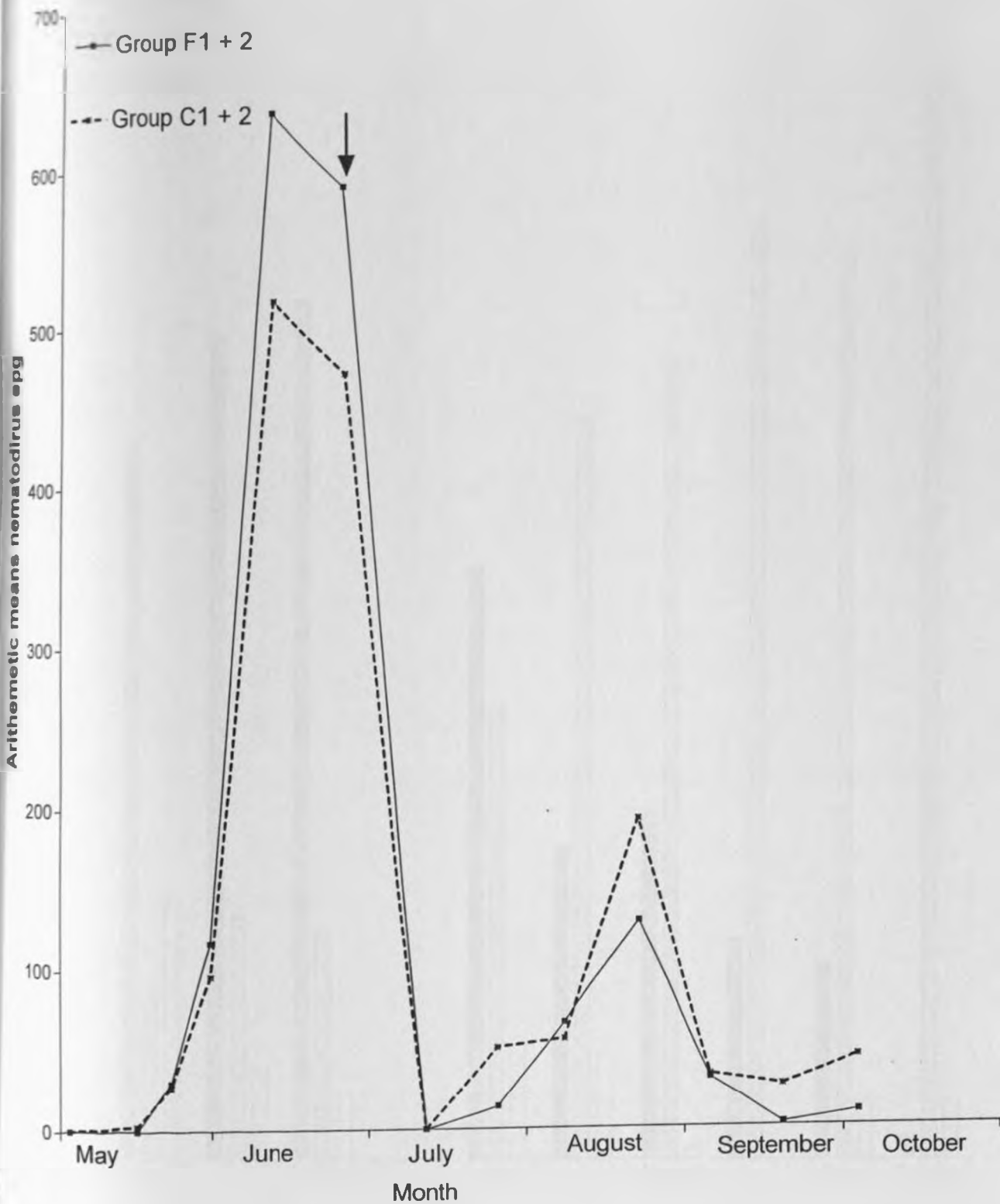


Figure 6.3: Arithmetic means *Nematodirus* spp faecal egg counts of lambs dosed with fungi (groups F1 + 2) and untreated lambs (groups C1 + 2). Arrow indicate anthelmintic treatment.



Figure 6.4: Percentage composition of various species of *Nematodirus* excreted by the lambs during the study period as identified by egg morphology.

season very similar to that of *Ostertagia* and *Trichostrongylus* (Figure 6.3).

### 6.3.3 Faecal larval cultures

The numbers of larvae of *Ostertagia/Trichostrongylus* spp. recovered from faecal cultures of groups F 1+2 in percentage of faecal egg counts of other strongyles were consistently lower than those of groups C 1+2 during the periods of fungi feeding (repeated measures ANOVA:  $p=0.02$ ) (Figure 6.5). The recovery of larvae, i.e. the proportion of eggs developing into the infective larval stage, was 1-20 % in the fungi-fed groups F 1+2 while it was between 26-76% in the untreated groups C 1+2 (Figure 6.5). When feeding with the fungus was terminated higher recovery rates were seen in F 1+2 than C 1+2 but for particularly *Nematodirus* spp. the egg counts were low in this period (range: 0-75 epg). The number of *Nematodirus* larvae (entirely *N. spathiger*) recovered in percentage of *Nematodirus* eggs increased over the study period in accordance with the egg counts (Figure 6.6). However, recovery rates did not seem to be affected by fungi-feeding. *Oesophagostomum* spp. constituted up to 20% of the infective larvae recovered in cultures on two occasions: in late June before anthelmintic treatment when the rate of development of *Oesophagostomum* was reduced in a degree similar to *Ostertagia/Trichostrongylus*, and in mid-September.

### 6.3.4 Pasture larval counts

From turnout until end of August, the number of *Ostertagia/Trichostrongylus* and *Nematodirus* spp. (*N. spathiger/N. filicollis*) infective larvae on herbage did not exceed 540 L3/kg dry herbage on paddocks grazed by the groups F 1+2 (Figure 6.7 and Figure 6.8). Following heavy rainfall in late August and in September (Figure 6.1) counts rose to 930 and 7,200 L3/kg for *Ostertagia/Trichostrongylus* and *Nematodirus* spp., respectively. In the paddocks grazed by groups C 1+2, a similar pattern was observed although counts were consistently higher. In September, maximum values of 4,400 L3/kg for *Ostertagia/Trichostrongylus* and 11,600 L3/kg

