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**Mitigating the effects of gastrointestinal nematode infection in
organic lambs through the use of hospital paddocks**

A thesis
submitted in partial fulfilment
of the requirements for the Degree of
Master of Agricultural Science

at
Lincoln University
by
Becca Moroka

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Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Master of Agricultural Science.

Mitigating the effects of gastrointestinal nematode infection in organic lambs through the use of hospital paddocks

by

Becca Moroka

This thesis investigated the use of a hospital paddock treatment comprised of chicory (*Cichorium intybus*), plantain (*Plantago lanceolata*) and red clover (*Trifolium pratense*) in place of anthelmintic treatment to provide a curative effect for gastrointestinal nematode infections in organic lamb production systems. Furthermore, in order to improve the agronomical restrictions caused by having large areas of farmland planted in these bioactive forages, a targeted selective treatment (TST) regime was used to identify only those individuals that were suffering from parasitism and thus expected to obtain a benefit at any point in time. The study was conducted at the Biological Husbandry Unit in Lincoln University, from December 2012 to April 2013 using sixty four, three month old Suffolk crossed lambs; thirty-two lambs each were allocated to graze one of two organic perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) paddocks infected either with *Teladorsagia circumcincta* (TCIRC) or *Trichostrongylus colubriformis* (TCOL). Within each parasite treatment, eight lambs were allocated to a drench control treatment to provide an estimate of the potential performance that could be achieved (TCIRC-D and TCOL-D), while the remaining twenty four lambs were subjected to a TST regime (TCIRC-TST and TCOL-TST). Individual live weight (LW) and faecal samples were monitored every two weeks. A pre-set LW gain of each lamb was calculated using the Happy factor system and at each decision time, TST individuals that did not achieve their target LW were removed from pasture and allowed to graze on the hospital paddocks for four weeks before being returned to pasture.

Overall, cumulative LW of TST individuals was lower than their drenched counterparts; being statistically similar on Day 0 – 51, with significant differences evident from Day 79 for the TCIRC-TST and from Day 65 for the TCOL-TST animals relative to their drenched controls. Consequently, an overall 32% and 39% reduction in growth; viz., 13.47 ± 1.06 cf. 19.97 ± 1.63 kg and 13.32 ± 0.65 cf. 21.96 ± 1.27 kg for the TCIRC-TST and TCOL-TST group, respectively was observed, which was generally associated with temporal variations in size of larval challenge and herbage mass, and the

interaction of these two factors with individual lambs. Performance of the TST lambs challenged with both parasite species improved to similar levels with those of the drench control after four weeks of hospitalisation. This was believed to be related to the quality of the bioactive forages as there was no evidence of a direct anthelmintic effect of the bioactive forages. Despite high FEC, mean LW from Day 79 was 39.1 kg and 36.2 kg for the TCIRC-TST and TCOL-TST lambs, respectively, suggesting that this approach was able to allow 80% and 58% of lambs to reach the required market weight of 38 kg without the use of anthelmintic. The overall observation supports the hypothesis that hospital paddock treatment using bioactive forages was able to partly mitigate the effects of infection and maintain reasonable lamb performance in an organic context. The benefit of the use of such approach appeared to be greater in abomasal than intestinal infections.

Keywords: Bioactive forages, gastrointestinal nematodes, lambs, organic, targeted selective treatment, *Teladorsagia circumcincta*, *Trichostrongylus colubriformis*

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This Master thesis is the final project for my Master's program in Agricultural Science at the Faculty of Agriculture and Life Sciences, Lincoln University, Canterbury, New Zealand. The purpose of the study was to evaluate the effectiveness of the targeted selective treatment (TST) regime in an organic setting without use of anthelmintic, but a hospital paddock, in maintaining performance of organic lambs to market or slaughter weight. This thesis has been completed in 9 months with the experimental work carried out in December 2012 to April 2013 at the Biological Husbandry Unit at Lincoln University and report writing completed in the latter months.

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Table of Contents

Abstract	ii
Acknowledgements	iv
Table of Contents	v
List of Tables	vii
List of Figures	ix
List of Abbreviations	xi
Chapter 1 Introduction	1
1.1 Aim and Objectives of the study	2
1.2 Hypothesis of the study	2
Chapter 2 Review of Literature.....	3
2.1 Importance of impact of GIN on sheep farming in New Zealand	3
2.2 Incidence and Distribution.....	3
2.3 Life cycle of gastrointestinal nematodes	4
2.3.1 GIN population dynamics.....	6
2.3.2 Impacts on the host	7
2.4 Host acquired immunity	11
2.5 Control options	13
2.5.1 Chemical control	13
2.5.2 Non-chemical control.....	15
2.5.3 Grazing management.....	15
2.5.4 Supplementary feeding.....	18
2.5.5 Use of bioactive forages.....	20
2.5.6 Chicory	26
2.5.7 Plantain	27
2.6 Integrated control of gastrointestinal nematodes	28
2.7 General summary.....	29
Chapter 3 Materials and Methods	31
3.1 Experimental design, pastures and animals	31
3.2 Treatments and measurements.....	32
3.2.1 Treatments.....	32
3.2.2 Happy factor system	Error! Bookmark not defined.
3.2.3 Herbage mass.....	33
3.2.4 Faecal Egg Count	34
3.2.5 Herbage contamination	34
3.2.6 Hospital paddock botanical composition.....	34
3.3 Data Analysis.....	35
Chapter 4 Results.....	36
4.1 Herbage measurements.....	37
4.1.1 Herbage Mass.....	37
4.1.2 Herbage composition of the hospital paddocks	38

4.1.3	L ₃ parasitic larvae contamination.....	39
4.2	Overall animal measurements.....	40
4.2.1	Faecal Egg Count (FEC).....	40
4.2.2	Live weight (LW) and live weight gain (LWG)	41
4.2.3	Number of animals reaching slaughter weight.....	42
4.2.4	Distribution of lambs at each time of selection	42
4.3	Performance attributes at time of selection	44
4.4	Performance attributes of hospitalized animals.....	46
4.5	Response to treatment (% change in HF)	52
Chapter 5 Discussion.....		55
5.1	Ability of the TST regime in maintaining lamb performance.....	55
5.1.1	Reductions in LW under the TST regime and larval challenge.....	56
5.1.2	Temporal variations in production loss under TST regime	58
5.2	Performance responses of hospital treatment.....	60
5.2.1	Effect of hospital paddock treatment on LWG	60
5.2.2	Re-admitted lambs.....	61
5.2.3	Anti-parasitic benefits of hospital treatment	62
5.2.4	Temporal variations in performance of hospital animals	63
5.3	Performance of lambs after their return to pasture.....	64
5.4	Predictors of parasitism	65
Chapter 6 Conclusion		66
References		67

List of Tables

Table 2.1: Geometric mean of worm counts of each species in groups of ten lambs slaughtered after one month on sheep pastures (Adapted from:Southcott and Barger, 1975).....	17
Table 2.2: Concentration of secondary plant compounds in temperate forage species with pastoral value for New Zealand farming systems (Extracted from Ramírez-Restrepo and Barry, 2005).....	21
Table 2.3: Negative effect of some plant secondary metabolites on the host animal.	23
Table 2.4: The consequences of PSM consumption on the performance of parasitized herbivores in relation to the performance of non-parasitized herbivores not given access to PSM* (Source: Athanasiadou and Kyriazakis, 2004).	24
Table 4.1: Number of lambs that reached slaughter weight of 38 kg, grazing either TCIRC or TCOL infected pasture, that were either exposed to a four weekly anthelmintic treatment regime (drenched) or a TST regime (TST) in which poor performing individuals were placed into respective hospital paddocks for four weeks before returned to pasture during the trial.	42
Table 4.2: Distribution of lambs grazing either TCIRC or TCOL infected pasture and subjected to a TST regime where lambs that failed to meet their target pre-set weight gains were placed respective hospital paddocks (-H), while lambs that reached their target weight gain were placed back on pasture (-P) at each selection time. The numbers in brackets indicate the number of lambs that were in the same paddock for the previous two week grazing period.....	42
Table 4.3: Mean LW (kg), LWG (kg/day) and FEC (epg) of lambs grazing TCIRC infected pastures at each weighing and selection time according draft; drenched or TST animals that remained on pasture (TST-Pasture), placed into the hospital paddock (TST-Hospital) or returned to pasture from hospital paddock (TST-Recovered).	44
Table 4.4: Mean LW (kg), LWG (kg/day) and FEC (epg) for lambs grazing TCOL infected pastures at each weighing and selection time according to draft; drench or TST animals that remained on pasture (TST-Pasture), placed into the hospital paddock (TST-Hospital) or returned to pasture from hospital paddock (TST-Recovered).	45
Table 4.5: Mean HF, LWG (kg/day) and FEC (epg) between drenched and TST-hospitalised lambs grazing TCIRC infected pastures for all weighing, selection and admittance times, at 1 st and 2 nd two week hospital grazing periods and at 1 st and 2 nd two week grazing periods after the hospitalised lambs returned to pasture, irrespective of when the hospitalised lambs were admitted.	46
Table 4.6: Mean HF, LWG (kg/day) and FEC (epg) between the drench and hospitalized lambs grazing the TCIRC infected pasture at time of admittance (0 days), at the 1 st (14 days) and 2 nd (28 days) two week hospital grazing periods and at the 1 st (42 days) and 2 nd (56 days) two week grazing period after the hospitalised animals returned to pasture for each weighing and selection time (Day 23-79).....	48
Table 4.7: Mean HF, LWG (kg/day) and FEC (epg) between drenched (Dren) and TST-hospitalised (Hos) lambs grazing TCOL infected pastures for all weighing, selection and admittance times, at 1 st and 2 nd two week hospital grazing periods and at 1 st and 2 nd two week grazing periods after the hospitalised lambs returned to pasture, irrespective of when the hospitalised lambs were admitted.	49
Table 4.8: Mean HF, LWG (kg/day) and FEC (epg) between the drench (Dren) and hospitalized (Hos) lambs grazing the TCOL infected pasture at time of admittance (0 days), at the 1 st (14 days) and 2 nd (28 days) two week hospital grazing periods and at the 1 st (42 days) and 2 nd (56 days) two week grazing period after the hospitalised animals returned to pasture for each weighing and selection time (Day 23-79).	51
Table 5.1: Apparent DM intake (kg DM/day) of lambs estimated from the pre- and post-grazing herbage mass and recovered larvae from herbage at each of the two week grazing	