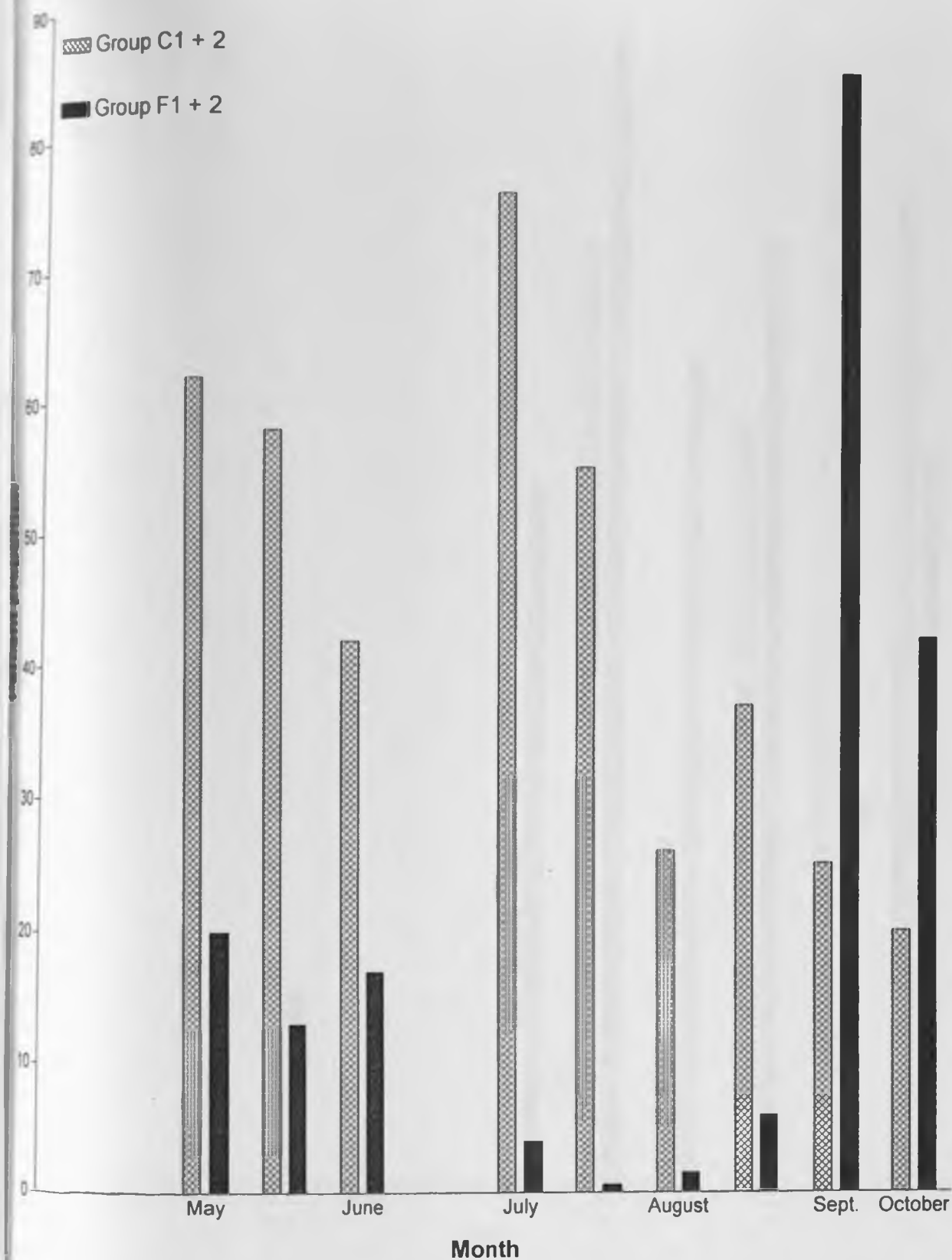
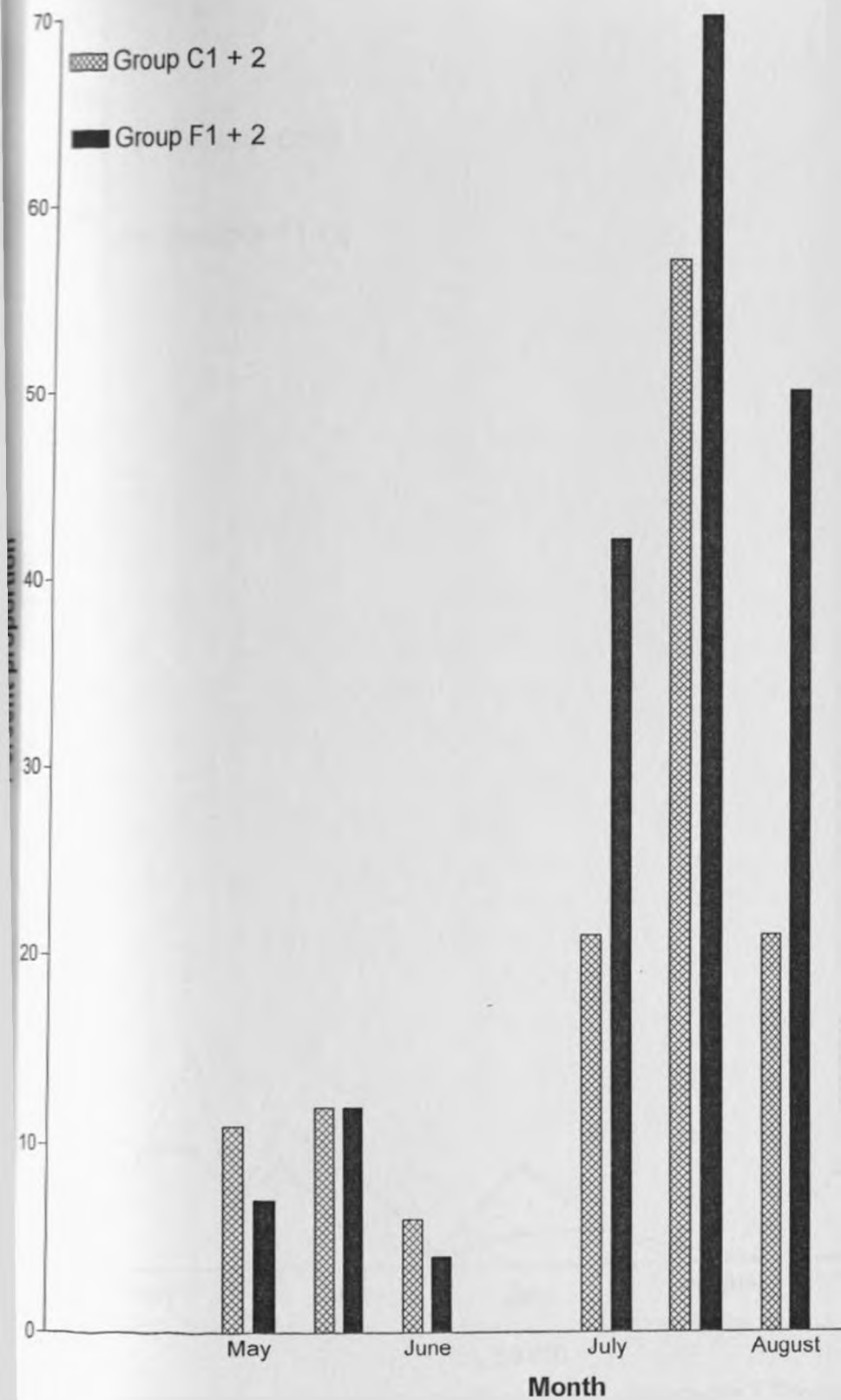


Figure 6.5: The proportion of eggs of *Ostertagia* and *Trichostrongylus* developing to infective stages in group faecal cultures of lambs dosed with fungi (groups F1 +2) and untreated lambs (C1 + 2).