<p>period, for both the pasture and hospital paddock for <i>T. circumcinta</i> (TCIRC-P & TCIRC-H) and <i>T. colubriformis</i> (TCOL-P & TCOL-H) throughout the entire trial.....</p>	56
<p>Table 5.2: Estimated daily larval intakes of lambs grazing the <i>T.circumcinta</i> and <i>T.colubriformis</i> infected pasture paddocks (TCIRC-P and TCOL-P) and their respective hospital paddocks (TCIRC-H and TCOL-H) at each of the two week grazing period throughout the trial.</p>	57

List of Figures

Figure 2.1: The basic lifecycle of a gastrointestinal nematode of sheep (Source: http://www.scops.org.uk/endoparasites-worm-species.html).	5
Figure 2.2: The interrelationship between pasture contamination by untreated ewes and lambs and the availability of infective larvae on pasture (Source: Vlassoff, 1982).	7
Figure 2.3: The typical time course to change in performance and in immune response of naive lambs exposed to chronic daily infection of larvae of the intestinal nematode <i>Trichostrongylus colubriformis</i> (Adapted from Kimambo <i>et al.</i> , 1988).	12
Figure 2.4: Mean body weight (in kg) of lambs infected daily with 3000 <i>T. colubriformis</i> larvae (SI, ▲), of lambs similarly infected but given abomasal infusion of protein (PI, ●), or energy (EI, Δ) and of their uninfected controls (C, ○) over 12 weeks (Source: Bown <i>et al.</i> , 1991b).	20
Figure 2.5: Back transformed mean of immature (■), total adult (□), male adult (▣) and female adult (▤) worm burdens recovered from the gastrointestinal tract of lambs infected with two single doses of 8000 L ₃ of <i>Teladorsagia circumcincta</i> on days 1 and 28 of the experiment and grazing one of the grass/clover, chicory, lotus and sulla sward. The vertical bars indicate the confidence intervals (95%) (Source: Tzamaloukas <i>et al.</i> , 2005).	25
Figure 3.1: Schematic diagram of the trial site at the Lincoln BHU organic farm.	31
Figure 4.1: Pasture mass (kg DM/ha) for TCIRC (—○—) and TCOL (—●—) infected pasture paddocks and TCIRC (—○—) and TCOL (—●—) hospital paddocks throughout the trial.	37
Figure 4.2: Percentage distribution of grass (■), chicory (▒), clover (▣), plantain (▤) and others (▥) in the TCIRC hospital paddock at each dissection throughout the trial. Note: Others category contains weeds and dead matter.	38
Figure 4.3: Percentage distribution of grass (■), chicory (▒), clover (▣), plantain (▤) and others (▥) in the TCOL hospital paddock at each dissection throughout the trial. Note: Others category contains weeds and dead matter.	38
Figure 4.4: The number of parasitic L ₃ larvae (L ₃ per kg DM/ha) recovered from the TCIRC (—●—) and TCOL (—○—) infected pasture paddocks, and the TCIRC (—●—) and TCOL (—○—) hospital paddocks throughout the trial.	39
Figure 4.5: Arithmetic mean FEC (epg) for animals grazing pastures infected with either TCIRC (—○—) or TCOL (—●—) that were drenched every four weeks, and animals grazing TCIRC (—○—) or TCOL (—●—) infected pastures that were not drenched, but exposed to a TST regime in which poor performing individuals were placed onto respective hospital paddocks for four weeks before returned to pasture. Bars represent the SEM.	40
Figure 4.6: Mean LW (kg) for animals grazing pastures infected with either TCIRC (—○—) or TCOL (—●—) that were drenched every four weeks, and animals grazing TCIRC (—○—) or TCOL (—●—) infected pastures that were not drenched, but exposed to a TST regime in which poor performing individuals were placed onto respective hospital paddocks for four weeks before returned to pasture. Bars represent the SEM.	41
Figure 4.7: Number of lambs subjected to a TST regime and grazing either TCIRC (■) or TCOL (▒) infected pastures that were re-admitted into the respective hospital paddocks at either two or four weeks after being returned to respective pastures.	43
Figure 4.8: Change in HF (%) of animals grazing the TCIRC infected pasture which were either subjected to a four weekly anthelmintic treatment regime (○) or to a TST regime (x) at two (A) and four (B) weeks after treatment relative to the pre-treatment happy factor values for all selection times. Each data point represents an individual animal.	53
Figure 4.9: Change in happy factor values (%) of animals grazing the TCOL infected pastures which were either subjected to a four weekly anthelmintic treatment regime (○) or to a TST regime (x) at two (A) and four (B) weeks after treatment relative to the pre-	

treatment HF values for all selection times. Each data point represents an individual animal.54

List of Abbreviations

ADD	Amino-acetonitrile derivatives
ANOVA	Analysis of variance
AR	Anthelmintic resistance
BHU	Biological Husbandry Unit at the Lincoln University, New Zealand
Ca	Calcium
cf.	Compare
Cm	Centimetre
COWP	Copper oxide wire particle
CT	Condense tannins
Cu	Copper
DM	Dry Matter
epg	Eggs per gram
FAMACHA	An anaemia evaluation system named after Dr. Francois “Faffa” Malan
FEC	Faecal Egg Count
GI	Gastrointestinal
GIN	Gastrointestinal nematode
g	Grams
≥	Symbol for greater than or equal to
ha	Hectare
HF	Happy factor
I	Roman numeral for number one
II	Roman numeral for number two
kg	Kilogram
<	Symbol for less than
≤	Symbol for less than or equal to
L	Litre
LW	Live weight
LWG	Live weight gain
L ₁	First stage of larval development on pasture
L ₂	Second stage of larval development on pasture
L ₃	Third stage of larval development on pasture
L ₄	Fourth stage of larval development inside the host
L ₅	Fifth (infective) stage of larval development inside the host
Mg	Magnesium
ME	Metabolisable energy
MEI	Metabolisable energy intake
ml	Millilitre
m ²	Square meter
NS	Not significant
/	Per
%	Percentage
P	Phosphorus
PSM	Plant Secondary Metabolites
pH	Abbreviation for the measure of the activity of the hydrogen ion

RCD	Randomized Complete Design
SEM	Standard error of mean
spp.	Species
TST	Targeted Selective Treatment
TCIRC	<i>Teladorsagia circumcincta</i>
TCIRC-D	An experimental treatment where the pasture was infected with <i>Teladorsagia circumcincta</i> and the lambs were subjected to a monthly anthelmintic drenching regime
TCIRC - TST	An experimental treatment where the pasture was infected with <i>Teladorsagia circumcincta</i> and the lambs were subjected to a targeted selective treatment regime.
TCOL	<i>Trichostrongylus colubriformis</i>
TCOL-D	An experimental treatment where the pasture was infected with <i>Trichostrongylus colubriformis</i> and the lambs were subjected to a monthly anthelmintic drenching regime
TCOL – TST	An experimental treatment where the pasture was infected with <i>Trichostrongylus colubriformis</i> and the lambs were subjected to a targeted selective treatment regime.
VFI	Voluntary feed intake
viz.	That is or namely

Chapter 1

Introduction

Gastrointestinal nematode (GIN) parasitism is a major animal health and welfare threat to small ruminant production systems throughout the world (Jackson and Coop, 2000; Waller, 1997a, 2006) through their impact on animal performance of which the two major contributing factors are reduction in feed intake and reduction in feed conversion efficiency (Coop and Holmes, 1996; Sykes, 1994; Sykes and Coop, 2001). Many studies have indicated level or size of challenge on pasture to be the major factor responsible for the reductions in feed intake and feed conversion efficiency leading to overall production loss, which in economic terms, translates to low profitability for the farmer or enterprise and a welfare threat to the animal concerned.

The increasing prevalence of anthelmintic resistance (AR) has stimulated research into other methods of GIN control of which the use of bioactive forages is one area. Grazing studies on use of bioactive forages have reported reductions in adult worm burdens and improved animal performance, suggesting that they may be a promising option for use in integrated control of nematode parasitism (Hoste *et al.*, 2006). Lambs exposed to naturally acquired infection had similar faecal egg counts (FEC), but significantly lower adult worm burdens when grazed on red clover than on ryegrass and white clover over a period of five weeks (Marley *et al.*, 2003). Similarly, artificially infected lambs grazing chicory over a period of two weeks had similar FEC, but significantly lower adult male worm burdens compared with those grazing ryegrass (Tzamaloukas *et al.*, 2005). In another study, lambs grazing red clover had reduced FEC and a higher daily growth rate than lambs grazing ryegrass over 56 days (Marley *et al.*, 2005). Despite the promising results, other studies did not observe any difference between lambs grazing chicory compared with ryegrass (Athanasiadou *et al.*, 2005). Deer grazed on chicory compared with pasture also had a faster growth rate (Hoskin *et al.*, 2003). These studies used monocultures swards and there is limited work done on mixed swards of these forages. A previous study using mixed sward of chicory, red clover and plantain observed no difference in FEC between lambs grazing the mixed sward and those grazing ryegrass and white clover, but performance of 80% of these lambs, in terms of live weight (LW), returned to normal after these lambs were exposed to the mixed sward over a period of four weeks (Lundberg, 2012).

Despite the beneficial effects of bioactive forages, these forages have weak agronomical properties; namely, poor persistent, delicate grazing management and poor matching of growth to animal demand due to their growth requirements (Ramírez-Restrepo and Barry, 2005). These qualities would make it impractical for farmers to dedicated large areas of their farm to growing bioactive

forages. Therefore, research is being conducted to determine appropriate strategies that would incorporate these forages into farming systems. This can either act as a sustainable substitute for chemotherapy in conventional farming systems to minimise the use of anthelmintics and thus preserve drug efficacy (Greer *et al.*, 2010; Greer *et al.*, 2009; Leathwick *et al.*, 2006), or to maintain reasonable production levels in organic farming systems (Lundberg, 2012). One of these strategies currently understudied is the targeted selective treatment (TST) strategy. This involves identifying and treating animals that are suffering from parasitism and are likely to benefit from treatment using either parasitological, pathophysiological or performance based indices (Kenyon *et al.*, 2009). In particular, performance-based systems using LWG as an indicator of parasitism in stock has recently been shown to provide appropriate parasite control while maintaining animal performance (Greer *et al.*, 2010; Greer *et al.*, 2009; Leathwick *et al.*, 2006). In these studies, only 20 – 70% of lambs required treatment at any one time (Greer *et al.*, 2013; Kenyon *et al.*, 2013). As such, this approach in combination with the use of bioactive forages may provide a means to overcome the agronomical issues surrounding having a large proportion of the farm in bioactive forages while providing adequate parasite control.

1.1 Aim and Objectives of the study

Accordingly, the research was aimed at determining whether the use of the TST regime with a hospital paddock treatment comprising of chicory (*Cichorium intybus*), plantain (*Plantago lanceolata*) and red clover (*Trifolium pratense*) in place of anthelmintic treatment would assist parasitized lambs in mitigating the effects of infection in an organic system. The main objectives were to raise lambs on organic pastures infected with *Teladorsagia (Ostertagia) circumcincta* and *Trichostrongylus colubriformis* for a period of four months utilising a TST regime to select only those animals that are likely to benefit the most to graze the hospital paddocks. This study also evaluated the suitability of such forages to provide a curative treatment and thus mitigate the impact of GIN infections in organic lambs.

1.2 Hypothesis of the study

It was hypothesized that moving poor performing lambs to graze the hospital paddock for a period of four weeks would assist them to recover from the effects of GIN infection and return to normal growth, hence maintain performance at an acceptable level without recourse to anthelmintic use.

Chapter 2

Review of Literature

2.1 Importance of impact of GIN on sheep farming in New Zealand

GINs remain a major threat to the health and welfare of small ruminants all over the world. They impair animal health and welfare by causing inappetence, diarrhoea, anaemia and in some cases death (Athanasiadou and Kyriazakis, 2004). These impacts occur as a consequence of infection on food intake, digestion, absorption and utilisation of absorbed nutrients. The ensuing results of these impacts are the reduction in animal performance and productivity and increased cost of control (Coop *et al.*, 1982). According to Jackson *et al.*, (2009), helminth infestations currently cost the global small ruminant industry many millions of dollars annually through their major effect on food intake, digestion, absorption, utilization and cost of control.

The negative impact of GIN parasitism on animal production and the high cost of control of their infections, suggest that GINs are of great economic importance, and at the same time, posing a threat to the health and welfare of small ruminants. In New Zealand, GIN parasitism is probably the most important factor limiting production in sheep farming systems (Familton and McAnulty, 1997; Leathwick *et al.*, 2001; Vlassoff *et al.*, 2001; West *et al.*, 2009b). According to Vlassoff and McKenna, (1994), production loss through GI parasitic infections has been a problem since the introduction of sheep into New Zealand in the early 1800s. The main production penalties identified are reduction in live weight gain and wool production at subclinical stage of infection (Vlassoff and McKenna, 1994). The ensuing effects are reduction in productivity, thus profitability of the enterprise. Diseases caused by internal parasites is a particular threat to the New Zealand sheep and wool industry; as these industries are the key export sectors of the country (Douch, 1990; Lorimer *et al.*, 1996).

2.2 Incidence and Distribution

The three most economically important GINs affecting small ruminants throughout the world belong to the *Haemonchus*, *Teladorsagia* and *Trichostrongylus* species (Jackson *et al.*, 2009; Kaplan, 2004; Papadopoulos, 2008; Waller, 2003). In New Zealand, twenty-nine species of GINs have been identified from sheep (Vlassoff and McKenna, 1994). The species that prevail in large numbers and are most closely associated with production losses and clinical diseases are *Haemonchus contortus*, *Ostertagia* spp. and *Trichostrongylus axei* in the abomasum, and *Trichostrongylus* spp., *Nematodirus* spp. and *Cooperia* spp. in the small intestine (Brunsdon *et al.*, 1975; Vlassoff *et al.*, 2001; West *et al.*,

2009a). Of these species, *Teladorsagia* and *Trichostrongylus* species are predominant in all districts in New Zealand, while *Haemonchus* and *Cooperia* species are abundant in the North Island.

Prevalence, distribution and abundance of nematodes depends on the biology and epidemiology of the nematode species concerned, the prevailing local climatic conditions and farm management practices (Vlassoff and McKenna, 1994; Vlassoff *et al.*, 2001). Seasonal variations in the country, for at least part of the year, is favourable for the development of the free-living stages of most of the GINs (Vlassoff and McKenna, 1994). Therefore, all these nematodes are found throughout the country; however, their relative abundance varies between districts (McKenna *et al.*, 1974). A study conducted by Herve *et al.*, (2003) on ewes of various ages observed high incidence of GINs in the most northern and southern parts of the country, compared with the central regions; indicating regional differences in nematode parasite epidemiology and host exposure to helminths. In this study, *Teladorsagia* spp. and *Trichostrongylus axei* were the most predominant species in the southern North, southern South and northern South Island regions, which reflects their ability to develop and survive at low temperatures. The observations obtained for the northern South Island regions concurs with results observed in a previous study on lactating sheep (McAnulty *et al.*, 2001). *Haemonchus* spp. was more abundant in the northern and southern North Island due to its requirement for higher range of temperatures for larval development (West *et al.*, 2009a). While the *Nematodirus* spp. was common throughout the country, due to their ability to survive in cold winters, development in the spring may be high and they can become pathogenic, especially in areas such as Canterbury, Otago and Southland (West *et al.*, 2009a).

2.3 Life cycle of gastrointestinal nematodes

GINs, with the exception of the *Nematodirus* spp., have a similar life cycle (Figure 2.1) (Vlassoff *et al.*, 2001; West *et al.*, 2009a). GINs do not multiply in the host and have a direct life cycle, as there is no intermediate host. Typically, there are two major stages to the life cycle; namely, the development of eggs into larvae on pasture (free-living, non-parasitic stage) and development of larvae into adults in the host (host, parasitic stage).