6.6: The proportion of eggs of *Nematodirus* (mainly *N. spathiger*) developed to infective stages in group faecal cultures of lambs dosed with fungi (groups F1 + 2) and control lambs (C1 + 2). <sup>1</sup>*Nematodirus* spp eggs were recovered in 1 su

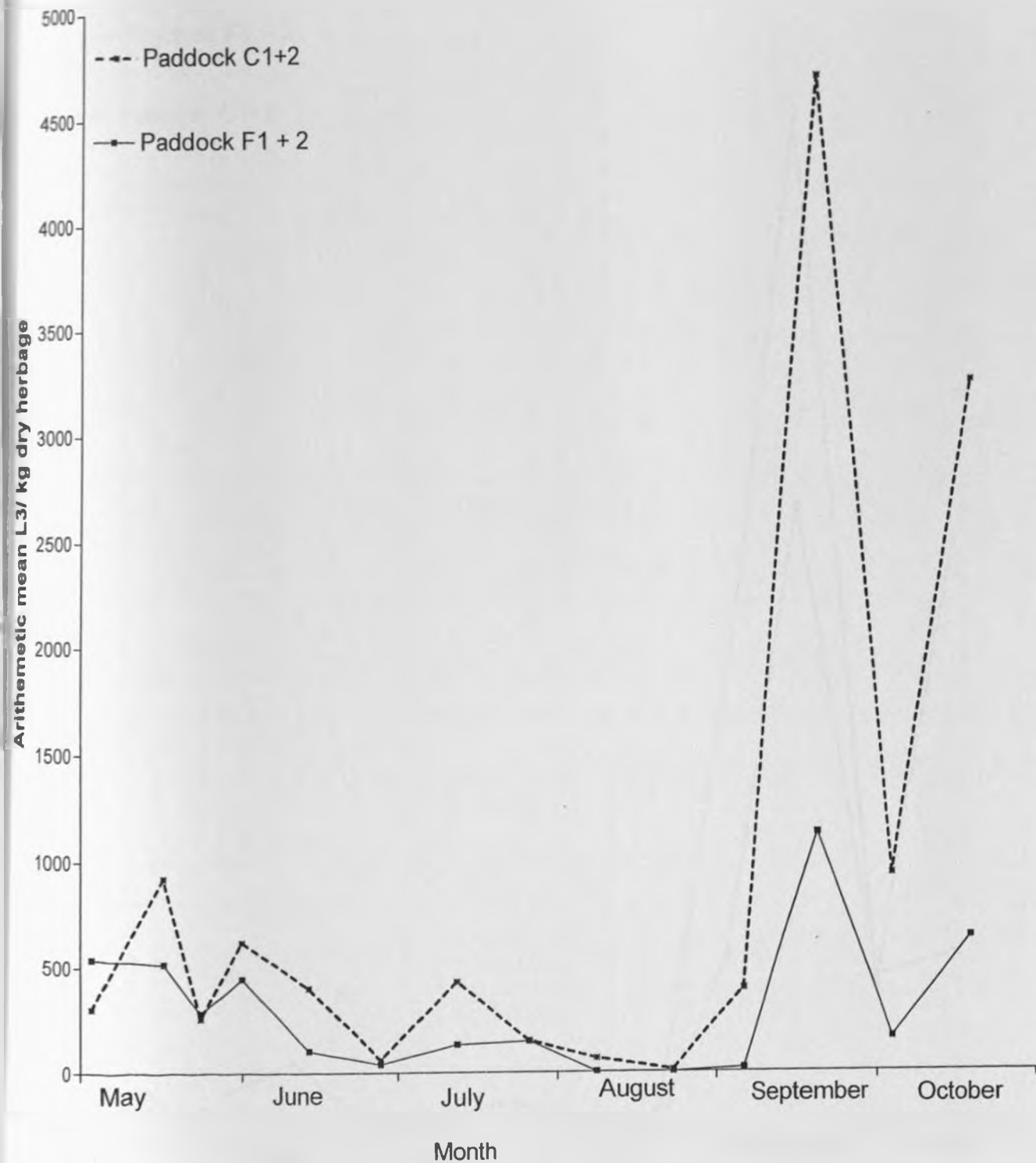


Figure 6.7: Arithmetic means pasture larval counts of *Ostertagia* / *Trichostrongylus* spp in paddocks grazed by lambs dosed with fungi (Paddocks F 1 + 2) and untreated lambs (paddocks C 1 + 2)