Adult female worms lay eggs in the host, which are excreted with the faeces. Eggs are protected from desiccation by the mass of faeces and, under appropriate conditions, undergo development through two microbivorous feeding and moulting larval stages (L₁ and L₂) to reach a non-feeding larval stage, L₃ (Familton and McAnulty, 1997; Vlassoff *et al.*, 2001). These two pre-infective stages require moist, warm conditions for successful development and providing there is adequate moisture, optimum development occurs between 15 and 30°C (West *et al.*, 2009a). The second moulting stage is an incomplete moult because the L₃ larvae retains the cuticle in the L₂ stage as a protection mechanism until it is ingested (Familton and McAnulty, 1997). Under adequate moisture levels, the L₃ larvae

migrates out of the faeces and up the grass where they remain until they are ingested by the sheep (Familton and McAnulty, 1997). At this stage and until ingested, the larvae cannot feed and is said to be at an arrested state of development, awaiting host encounter (Familton and McAnulty, 1997; Sutherland and Scott, 2010). In unfavourable conditions, especially dry summers, the L₃ larvae goes into an arrested state of development and can survive for as long as 12 months in the ground (Familton and McAnulty, 1997). The length of time of development of eggs to infective larvae on pasture is variable and dependent on climatic conditions, but generally ranges from 2 – 12 weeks (West *et al.*, 2009a).

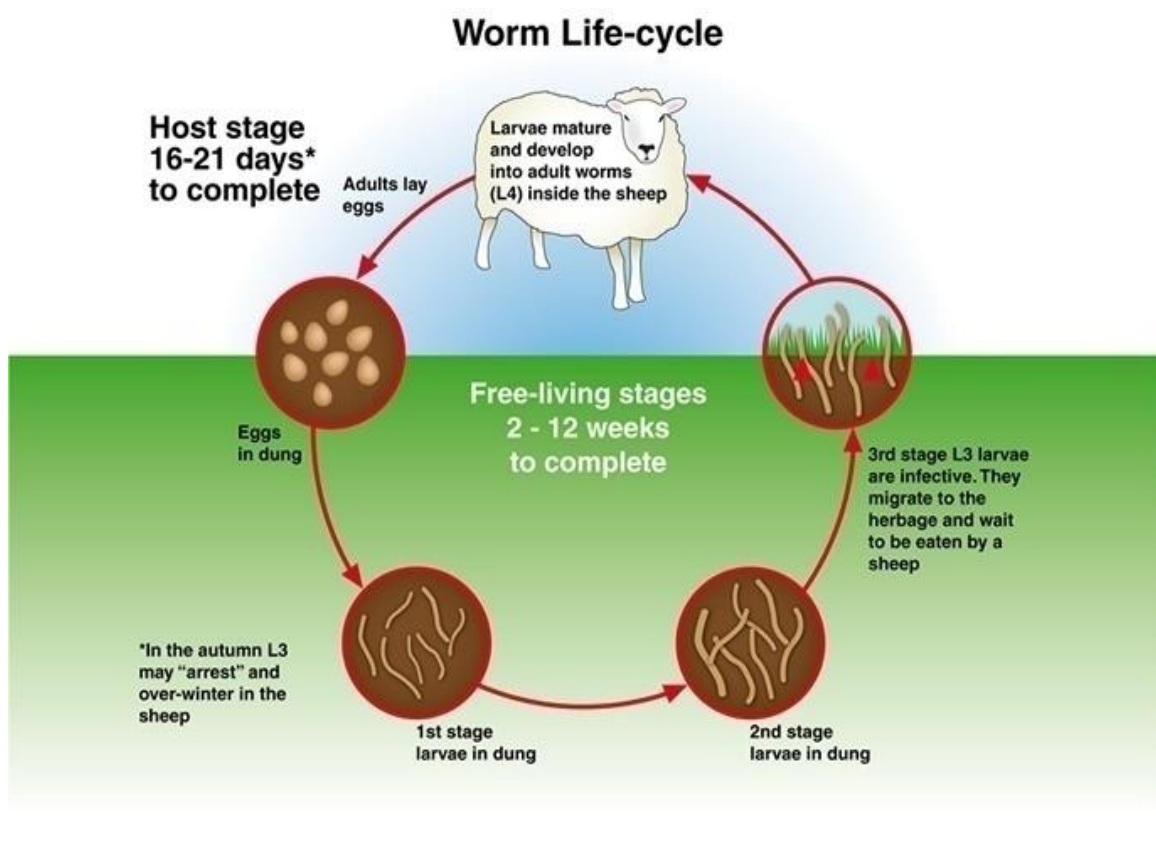


Figure 2.1: The basic lifecycle of a gastrointestinal nematode of sheep (Source: <http://www.scops.org.uk/endoparasites-worm-species.html>).

Once the L₃ larvae enters the digestive tract, they exsheath and remove the retained cuticle (Sutherland and Scott, 2010). The stimulus to exsheath normally occurs in the section of the digestive tract anterior to the site of infection, and usually occurs in response to changes in the carbon dioxide level, temperature and pH of the site (Familton and McAnulty, 1997; Sutherland and Scott, 2010). Consequently, abomasal parasites (*Teladorsagia circumcincta*) exsheath in the rumen, while the small intestinal parasites (*Trichostrongylus colubriformis*) exsheath in the abomasum. After exsheathing, the L₃ larvae move into the preferred area of development, which for *Teladorsagia* spp. is the abomasal glands and *Trichostrongylus* spp., the intestinal mucosa. Once there, the L₃ larvae moults and becomes L₄ larvae. The L₄ larvae feed on the abomasal glands and intestinal mucosa, thus

growing rapidly and within a few days, cause loss of function in the abomasal glands (*Teladorsagia* spp.) and intestinal mucosal damage (*Trichostrongylus* spp.) (Familton and McAnulty, 1997). After about 8-10 days, the L₄ larvae moult into immature adults (L₅), which mature and become sexually active over 7-10 days. Typically, it takes around 15-21 days from ingestion of the infective L₃ larvae to the appearance of worm eggs in the faeces; period known as the pre-patent period (Vlassoff *et al.*, 2001; West *et al.*, 2009a). The male and female adult worms in the host animal mate, the female produces fertile eggs which are passed out in the faeces of the host animal, and the cycle continues.

2.3.1 GIN population dynamics

GIN infections of sheep follow a common pattern from year to year. The pattern of faecal egg output closely follows the level of contamination on pasture (Figure 2.2) (McKenna, 1981). Generally, worm burdens in pasture build up to reach a minor peak in spring and a major peak in autumn (Vlassoff, 1982; West *et al.*, 2009a), although this is dependent on prevailing weather conditions that suit development. Breeding ewes are the initial source of pasture contamination and rise in FEC in spring (Figure 2.2). During this period, the ewe's immune system relaxes, partly due to repartitioning of ingested nutrients to accommodate the stress of pregnancy and lambing, resulting in them becoming susceptible again to infection (Brunsdon, 1971; Sykes, 1994; Vlassoff *et al.*, 2001). About four weeks after lambing, ewes regain their immunity, thereby becoming resistant to re-infection. However, the matured adult nematodes from the infective larvae ingested during the peri-parturient period may remain in the ewes for several weeks longer resulting in a minor peak in FEC about 6 – 8 weeks post-parturition (spring to early summer) before the majority of the adult worms are expelled (Figure 2.2) (Brunsdon, 1971; Vlassoff *et al.*, 2001).

The overwintered L₃ larvae and those from the spring peak give rise to the first generation of worms which accumulate in lambs over summer (refer to point 2 to 3 in Figure 2.2), due to their initial low intake of herbage and their inability to resist infection at young ages. The L₃ from the autumn peak produces the second generation of worms in lambs, resulting in further increase in FEC, reaching a peak in late summer to early autumn. The lambs then become the main source of pasture contamination in autumn (refer to points 3 and 4 in Figure 2.2). After peaking in autumn, larval challenge in lambs and on pasture rapidly declines in late autumn to winter. This is associated with the declining temperatures resulting in a decreasing proportion of eggs surviving on pasture, and also with the development of a significant immune capacity when the lambs are 10 -12 months of age (Figure 2.2) (Vlassoff and McKenna, 1994; West *et al.*, 2009a). After the elimination of most of their worm burdens, the lambs tend to remain resistant to re-infection from infective larvae, provided a constant level of exposure is maintained (Sykes, 1994; Vlassoff *et al.*, 2001).