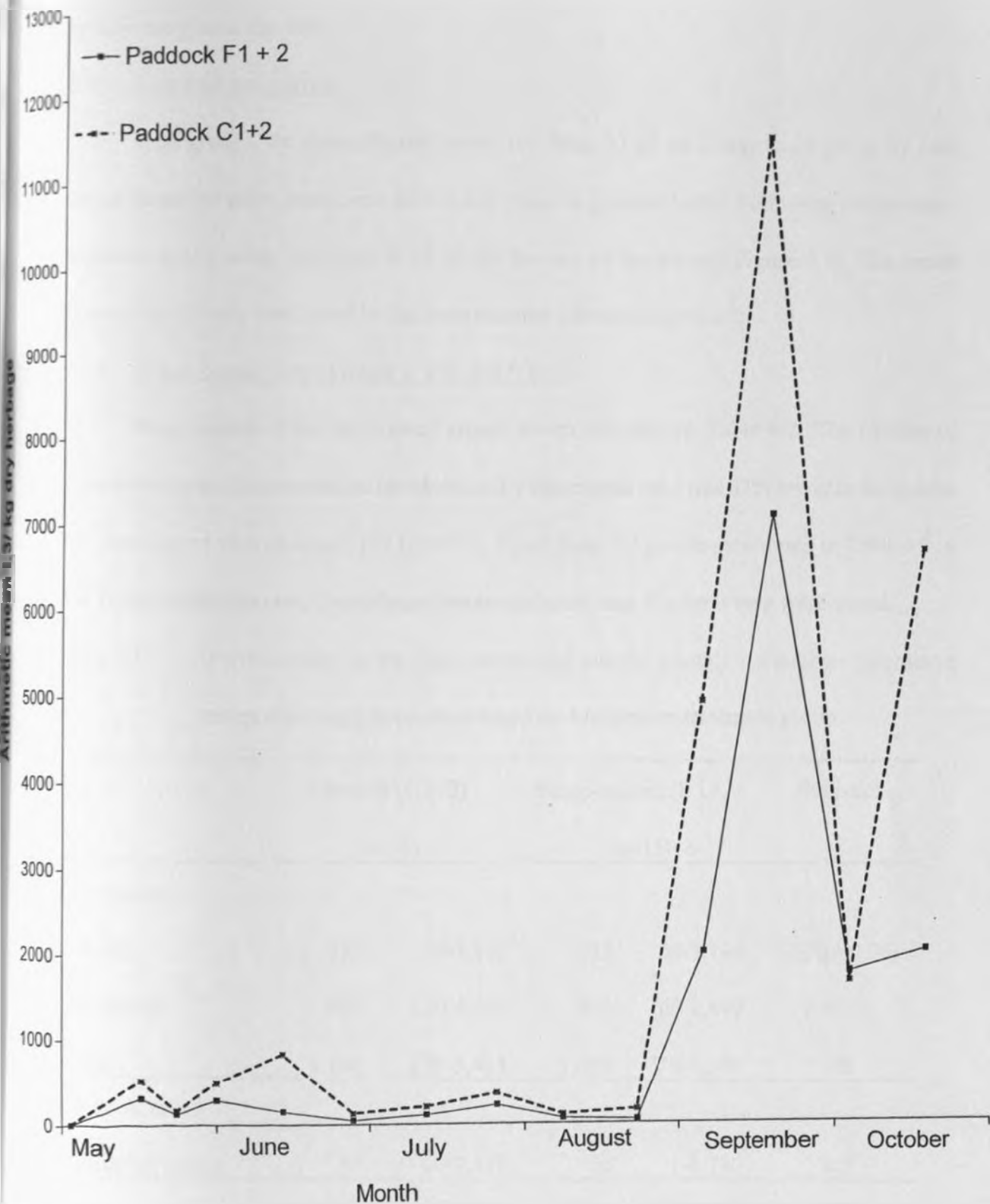


Figure 6.8: Arithmetic means pasture larval counts of *Nematodirus* spp in paddocks grazed by lambs dosed with fungi (paddocks F 1 + 2) and untreated lambs (paddocks C 1 + 2).

for *Nematodirus* spp. were observed (Figures 6.7 and 6.8). *N. battus* was not identified in the herbage samples at any time.

6.3.5. Serum albumin levels

In all groups, the mean albumin levels fell from 33 g/l on 2 May to 26 g/l on 27 June during the period when lambs were affected by parasitic gastroenteritis. Following anthelmintic treatment, mean levels stabilized at 28 g/l for the rest of the season (Figure 6.9). The serum albumin levels were unaffected by the experimental treatments ( $p=0.27$ ).

6.3.6. Worm counts of the groups C 1+2 and F 1+2

Worm counts of the set stocked groups are summarised in Table 6.1. The number of immature stages in the abomasum (predominantly *Ostertagia* spp.) was 62% lower in the groups F 1+2 compared with groups C 1+2 ( $p<0.05$ ). Apart from the genera mentioned in Table 6.1, a few *Trichostrongylus axei*, *Oesophagostomum radiatum* and *Trichuris ovis* were found.

Table 6.1. Worm burdens for the fungi-treated and control animals at slaughter (geometric means and range) Speciation based on 4 lambs per treatment group.

|                         | Controls (C1+2) |            | Fungi-treated (F 1+2) |           | Statistics               |
|-------------------------|-----------------|------------|-----------------------|-----------|--------------------------|
|                         | (n=16)          |            | (n=15)                |           |                          |
| Abomasum                |                 |            |                       |           |                          |
| Adults <sup>1</sup>     | 221             | 10-1,815   | 613                   | 50-3,140  | NS <sup>2</sup> (p=0.06) |
| Immatures               | 809             | 120-4,760  | 301                   | 60-1,440  | p<0.05                   |
| Total                   | 1,140           | 130-5,435  | 1,065                 | 310-3,340 | NS                       |
| Small intestine         |                 |            |                       |           |                          |
| <i>Nematodirus</i> spp. | 57              | 0-19,435   | 56                    | 1-5,780   | NS                       |
| Total                   | 1,599           | 133-24,010 | 1,321                 | 312-7,145 | NS                       |

<sup>1</sup> comprising 96-97% *Ostertagia* spp.      <sup>2</sup> NS = Not significant

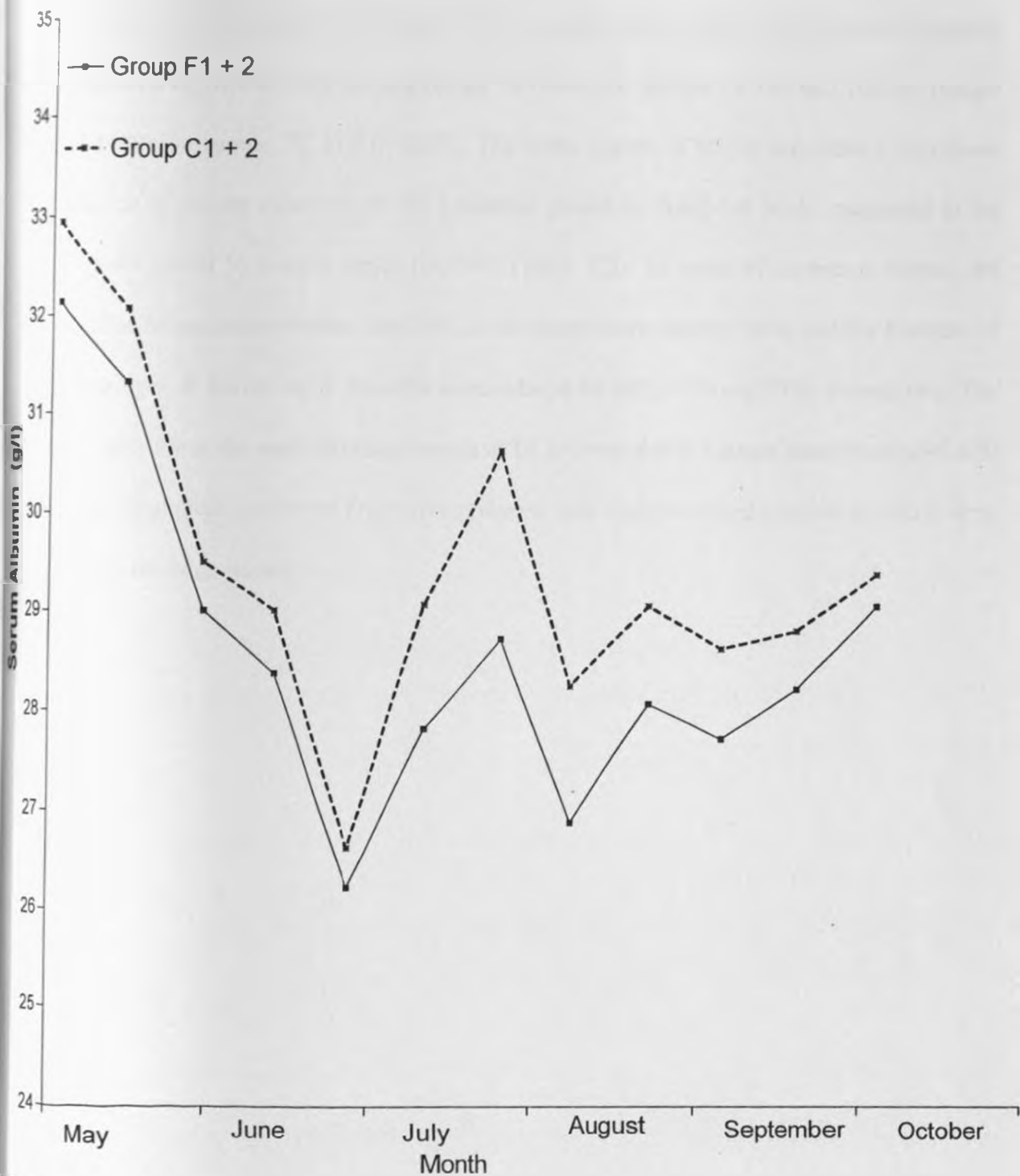


Figure 6.9: Arithmetic means serum albumin levels of lambs dosed with fungi (groups F 1 + 2) and untreated lambs (groups C 1 + 2).

#### 6.3.7. Tracer lambs

Following 3 weeks on pasture, mean serum pepsinogen levels ( $\pm$  s.d.) were  $0.4 \pm 0.2$  i.u./l and  $0.2 \pm 0.1$  i.u./l in groups TC 1+2 and TF 1+2, respectively ( $p < 0.05$ ). At the time of slaughter, mean faecal egg counts were 283 epg (range: 40-1040) for groups TF 1+2 and 708 epg (range: 140-1400) for groups TC 1+2 ( $p < 0.05$ ). The worm counts of tracers indicated a significant reduction in pasture infectivity in the paddocks grazed by fungi-fed lambs compared to the paddocks grazed by control lambs ( $p < 0.05$ ) (Table 6.2). In terms of geometric means, the reduction in total worm burden was 86%, in abomasal worm burden 68%, and the numbers of *N. spathiger*, *N. battus* and *N. filicollis* were reduced by 98%, 97% and 95%, respectively. The large variation in the small intestinal counts of TF 1+2 was due to a single lamb housing 47,630 worms. Negligible numbers of *Trichostrongylus vitrinus* were recovered whereas no worms were found in the large intestine.

Table 6.2. Worm burdens of tracer lambs grazing paddocks of previously untreated (group TC 1+2) and fungi treated lambs (group TF 1+2)(geometric means and range). Differences between group means were tested by analysis of variance on log-transformed counts.

|                        | Group TC 1+2 |              | Group TF 1+2 |            | Statistics      |
|------------------------|--------------|--------------|--------------|------------|-----------------|
|                        | (n=4+4)      |              | (n=4+4)      |            |                 |
| Abomasum               |              |              |              |            |                 |
| <i>O. circumcincta</i> | 577          | 117-2,240    | 246          | 40-1,250   | NS <sup>1</sup> |
| <i>O. trifurcata</i>   | 89           | 23-360       | 10           | 0-79       | p<0.05          |
| <i>T. axei</i>         | 4            | 0-360        | 1            | 0-39       | NS              |
| Immatures              | 655          | 100-3,050    | 111          | 17-1,900   | p<0.01          |
| Total                  | 1,449        | 390-5,560    | 458          | 140-3,240  | p<0.05          |
| Small intestine        |              |              |              |            |                 |
| <i>N. spathiger</i>    | 1,279        | 0-10,260     | 20           | 0-26,197   | p<0.05          |
| <i>N. battus</i>       | 1,538        | 660-4,750    | 51           | 0-2,657    | p<0.01          |
| <i>N. filicollis</i>   | 2,384        | 0-15,358     | 124          | 0-20,957   | NS              |
| Total                  | 9,966        | 660-23,270   | 815          | 20-47,630  | p<0.05          |
| Total                  | 12,246       | 1,220-25,690 | 1,693        | 287-50,870 | p<0.05          |

<sup>1</sup>NS = Not significant

### 6.4 DISCUSSION

The present study showed that daily dosing of grazing lambs with the fungus *D. flagrans* for 3½ months affected the development and spreading of free living stages of ovine nematodes on pasture thereby reducing pasture infectivity. The lower pasture infectivity was evident in the late season by lower pasture larval counts and was confirmed by significantly lower worm burdens of tracer lambs in paddocks earlier grazed by fungi dosed lambs compared to control

paddocks. To our knowledge this is the first report demonstrating an effect of bio-control on ovine nematodes in a natural epidemiological context. The results are in accordance with the findings in grazing calves being fed with *D. flagrans* for two and three months, respectively (Grønvold *et al.*, 1993; Larsen *et al.*, 1995; Nansen *et al.*, 1995) but there is also close agreement with the recent field studies in monogastric animals (Larsen *et al.*, 1996; Nansen *et al.*, 1996).

This study demonstrated that the nematode-destroying ability of the fungi against *Ostertagia* spp., *Trichostrongylus* spp. and *Haemonchus contortus* found in faecal cultures by several workers (Grüner *et al.*, 1985; Peloille, 1991; Larsen *et al.*, 1994; Waller and Faedo, 1993; Waller *et al.*, 1994) may be reflected in the reduced pasture contamination under field grazing conditions. However, our study also raises some questions and potential problems.

It was evident that the ovine nematode species were affected differently by the fungal treatment. The *in vitro* faecal assays, conducted along with the field experiment, showed a significant reduction in the numbers of infective larvae of predominantly *Ostertagia* spp. whereas no reduction could be observed in case of *N. spathiger* larvae after the two week incubation period. In the genus *Nematodirus* the development to infective stage takes place within the egg, and as spontaneous hatching of *N. spathiger* normally takes place after 10 days incubation at room temperature (Anon., 1986), this may fail to coincide with the fungal trapping activity. Since *N. battus* and *N. filicollis* require special conditions for hatching, including up to 4-6 weeks incubation periods (Thomas, 1959; Anon. 1986), it was not expected that the faecal culture assay used here would give any indications as to the potential effect of the fungus against these two parasites. The high recovery of other strongyles from faecal cultures observed in the fungi-treated animals after termination of the fungi feeding was most likely due to a better hatchability of the eggs in relation to the lower level of immunity in these lambs compared to the control lambs. However, this observation has not been made in previous studies.

The reduction effect against *Ostertagia*/*Trichostrongylus* spp. larvae observed *in vitro* was confirmed in the field by reduced herbage larval counts (statistically significant) and later by the worm counts of tracer lambs in which almost complete predominance of *Ostertagia* spp. was found. In contrast to the findings of the *in vitro* studies, a clear effect of the fungus against *N. spathiger* was demonstrated in the field study. The numbers of *Nematodirus* spp. on pasture were reduced, and these reductions were confirmed by significantly lower worm counts of *N. spathiger* in the tracers on pastures of fungus-fed lambs compared with tracers on pastures previously grazed by the untreated lambs.