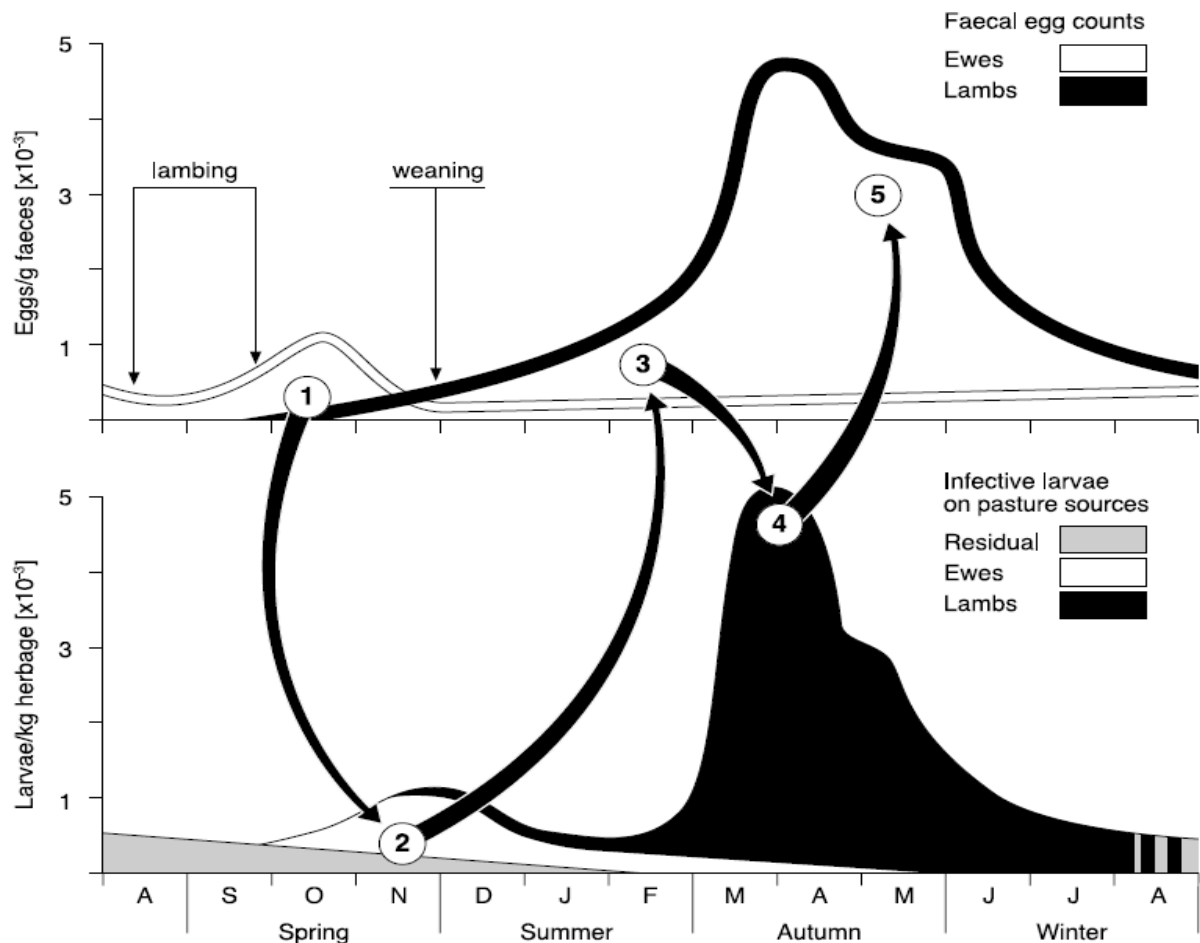


Figure 2.2: The interrelationship between pasture contamination by untreated ewes and lambs and the availability of infective larvae on pasture (Source: Vlassoff, 1982).

2.3.2 Impacts on the host

GINs impact small ruminants either directly or indirectly. The direct effects are associated with the pathological damage inflicted by the nematode infections on the gastrointestinal tract (GI). Indirect effects occur as a consequence of the host's response to the presence, level and effect of parasitism in the tract; of which, majority of the negative effects are associated with (Sutherland and Scott, 2010). The degree of damage caused by GINs to the GI tract is influenced to a large extent by the numbers and species of parasites present (level of parasitic challenge) and also by host factors including age, immunity, general health and plane of nutrition (Jackson and Coop, 2007; McKenna, 1997; Sykes, 1994).

The impact of GIN parasitism on the host animal is determined in studies to be closely associated with the size of larval challenge. According to McKenna, (1981), the sole presence of 5000 *Teladorsagia circumcincta* or *Trichostrongylus colubriformis* worms/kg of herbage is regarded as sufficient to cause clinical disease. In contrast, Steel *et al.*, (1980) observed that the threshold level of exposure for lambs to *Trichostrongylus colubriformis* infections for a reduction in LWG to be evident was between 950 and 3000 L₃/ week. A similar study carried out by Symons *et al.*, (1981) for

Teladorsagia circumcincta infections observed 12000 to 37500 L₃/week to be the threshold level of exposure of lambs for a reduction in LWG to be evident indicating species of parasite can have different levels of pathogenicity. The differences in size of challenge required to impair production in animals observed in the mentioned studies indicates that other biotic and abiotic factors surrounding the host animal could also play a part in influencing performance of animals in relation to the size of challenge.

GINs cause parasitic gastroenteritis in small ruminants (Jackson and Coop, 2007), which generally reflects the mixed infection status in grazing situations (Sutherland and Scott, 2010) and is usually accompanied by production loss. Impaired production in grazing animals is usually associated with clinical and sub-clinical parasitism (Steel, 1978; Sykes and Coop, 1976), with poor growth rates in young animals and loss of body weight in older animals (MacRae, 1993). Parasitic infection ranges from acute infection, which frequently result in high rates of mortality to chronic or clinical infection, which result in various degrees of morbidity and premature culling, to subclinical infection, with sheep appearing healthy but performing below potential (Jackson and Coop, 2007).

2.3.2.1 Direct impact of *Teladorsagia circumcincta*

Teladorsagia circumcincta, formerly referred to as *Ostertagia circumcincta*, are brownish, thread-like, and usually 1-2 centimetre long abomasal parasites of small ruminants (Familton and McAnulty, 1997; West *et al.*, 2009a). These worms inhabit and feed on the abomasal mucosal glands by burrowing into them. This causes damage to the acid producing cells of the mucosa, which can increase the pH of the abomasal fluid to values that interferes with protein digestion (Familton and McAnulty, 1997; Jackson and Coop, 2007). Consequently, there is a loss of macromolecules such as pepsinogen and plasma protein into the gut lumen, leading to hyperalbuminemia and increased levels of pepsinogen in the plasma (Jackson and Coop, 2007). These worms cause the disease teladorsagiosis in sheep. There are two type of teladorsagiosis; type I and II. Acute *Teladorsagia* infection (Type I), occur in lambs during their first season at grass as a result of ingestion of large number of infective larvae over a short time; with clinical symptoms such as watery diarrhoea, dehydration, inappetence and weight loss observed (Jackson and Coop, 2007). Sub-acute or chronic infections (Type II), occurs in ewes' and hoggets' in late winter/early spring, with affected sheep showing intermittent diarrhoea and progressive loss of body condition and weight (Jackson and Coop, 2007).

2.3.2.2 Direct impact of *Trichostrongylus colubriformis*

Trichostrongylus colubriformis are black, hair-like and usually less than 1 cm long intestinal parasites of small ruminants (Sutherland and Scott, 2010). These worms inhabit and feed on the intestinal

mucosa by penetrating the epithelial glands forming tunnels in the intestinal mucosa, which rupture resulting in oedema and loss of plasma proteins into the lumen of the intestines (Familton and McAnulty, 1997; Sutherland and Scott, 2010). These worms cause the disease trichostrongylosis in sheep. The clinical signs of acute infection by *Trichostrongylus* spp. displayed by the infected sheep include anorexia, persistent diarrhoea and weight loss, with varying degrees of hypoalbuminemia, damaged fleece and wool (Jackson and Coop, 2007). Common signs of sub-clinical infections are inappetence, poor growth rates and sometimes soft faeces.