In the present study, the significantly lowered number of *N. battus* in the tracers from fungi-paddocks was not anticipated, due to the original conception of the factors controlling the development and hatching of larvae in this species in northern Europe. Thus, the environmental conditions conducive to hatching of the *N. battus* are temperatures above 10°C following a period of prolonged cold weather, conditions that typically appear in early spring in the temperate climate after deposition of eggs in the previous year (Thomas, 1959). However, one explanation for these observations could be that we, as has already been documented by other researchers in Europe and USA (Borgsteede, 1983; Hollands, 1984; Rickard *et al.*, 1987; 1989), are dealing with a strain or type of *N. battus* where the larvae hatch during the same season that eggs are deposited. If so, these larvae were also exposed to the trapping fungus before transmission to the pasture. As hatching would not be anticipated within 4 weeks on pasture (Thomas, 1959), the reduction in *N. battus* worm burdens observed in this study indicate a long period of the fungal activity within the sheep pellets on pasture. The same applies to a lesser degree of fungal activity over *N. spathiger* which has to take at least 10 days before hatching, as mentioned above. The desiccation of the faeces and chlamydospores during the drought period in July and most of August may have contributed to a longer survival of the fungi.

The clinical disease observed during June in lambs of all groups irrespective of fungi feeding was probably related to the recent weaning and uptake of overwintering larval infection although some auto-infection cannot be ruled out. The lack of statistically significant differences in faecal egg counts and worm burdens (apart from recent intake of *Ostertagia* larvae) in the permanently grazed weaners may thus be explained by the elimination of the established overwintering larval infection by the June anthelmintic treatment and the subsequent very dry spell where no or little translation took place until the rains started in late August. This could also explain the lack of a response of treatments on the weight gains. The trend towards higher weight gains in the control groups could possibly be related to the higher energy supply from the supplements given to these groups although the relative importance is difficult to assess due to the unknown intake of grass.

This study clearly shows that the strategic use of nematophagous fungi will not eliminate the risk of clinical or subclinical disease in early spring due to a heavy overwintering larval infection nor overcome typical spring nematodiosis. Control of helminths in sheep enterprises should be based on an integrated strategy where biological control agents such as *D. flagrans*, grazing management and/or anthelmintics each play a part. The use of a fungal bio-control agent is a strict preventive measure reducing reinfection with larvae developed from eggs excreted after turnout. This may reduce the otherwise pathogenic worm burdens in the latter part of the season to such a low level that both clinical and severe subclinical effects can be avoided, and at the same time the animals build up their natural immunity by a low, but sufficient level of larval exposure.

## **7.0. General discussion, conclusions and significance of the study.**

### **7.1. General discussion and conclusions.**

Gastrointestinal nematodes are considered a serious economic problem affecting the livestock industry especially in tropical and subtropical areas of the world (Bejsovec, 1991, Tembely and Hansen, 1996). Hematological disorders (Hayat *et al.*, 1996), loss of appetite, slow growth, malnutrition, susceptibility to other diseases and loss of weight are the most common symptoms observed in infected animals (Charleston, 1994; Sauer, 1996). Control of these parasites helps to reduce mortalities and improve production.

Different control measures have been proposed against these parasites. The most common method of control is administering anthelmintic drugs to animals in a systematic or strategic way. The use of these chemotherapeutic agents helps to reduce the parasitic burden in animals. There are however, problems associated with the current excessive dependency on anthelmintics for parasite control in livestock such as development of drug resistant nematode populations particularly in small ruminants (Craig, 1993; Bjørn, 1994; Waller *et al.* 1996) and the potential for ecotoxicity of some endectocides eliminated in the environment (Herd *et al.*, 1993; Herd, 1995). There is also the increasing consumer demand for animal products and pastures free from chemical residues and their effect on international trade (Woolaston and Baker, 1996).

These problems have called for intensified research with the objective of developing alternative strategies for worm control. Of those which are currently under consideration, breeding for resistance (Gray and Gill, 1993), nutritional supplementation (Knox and Steel, 1996) and improved grazing management (Barger, 1996) offer immediate prospects of application to complement the strategic use of anthelmintics. Vaccination against ruminant gastrointestinal nematodes remains elusive. There is increasing possibility for exploitation of the nematode trapping fungi as biological control agents (Larsen, 1999).

For all these methods of control to be successful and effective, a clear understanding of the epidemiology of the gastrointestinal nematodes is desirable for each region, farming system and host and species of nematode. In this thesis, the epidemiology, prevalence and control of gastrointestinal nematodes of goats on small scale farms in a marginal low potential area of Kenya was studied. A study on the impact of natural gastrointestinal nematode infections in young set stocked goats in this region was also carried out.

Alternative methods of control of gastrointestinal nematodes in weaner lambs without the use of anthelmintics was studied in Denmark. This was done by the use of grazing management strategies and dosing with the nematode destroying fungus *Duddingtonia flagrans*.

The epidemiological survey showed that goats of all ages are infected with gastrointestinal nematodes. The levels of infection varied with seasons, being high during the rainy seasons. There was also variation in the level of infection between farms. This seasonal variation in intensity of gastrointestinal nematode infections has been reported by Lutu, (1983), in dairy goats on a farm in the high rainfall area of Kenya highlands. It has also been reported in the high veld of Zimbabwe (Pandey *et al.*, 1994), in Malaysia (Dorny *et al.*, 1995) and in the semi desert of Mauritania (Jacquiet *et al.*, 1995). In most of these reports, the prevalence and intensity of infection measured by either faecal egg counts (EPG) or total worm counts followed the rainfall patterns and there was a direct relationship between the amount of rainfall and intensity of infection with gastrointestinal nematodes. In southern Mauritania, season was the most important factor influencing strongyle egg output among sheep and goats, the rainy season being the most favourable and the dry season least favourable. The rainy season leads to improved egg hatching, larval survival due to improved vegetation cover (Dinnik and Dinnik, 1961), higher intake of infective larvae which leads to heavier worm burdens and increased faecal egg counts as observed in the present study.

The intensity of infection with gastrointestinal nematodes did not vary significantly across the age groups of the goats and age immunity to gastrointestinal nematodes does not seem to occur in goats in these areas. This is similar to the observations by Maingi *et al.*, (1993), in goats on four farms in four districts of Kenya. Silva *et al.*, (1998), observed that older goats had higher gastrointestinal parasite burdens than young goats in Brazil. Similar observations were also made in Nigeria by Anene *et al.*, (1994). In the study reported in this thesis, differences in the intensity of infection related to age were only demonstrated during the dry season when the kids were shedding more eggs than the older goats. In the semi desert climate of Mauritania, Jacquet *et al.*, (1995) reported that differences in gastrointestinal nematode infections related to age were only evident during the dry season when the older animals were shedding more eggs. This difference from the observations made during the present study may be due to the differences in the climatic conditions.