2.3.2.3 Indirect impacts on the host

The indirect effects of the pathophysiological damages caused by GINs are reduction in feed intake and feed conversion efficiency; the two main factors contributing to reduced performance, mainly in LWG, milk production and reproduction in parasitized animals (Coop and Holmes, 1996; Coop *et al.*, 1982; Holmes, 1987; MacRae, 1993; Sutherland and Scott, 2010; Sykes, 1994; Sykes and Coop, 1977, 2001; Sykes and Greer, 2003). The reduction in feed intake occurs as a result of reduction in appetite, which in part, may reflect an indirect consequence of the feeding activity of the parasite in the GI tract (Coop *et al.*, 1982; Sutherland and Scott, 2010; Sykes and Coop, 1977). However, more recent studies have implicated compounds associated with the developing immune response to be responsible for the lack of appetite in sheep (Greer *et al.*, 2005; Greer *et al.*, 2008). Studies have reported a 20-50 % reduction in food intake in young lambs having subclinical infection, while total lack of appetite was observed in similar aged animals with acute infection status (Coop and Holmes, 1996; Kimambo *et al.*, 1988; Sykes and Coop, 1976, 1977; Sykes and Greer, 2003; Sykes *et al.*, 1988). Exposure of adult ewes to subclinical infection during pregnancy and lactation resulted in a reduction in food intake of 25 -30% only during lactation (Leyva *et al.*, 1982). This was suggested to be associated with their reduced intake and the relaxation of their immune system as a result of stress imposed during the parturition and lambing period (Sykes, 1994; Vlassoff, 1982). Reduction in feed conversion efficiency occurs as a result of reduction in the efficiency of use of absorbed nutrients (Coop and Holmes, 1996; Sykes and Coop, 1976, 1977). Gross efficiency of use of metabolisable energy (ME) for growth was observed to be reduced by 19-31% when growing lambs were exposed to *Ostertagia circumcincta* larval intakes of 1000 L₃/kg DM and over (Coop *et al.*, 1982). The impact of reduced feed conversion efficiency in lambs, as a result of high parasitic challenge, was found to be greatest on intestinal infections as opposed to the abomasal infections (Sykes *et al.*, 1988). Despite obvious impacts on the host animal, the underlying mechanisms of parasite induced inappetence and depressed feed conversion efficiency is not well understood to date (Sutherland and Scott, 2010), although components associated with the developing immune response have been implicated (Greer *et al.*, 2005; Greer *et al.*, 2008)

2.3.2.4 Metabolic consequences of infections

Depression of feed intake and feed conversion efficiency as a result of GIN parasitism, in turn, affects metabolism and partitioning of nutrients throughout the animals' body. As a result, energy, protein and mineral metabolism and utilization are compromised, leading to impaired soft tissue deposition, skeletal growth and meat, milk and wool production (Coop *et al.*, 1982; Holmes, 1993; McAnulty *et al.*, 1982; Poppi *et al.*, 1990; Sykes, 1983, 1994; Thamsborg and Agergaard, 2002).

GIN infections have been reported to affect protein (nitrogen) digestion and absorption. Generally, GIN infections induce protein deficiency in the host animal by increasing the demand for amino acid in the alimentary canal while reducing supply through the depression of appetite (Bown *et al.*, 1984, 1991a, 1991b; Kyriazakis *et al.*, 1996a; Poppi *et al.*, 1986; Sykes and Coop, 2001). The direct damage of the worms on the abomasum and intestine results in a 2-3 fold increase in leakage of plasma protein, secretion of mucus, together with sloughing of epithelial cells, consequently resulting in increased flow of protein into the proximal intestine for the purpose of repairing the gastrointestinal tract, synthesizing plasma proteins and producing mucoprotein (Bown *et al.*, 1991b; Poppi *et al.*, 1986; Sykes and Greer, 2003). The associated cost of this consequence is the reduction in ration digestibility, hence, the metabolisable energy (ME) available to the infected animals (Coop and Holmes, 1996; MacRae, 1993).

GINs have also been demonstrated to affect mineral metabolism. The presence of parasites was observed to reduce the absorption of phosphorus (P) from the small intestine (Bown *et al.*, 1989; Poppi *et al.*, 1985), resulting in inhibition of bone mineralisation, hence reduced bone formation (Sykes *et al.*, 1979), low plasma P levels and reduced salivary P secretions, which is suggested to be responsible for inducing lack of appetite (MacRae, 1993; Milton and Ternouth, 1982). The mechanisms for this in sheep dosed with *Trichostrongylus colubriformis* larvae are not fully understood, but were suggested to be related to reduced intestinal motility in the upper regions of the duodenum (Gregory *et al.*, 1985; Poppi *et al.*, 1990), and the consequent precipitation of the digesta P out of solution as calcium and magnesium complexes at pH 6-8 in the upper small intestine (Poppi *et al.*, 1985). Copper (Cu) metabolism in sheep infected with *Teladorsagia circumcincta* was demonstrated to be affected, primarily through the elevation of the abomasal pH, which decreased the solubility of the copper oxide wire particles (COWP) resulting in lowered uptake of Cu by the liver (Bank *et al.*, 1990a).

The many studies investigating impacts of GIN parasitism on the host used mono-specific infection. One major factor impacting host in these studies, is the reduction in feed intake, which in naive lambs, appears to be proportional to larval intake up to about 5000 larvae/day; being consistent across the major nematode species (Sykes and Greer, 2003). Mixed infections are typical in field

situations and studies have shown that effects of mixed infection on growth rate with *Trichostrongylus colubriformis* and *Teladorsagia circumcincta* parasites are multiplicative rather than additive (Steel *et al.*, 1982; Sykes *et al.*, 1988). Thus, results obtained from mono-specific infection studies could provide an underestimation of the effects of mixed infection on the host in field situations.

2.4 Host acquired immunity

Host immunity refers to the state of having sufficient biological defences within an animal to avoid or resist infection and disease. Development and acquisition of host immunity is influenced by exposure to parasite infection, level of challenge, nematode species present in the system and the host plane of nutrition. New-borns of all farmed animal species are born without the innate ability to resist all types of infections and diseases (Sutherland and Scott, 2010; Sykes, 1994). Consequently, GIN parasitism is most severe in this age group of animals. However, with aging and continuous exposure of naïve animals to infection, the negative impacts of infection decrease as host immunity is acquired (Sykes, 1994). Host immunity is generally complete after puberty, but is suggested to be maximal after the first partum (Sykes, 1994).

In the case of young lambs, host immunity to *Trichostrongylus colubriformis* and *Teladorsagia circumcincta* develops within 3-5 months following continued exposure to parasitic challenge (Kimambo *et al.*, 1988; Sykes, 1994). For *Trichostrongylus colubriformis*, the lambs develop immunity against incoming infective L₃ larvae first, enabling them to resist re-infection, followed by expulsion of most of the established adult worm burden (Dobson *et al.*, 1992). For *Teladorsagia circumcincta*, host immunity against resident adult worms develops first, resulting in expulsion of most of the adult worm burden and immunity against adult worm fecundity after some weeks. The newly ingested or arrested L₃ larvae in the abomasal glands develop into mature adults, where most are expelled resulting in acquired immunity against incoming infective larvae (Dobson *et al.*, 1992).

The time course of infection in naive lambs suddenly exposed to significant levels of infection was well established in a study by Kimambo *et al.*, (1988) (Figure 2.3). Infection of naive lambs with *Trichostrongylus colubriformis* resulted in a severe growth check from weeks 4 to 14 of infection, but the animals subsequently returned to a normal growth pattern after 14 weeks, which was associated with acquisition of immunity despite continued larval intake (Figure 2.3) (Kimambo *et al.*, 1988; Kyriazakis *et al.*, 1996b; MacRae, 1993; MacRae *et al.*, 1982; Poppi *et al.*, 1986; Sykes and Greer, 2003). The reduction in live weight gain from weeks 4 to 14 of infection was mostly associated with some loss of appetite (MacRae, 1993) and was proportional to the increase in FEC (level of parasitism), while the return to normal growth rate was associated with the increasing eosinophil counts (Figure 2.3). Eosinophils, mast cells and their secretory products are frequently associated

with developing resistance or immunity to GINs (Huntley *et al.*, 1987; Rothwell *et al.*, 1993); however their exact role has not yet been determined (Dobson *et al.*, 1992). The natural immunity of sheep accumulated during growth can fail at times, making them susceptible to re-infection. Immunity of the host animal is compromised when the animal is nutritionally stressed or ill, regardless of age, and in breeding ewes during parturition and lambing (Brunsdon, 1971; Sykes, 1994; Vlassoff *et al.*, 2001).