Explanations to this lack of differences in the level of infection with age in goats have been given by several researchers. Le Jambre and Royal, (1976), suggested that goats as a consequence of their browsing habit, have evolved a less efficient acquired resistance than sheep. Dorny *et al.*, (1995), reported that when left freely to browse, goats have been thought to pick fewer larvae than sheep and the slower build up of immunity with age may be the result of less contact with gastrointestinal nematode parasites. Dorny *et al.*, (1995) and Vlassolf *et al.*, (1999) showed that the development of immunity to gastrointestinal nematodes is slower in goats compared to sheep. Another explanation for the lack or slow development of immunity in goats especially in the tropics is the practice of “lying out” or “planting” by goat kids where kids are left in “camps” or penned around the homestead while the mothers go out feeding (Peacock, 1996). This is unlike in sheep where the lambs accompany and are usually beside the ewes when feeding. In the present study, the kids were kept around the homestead or in houses for up to 3

months. This behaviour delays contact with the infective larvae. Vlassoff *et al.*, (1999), observed that resistance to nematode establishment in goats develops from 6 to 12 months of age and there is no marked increase in resistance beyond this age. All these factors may have played a role in the observations made in the present study.

*Haemonchus contortus* was the main nematode recovered from the coprocultures and slaughtered goats. This observation is similar to those of other workers (Lutu, 1983; Maingi *et al.*, 1993; Githigia *et al.*, 1996; Shavulimo and Semenye, 1990; Gatongi *et al.*, 1998) who examined the prevalence of gastrointestinal nematodes in sheep and / or goats. *H. contortus* is also the most important nematode in Kenya (Waruiru *et al.*, 1991; Wanyangu *et al.*, 1996). Larvae of this nematode have been shown to undergo hypobiosis in sheep and goats during unfavourable conditions in the semi-arid regions of Kenya (Gatongi *et al.*, 1998), Zimbabwe (Pandey *et al.*, 1994) and Mauritania (Jacquet *et al.*, 1995). A sudden rise in faecal egg counts and parasitic gastroenteritis was observed in goats in the present study before and immediately after the onset of the rainy season. This is likely to have resulted from resumption of development of hypobiotic larvae.

The number of individual salvage anthelmintic treatments given in the study did not vary with the age groups of the goats as the infection levels did not vary with the age groups. However, where diagnostic facilities and technical skills are available, individual anthelmintic treatments are recommended in order to prevent or delay the development and spread of anthelmintic resistance.

Results of the study on the impact of natural gastrointestinal nematode infection on set - stocked goats, showed that natural gastrointestinal nematodes led to reduced productivity through subclinical and clinical parasitic gastroenteritis. They also led to mortalities, reduced weight gains and weight loss especially in young goats. Such goats never reach their full

production potential. These observations agreed with those of Lutu, (1983); Shavulimo *et al.*, (1988) and Shavulimo, (1993) in Kenya, and those of Joshi, (1994), in Nepal, Costa and Vieira, (1983) in Brazil, Saithanoo, (1996), in southern Thailand, Mostofa, *et al.*, (1996) in Bangladesh and Hoste and Chartier, (1993), in France.

During the present study, death from parasitic gastroenteritis occurred in goats with EPG counts of between 3500 - 6500. In the study on dairy goats, Lutu, (1983), observed that goats dying from parasitic gastroenteritis had strongyle egg counts of between 12,200 and 50,000. The difference in the two observations may have arisen from the breed of goats, age and physiological status of these goats.

Hypobiosis was recorded in this study and occurred at the end of the short rainy season. There was no evidence of hypobiotic larvae at the end of the dry season but there was evidence of increased faecal egg counts possibly due to resumed development and maturation of such hypobiotic larvae just before the rains. Gatongi *et al.*, (1998), reported the occurrence of hypobiotic larvae in goats in a semi arid area of Naivasha, Kenya. They observed that *Haemonchus contortus* survives the dry season in this area as hypobiotic larvae. They also observed that the development and maturation of the hypobiotic larvae depended on the amount and duration of rainfall and adult worms and hypobiotic larval populations may occur together. In the study by Gatongi *et al.*, (1998), most of the hypobiotic larvae were found in the abomasal luminal contents and few embedded in the mucosa. This was also observed in the present study. In the present study, larvae picked at the end of the short rainy season underwent hypobiosis. Whether or not larvae undergo hypobiosis may be a mechanism inherent in the worm for survival which responds to weather changes in a pre-programmed pattern (Gatongi *et al.*, 1998).

Frequent suppressive anthelmintic treatment reduces production losses especially during periods of drought. However, this frequent repeated treatment with the same anthelmintic drug

may have led to selection of anthelmintic resistant nematodes in the present study. This was evident from the low egg counts in the treated goats and the total number of worms recovered at the end of the study in the suppressively treated groups.

This study confirmed the earlier observation from coprocultures that *H. contortus* is the main nematode infecting goats in the low marginal areas of central Kenya. This species composed almost 100% of the worm counts in all the animals. An interesting finding in this study was the absence of worms in the small intestines and the abomasal worm burden (100% *H. contortus*) accounting for 100% of the total worm burdens in almost all the animals. *Haemonchus contortus* is a voracious blood sucker and due to its pathogenicity, control failure is easily noticeable and has been associated with anthelmintic resistance in small ruminants in the tropics and sub tropics (Dorny *et al.*, 1994; Waller, 1997a; Maingi *et al.*, 1998; Chandrawathani *et al.*, 1999).

The study on integrated grazing management showed that it is possible to achieve sufficient gastrointestinal nematode control and good production levels in weaned lambs without the use of anthelmintics. This is possible through a strategy where the lambs are weaned at the beginning of July and moved to clean pasture where they should not stay for more than 3 months. The clean pasture should have been spelled for at least one year / grazing season before the lambs are introduced. This compared to the earlier method recommended by Eysker *et al.*, (1998a) in cattle where the animals were moved to a clean pasture at the beginning of July. In their study, monthly moves were recommended up to the housing period or to the end of the grazing season unlike in the present study where a move was recommended after 3 months on the clean pasture. In Britain, Coles and Roush, (1992), recommended that ewes and lambs be moved at the beginning of July and lambs share the same pasture with the ewes after weaning. The lambs are then moved to a clean pasture with or without anthelmintic treatment before housing or slaughter.

The strategy recommended in the present study is compatible with non- chemical pest management control methods of organic farming which is being emphasized in countries in the European Union. However, this strategy will not work in the tropics like Kenya where there is no well defined grazing seasons. The strategy will also not apply in the pastoral and the communal grazing areas of the tropics especially in Africa. In such areas provision of safe pasture is only possible through rapid rotational grazing as earlier recommended for the Pacific Islands (Barger, *et al.*, 1994). This system is readily adaptable to more traditional husbandry methods such as tethering and shepherding which are commonly used by small holders in developing countries. Shepherding is the most common type of husbandry in the semi arid areas and on large farms / ranches in Kenya while tethering is common among small scale farmers in the high potential areas of Kenya where land sizes are limited.

Other methods which can be applied for integrated gastrointestinal nematode control in Kenya include the use of medicated urea molasses block (research already under field conditions at KARI) and exploring the potential of biological control using nematophagous fungi.

The studies to examine the potential of the nematode destroying fungi to control naturally acquired gastrointestinal nematode infections in sheep showed that the fungus is able to reduce the acquisition of infective larvae from pasture. Daily dosing of grazing lambs with *Duddingtonia flagrans* for 3½ months affected the development and spread of parasitic larvae of ovine nematodes thereby reducing pasture infectivity. This was evident by the lower pasture larval counts in the late season and confirmed by significantly lower worm burdens of tracer lambs that had grazed paddocks which had been earlier grazed by fungi dosed lambs. The present study was the first demonstration of biocontrol of ovine nematodes under a natural epidemiological context. These findings agree with the similar earlier studies in calves (Grønvold *et al.*, 1993; Larsen, *et al.*, 1995; Nansen *et al.*, 1995) and closely agree with the recent findings that have

been observed in pigs (Nansen *et al.*, 1996) and horse (Larsen *et al.*, 1996; Fernandez *et al.*, 1997). The study further demonstrated that the nematode destroying effects of the fungi against *Ostertagia spp*, *Trichostrongylus spp* and *Haemonchus contortus* found in sheep faecal cultures (Larsen, *et al*, 1994; Waller and Faedo, 1993; Waller *et al.*, 1994) may be reflected in reduced pasture contamination under field grazing conditions.

The present study has shown that ovine nematode species are affected differently by the fungus due to the larval biology of the pre-parasitic larvae. *Nematodirus spp* require special conditions for hatching and develop in the egg up to L3 as compared to *Ostertagia spp*, *Trichostrongylus spp* and *Haemonchus spp* which hatch as L1 and are exposed to the fungus when fungal traps are active. This study however, showed a significant reduction in the number of larvae of *Nematodirus spp* in pasture and in the total worm burdens. These results show that the *Nematodirus spp* infecting sheep were hatching within the same season and the reduced worm burdens indicated a prolonged period of fungal activity within the sheep pellets on pasture.

The clinical parasitic gastroenteritis observed in the present study due to heavy overwintering larval infection in June clearly shows that nematophagous fungi reduce the number of infective larvae developing in the faeces but have no effect on larvae that have already passed to the vegetation or on worm burdens in the host animals. Therefore, to maximize the effect of the nematophagous fungi, or any other biological control agent, it is important first to secure a low level pasture infectivity by grazing management strategies or by strategically applied anthelmintic treatments. It is hoped that after animals have been dosed for a couple of seasons, the larval population on pasture will be diminished and the need for anthelmintic intervention reduced. The use of a fungal bio-control agent is a strict preventive measure for reducing re-infection with larvae which have developed from eggs shed after turnout. This may reduce the otherwise pathogenic worm burdens in later part of the season.

From this study it is observed that gastrointestinal nematode control in sheep enterprises should be based on an integrated strategy where biological control agents such as *Duddingtonia flagrans*, grazing management and/or anthelmintics each play a part.

At present impediments to the adoption of nematophagous fungi in practical control schemes include a lack of suitable application systems, assessment of long term environmental effects and finally, acceptance of the principle by farmers. Mixing the fungal chlamydospores in feed supplement has been shown to be a realistic option for long term application in intensive production systems (Thamsborg *et al.*, 1999). In more extensive systems, the incorporation into feed blocks, mineral licks or ruminal bolus may be potential options which need to be investigated.

In Kenya, potential for biological control by daily feeding of animals with a supplement containing the fungus can be applicable in intensive grazing systems like zero grazing, in systems where daily gathering of animals is part of the farm routine activities such as milking time in dairy farms or night housing for most small ruminant enterprises.

## **7.2: Significance of the study.**

1. This study provided important insights into the epidemiology and intensity of natural gastrointestinal nematode infections in goats in the marginal low potential areas of Central Kenya. The intensity of infection with gastrointestinal nematodes does not vary with age of goats. This confirms the point prevalence observation by Maingi *et al.*, (1993), on a few farms studied in Kenya. The results of this study have shown that the blood sucking nematode *H. contortus* is the main nematode in goats in this area contributing almost 100% of the total worm burdens. This information will help the policy makers in deciding the type of anthelmintics to be used in strategic flock treatments in this area. Narrow spectrum anthelmintics like closantel which act against

*H. contortus* may be preferable. This information has also shown that there is a potential for the application of the FAMACHA technique as a tool for diagnosis as *H. contortus* the main nematode is a blood sucking nematode.

2. This study has shown that mortalities and clinical parasitic gastroenteritis occurs in kids and lactating does during times of drought and feed scarcity. This information may help in developing a strategic control programme for goats in these areas where anthelmintic treatments and supplementary feeds are given during the dry season especially to pregnant, lactating and weaner goats. This study has also established that hypobiosis involving *H. contortus* occurs during the dry season and maturation and development of hypobiotic larvae occur at the end of the dry season and beginning of the rainy season. The results of this study may help in developing a strategic control programme for goats in these areas where flock anthelmintic treatments are given at the end of the dry season.
3. This study has shown that natural gastrointestinal nematode parasites affect the productivity of set - stocked goats leading to weight loss and mortalities. The effects are more profound during drought. *H. contortus* is the cause of these losses. The results of this study have shown that set-stocked goats in this area will require supplementation and strategic anthelmintic treatments during the dry season. This information will help farmers, government and non governmental organizations who may want to keep set-stocked goats in these areas for various development programmes.
4. This is the first study that has shown that it is possible to achieve good parasitological nematode control and improved production in weaner lambs by moving them to clean pastures at the beginning of July in Denmark without the use of anthelmintics. This information may help in developing and designing of alternative methods of nematode control in sheep in this country. The results of this study have shown the potential

application of grazing management in integrated gastrointestinal nematode control strategies in Kenya in various enterprises. This information will help the resource poor farmers who may not afford anthelmintics but have the required land resources for grazing management practices such as rapid rotational grazing and tethering.

5. This is the first study that has shown the effect of the nematode destroying fungus *Duddingtonia flagrans* under field conditions on naturally acquired ovine gastrointestinal nematodes. It is the first report demonstrating an effect of bio-control on ovine nematodes in a natural epidemiological context. The study has shown that the use of fungal biocontrol agent is a strict preventive measure reducing reinfection with larvae developed from eggs shed after turnout. This study has also shown that ovine nematode species are affected differently by the nematode destroying fungus depending on their larval biological development. This information has shown that gastrointestinal nematode control in sheep enterprises should be based on an integrated strategy where biological control agents such as *D. flagrans*, grazing management and/or anthelmintics each play a role. The results of this study have shown the possibility of biological control using nematode destroying fungi in Kenya in husbandry enterprises where daily gathering of animals, night housing and supplementary feeding is part of the farm routine. This will be an additional tool in the fight against the development and spread of anthelmintic resistant nematodes in small ruminants.

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