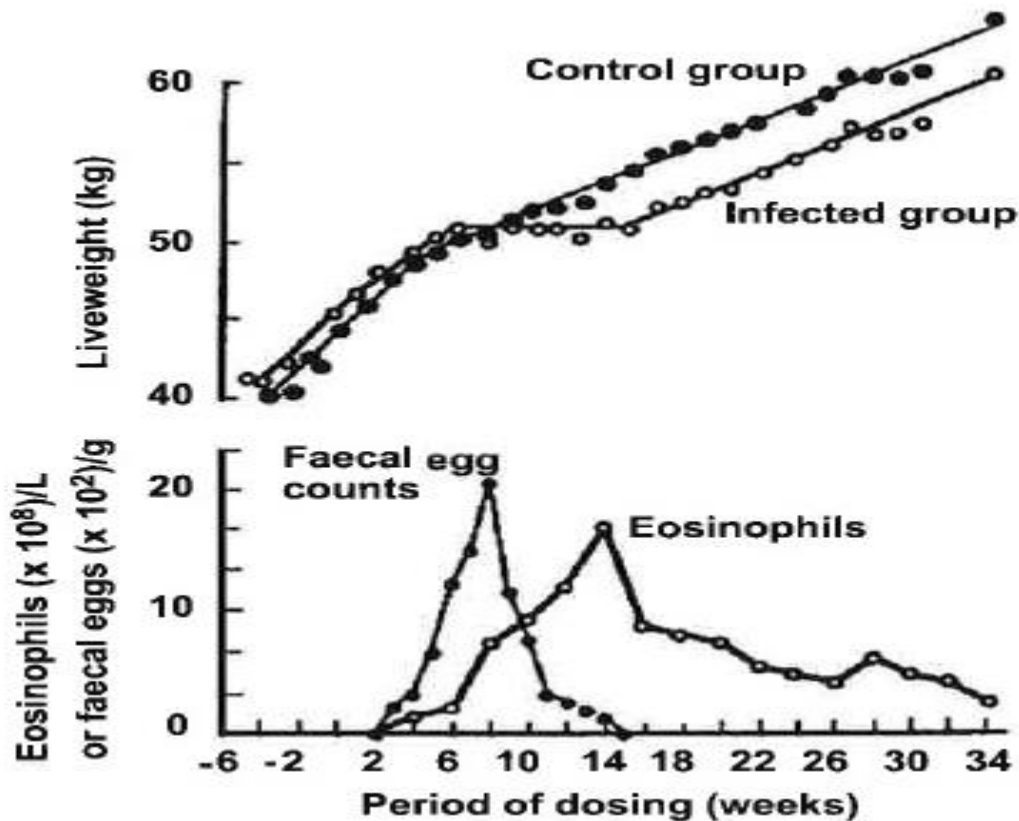


Figure 2.3: The typical time course to change in performance and in immune response of naive lambs exposed to chronic daily infection of larvae of the intestinal nematode *Trichostrongylus colubriformis* (Adapted from Kimambo *et al.*, 1988).

Acquisition of full immunity against GINs comes with a cost. Several authors have estimated an associated nutritional cost to be 15% of maintenance requirements in a mature animal (Greer *et al.*, 2005; Sykes, 1994) and 20-50% reduction in nutrient utilisation in grazing lambs (Greer *et al.*, 2005; Greer *et al.*, 2008). The major metabolic cost of immunity development is associated with parasite induced inappetence and is suggested to be the major cause of reduced feed intake, therefore LWG (Kyriazakis *et al.*, 1996b; Sykes and Greer, 2003). In order to maintain the stability of a production system, a balance of some sort has to be maintained between preventing excessive pathological damage that will result in reduced animal productivity and allowing the lambs sufficient exposure to infestation to stimulate development of immunity (Sykes, 1994).

2.5 Control options

Since eradication cannot be achieved, the aim of control measures is to maintain the nematode parasitic populations on pasture and the host at levels that are compatible with economic production (Vlassoff *et al.*, 2001; West *et al.*, 2009a). An essential prerequisite for the design of control measures against nematodes is an adequate understanding of their epidemiology (Jackson *et al.*, 2009; Nansen, 1987). Since there is a wide regional variation between sheep management systems and the type of parasites found on them, it is difficult for regional control measures to be developed and implemented (Jackson *et al.*, 2009). Researchers, farmers and their advisors have to decide upon which control measure will be suitable for each type of farming system, considering the environment, farm management in place and the epidemiology of the nematodes present in the system.

Anthelmintics constitute the classical control technique for mitigation of infections caused by GINs in conventional farming systems and are aimed at curbing or reducing helminth infections (Jackson *et al.*, 2009; Lawrence *et al.*, 2006; Leathwick *et al.*, 2001; McKenna, 1994; Nansen, 1987). On the other hand, the main control option to mitigate infections in organic farming systems lie in grazing management, which requires thorough knowledge of the farm system, the parasites on it and the epidemiology of the parasites. The widespread prevalence of AR, together with increased public concern about high chemical residues entering the food chain in conventional systems and an increasing consumer demand for organic products has stimulated investigation into alternative control measures other than chemotherapy (Athanasidou and Kyriazakis, 2004). Some of these approaches currently under investigation include breeding livestock for nematode resistance (Gray, 1997), biological control of nematodes using nematophagus fungi (Larsen, 1999), development of vaccines against helminths (Smith, 1999), nutrient supplementation of parasitized herbivores (Houdijk *et al.*, 2001) and use of bioactive forages (Coop and Kyriazakis, 1999).

2.5.1 Chemical control

At present, the main method of control of GINs throughout the world is through the use of anthelmintics (Jackson *et al.*, 2009; Kaplan, 2004; Leathwick *et al.*, 2001; Wolstenholme *et al.*, 2004). Anthelmintic drugs are the most effective control method of removing existing worm burdens and preventing or reducing the establishment of ingested L₃ (Kaplan, 2004; Lawrence *et al.*, 2006; Nansen, 1987; Sutherland and Scott, 2010) which constitute the two main aims of chemical control. Broad spectrum anthelmintics available for veterinary use are divided into five major classes based on their chemical structure and mode of action. These classes are namely, 1) benzimidazoles, 2) imidathiazoles/tetrahydropyrimidines, 3) macrocyclic lactones, 4) amino-acetonitrile derivatives (AAD) and 5) spiroindole. By combining two or more anthelmintic families into one product, it is

possible to extend the spectrum of activity and reach an improved efficacy (Sutherland and Scott, 2010). In New Zealand, farmers have continued using anthelmintics to successfully control nematode infection, despite reports of anthelmintic resistance. However, this has led to the increasing prevalence of resistant worm populations to the three previously available broad spectrum anthelmintics (Lawrence *et al.*, 2006; Leathwick *et al.*, 2001; Waghorn *et al.*, 2006). Over the last three decades, the animal farming community had assumed that the animal health industry could and would keep producing new anthelmintics with novel modes of action. However, that has not been the case, since after the introduction of macrocyclic lactones in the 1980's, there was a lapse of 25 years before the introduction of monepantel (in AAD class) in 2009 (Good *et al.*, 2012; Jackson *et al.*, 2009; Kaminsky *et al.*, 2008), which was closely followed by the release of the spiroindole class in 2012 (Good *et al.*, 2012). Chemotherapy for prevention or suppression of nematode populations in parasitized animals is now viewed as an unsustainable method of control due to the widespread prevalence of AR, and also because introduction of new drugs for continuation of chemical control cannot be relied upon (Jackson *et al.*, 2009).

With the increasing prevalence of AR, coupled with the uncertainty as to the availability of new drugs in the foreseeable future (Jackson and Coop, 2000; Kaplan, 2004), it is now widely accepted that sustainability of chemical-based worm control strategies will depend upon ensuring that some proportion of the nematode population remain in refugia (Besier, 2008; Jackson and Waller, 2008; Jackson *et al.*, 2009; van Wyk, 2001). Refugia, refers to the proportion of a nematode population that is free-living on pasture or the proportion of untreated animals in a flock (Besier, 2008; van Wyk, 2001; van Wyk *et al.*, 2006). The main idea behind the concept of refugia is to maintain susceptible nematode genotypes in the system which will dilute the population of resistant genotypes that evolve following application of anthelmintics, hence slow down the development of AR.

Consequently, most current recommendations to combat AR have pushed for the adoption of control measures with reduced treatment frequency (Besier, 1997; Jackson and Coop, 2000; Prichard *et al.*, 1980; Waller, 1985); however loss in production will still be observed. The only realistic option for sustainable GIN control is to develop novel non-chemical approaches that decrease the need for anthelmintic treatment, resulting in less use of anthelmintics (Hein *et al.*, 2001; Waller, 1997a, 1997b), but these approaches would not be as effective as anthelmintics (Kaplan, 2004). Therefore, there is a need for research to be conducted on systems or regimes that would reduce frequency of anthelmintic treatment and at the same time maintain animal production levels.

Several strategies are currently understudied; namely, targeted selective treatment (TST), targeted treatment and dilution strategy. For the purpose of this research, only the TST regime will be briefly reviewed. In the TST regime, only animals that would benefit most from the treatment are selected and treated at epidemiologically appropriate times, leaving the whole flock untreated (Besier, 2008;

Jackson and Waller, 2008; van Wyk *et al.*, 2006). Sreter *et al.*, (1994) observed that GI populations in grazing sheep were highly aggregated and over dispersed, with 80% of nematodes found in only 20-30% of the hosts, whilst the remainder of the flock had lower worm burdens. The susceptible nematode genes are therefore, maintained through the untreated flock. Since this strategy is aimed at disease susceptible or pasture contaminating animals, it requires the ability to identify these individuals within the flock. According to Greer *et al.*, (2009), identifying individuals requiring anthelmintic treatment using this strategy is the main problem hindering its adoption and use, especially in the temperate regions, where there are very few parasite specific indicators.

According to Kenyon *et al.*, (2009), an ideal indicator for use in a TST or target anthelmintic treatment regime should be cost-effective, simple to use, require minimal operator training and allow treatment decisions to be made sheep-side. Several indicators, proposed and used for the selection of disease susceptible animals, are classified into three broad groups; namely, pathophysiological markers such as anaemia and dag score; parasite-based markers such as FEC and production parameters such as live weight gain, body condition score and milk production (Besier, 2008; Kenyon *et al.*, 2009; van Wyk *et al.*, 2006). Studies have suggested that pathophysiological markers such as anaemia and dag scores are not suitable indicators for temperate countries, as there are no devised systems in place for anaemic assessment and also by the time animals are identified using dag scores, performance would have been jeopardized (Besier, 2008). Parasite-based markers such as FEC, on the other hand, has been and is currently the indicator for parasitism in all small ruminant farming systems; however, there are limitations with its practical use; namely, decision to select and treat animals is not sheep-side, different treatment threshold for different parasite species and higher producing parasite species (*Haemonchus contortus*) tend to mask the lower producing species (*Teladorsagia circumcincta* and *Trichostrongylus colubriformis*) (Kenyon *et al.*, 2009). In the case of production indices, live weight gain is considered to be a sensitive marker for selection of individuals in temperate areas. Two approaches for use of live weight gain as a selection indicator have been explored; namely to leave a proportion of the heaviest animals untreated at each time of drenching (Leathwick *et al.*, 2006) and to predict the weight gain of each animal and treat animals that fail to reach that pre-set live weight gain (Greer *et al.*, 2009).

2.5.2 Non-chemical control

2.5.3 Grazing management

For over 40 years, pasture rotations and grazing management in various guises, have been used to minimise the threat of GIN infestations in sheep production systems (Jackson *et al.*, 2009). Grazing management plays the role of reducing anthelmintic use and improving helminth control (Barger, 1997), with the main aim of minimizing the level of parasitic challenge on pasture, thereby providing

a clean pasture where stock can graze safely (Barger, 1999; Hein *et al.*, 2001; Jackson *et al.*, 2009; Nansen, 1987). In order for grazing management to be effective in reducing nematode infections in small ruminants, one must have a thorough understanding of the farm management system, the type and epidemiology of the parasites on it and its interactions with the host, the climatic conditions, together with the demands that grazing management will make upon the type of stock and the use of pasture (Barger, 1999; Jackson *et al.*, 2009). Some of the important epidemiological factors that are taken into consideration when using grazing management as a control option are infective larval availability on pasture on a daily basis, timing of peaks in larval availability, factors influencing survival of larvae on pasture at different times of the year and environmental requirements for egg hatching and larval development for the parasite concerned (Barger, 1999). When grazing management is used in conjunction with anthelmintics, care must be taken to maintain a proportion of the parasite population in refugia (Jackson *et al.*, 2009).

Some of the grazing management strategies that were practiced included the dose and move strategy (Michel, 1969), alternating hosts (Hein *et al.*, 2001; Southcott and Barger, 1975), use of cropping and aftermaths (Brunsdon, 1980), “clean grazing” (Mitchell and Fitzsimons, 1983; Mitchell *et al.*, 1984) and rotational grazing (Barger, 1999; Hein *et al.*, 2001). These strategies have been classified into three major classes; namely preventive, evasive and dilution strategies. In all these strategies, the main intention is to prevent contamination of clean pastures with worm eggs and anthelmintics is used less frequently (Barger, 1997).

Preventive strategies rely on putting worm-free animals on a 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 (Barger, 1997; Barger, 1999; Thamsborg *et al.*, 1999). An example of the preventive strategy used by Australian sheep producers for the control of GINs is to treat the lambs at weaning and then move them onto a clean or safe pasture, with a further two or three anthelmintic treatments at eight week intervals (Barger, 1997; Barnes *et al.*, 1995). This strategy is similar to the “Dose and move system”.

Another extreme form of preventive strategy is to alternate different species of host animals on the same pasture at intervals of 2 – 6 months, with anthelmintic applied when necessary and usually at the time of alternation (Donald *et al.*, 1987; Nansen, 1987; Thamsborg *et al.*, 1999). In this system, host specificity is used to prevent contamination and not anthelmintic, therefore the right host species should be used (Barger, 1997). Alternation of sheep and goat will probably not be beneficial, as they share the same parasite species (Barger, 1999). However, alternation of sheep and cattle is beneficial, as observed in some studies. Southcott and Barger (1975) observed 93 – 99% reduction in the number of *Haemonchus contortus* and *Trichostrongylus colubriformis* worm burdens when sheep

were alternately grazed with cattle for 6, 12 and 24 weeks during summer (Table 2.1). The authors also observed a 96% reduction in *Teladorsagia circumcincta* worm burden after 12 and 24 weeks, but not at 6 weeks of alternate grazing (Table 2.1). A later study by the same authors also observed greater LWG, 27% more wool production, decreased animal mortality of 34% and low numbers of *Trichostrongylus colubriformis* and *Ostertagia circumcincta* in sheep grazed under a cattle and sheep six monthly interchange grazing system for three years (Barger and Southcott, 1978). The observations of both studies suggest that cattle can be used to prepare cleaner pastures for safe grazing by sheep. Using this strategy, Barger and Southcott, (1978) and Donald *et al.*, (1987) observed no significant difference in parasitism and production of lambs drenched only once or twice compared with those receiving regular treatment over a three year period. These observations suggest that this strategy can be used to mitigate the effects of GIN infections and at the same time reduce the frequency of use of anthelmintic, with minimal loss in production.

Table 2.1: Geometric mean of worm counts of each species in groups of ten lambs slaughtered after one month on sheep pastures (Adapted from:Southcott and Barger, 1975).

Treatment	Species		
	H	O	T
6 weeks cattle	179	23	365
6 weeks sheep	6289	20	5947
Significance	***	NS	***
12 weeks cattle	100	1	342
12 weeks sheep	1340	27	7327
Significance	***	**	***
24 weeks cattle	1	36	101
24 weeks sheep	2378	855	5727
Significance	***	**	***

Keywords:

H – *Haemonchus contortus*; O – *Ostertagia circumcincta*; T- *Trichostrongylus colubriformis*
 NS- $P > 0.05$; * $P < 0.05$; ** $P > 0.01$; *** $P > 0.001$

The leader/follower system where susceptible calves are grazed rotationally on permanent pastures, followed by 2-3 year old heifers or cows, is also another form of preventive strategy (Downey and Fallon, 1973; Leaver, 1970). The idea behind this system is that young animals will only harvest the upper succulent part of the fresh herbage and then move to the next paddock; thus avoiding the infective larvae which are mainly localised in the lower growth to be eaten by the subsequently introduced older and immune animals (Nansen, 1987). This system was however, not widely adopted and used because it required careful grazing management and follower animals were usually exposed to high parasitic challenge on pasture (Nansen, 1987).

Evasive strategies rely on movement of livestock to another pasture just before the larvae resulting from eggs in the contaminated pasture are likely to appear in significant numbers (Barger, 1997;

Thamsborg *et al.*, 1999). The “Dose and move system” of Thomas and Boag for ewes and lambs is one example of an evasive strategy (Boag and Thomas, 1973). This system is based on the movement of animals to parasitologically safe pasture in mid-summer, and is usually combined with anthelmintic treatment (Michel, 1969; Nansen, 1987). Rotational grazing is also an evasive strategy, which is a grazing technique that involves intensive subdivision of pasture into paddocks which are grazed for short periods of time and then spelled for a relatively longer period (Barger, 1999). A rotational grazing study carried out by Burke *et al.*, (2009) on organically reared Katahdin lambs fed Bermuda grass under continuous and rotational grazing systems observed a greater requirement for de-worming in continuous stocked than animals under rotational grazing. Overall, rotational grazing systems in temperate climates are considered ineffective for the control of parasitic nematode because of the long survival times of infective larvae on pastures (Barger, 1999; Gibson, 1973).

Dilution strategies, on the other hand, rely on the use of a small population of susceptible and a larger population of resistant animals of the same or different species to reduce the herbage infestation resulting from their combined faecal output of worm eggs (Barger, 1997; Thamsborg *et al.*, 1999). The effectiveness of this strategy depends on the proportion of the susceptible and resistant animals in the grazing system (Thamsborg *et al.*, 1999). According to Barger (1997), there has been little work done on dilution strategies as a means of worm control, but they are widely used in practice and are often, but not always, aimed at giving younger animals a greater choice in diet selection. A mixed grazing study by Jordan *et al.*, (1988) observed 25% lower helminth infestations, with a 6.4% more LWG in lambs grazed with cattle and sheep compared with those grazed under the sheep only grazing system. The ewes and cattle were the resistant animals that provided the dilution effect and the lambs were the susceptible animals that benefited from this effect.

2.5.4 Supplementary feeding

Immunological management, is another approach of mitigating the impact of GIN on small ruminants without frequent use of anthelmintics (Jackson *et al.*, 2009). This can be achieved through optimized nutrition to improve the host plane of nutrition (Coop and Kyriazakis, 1999; Van Houtert *et al.*, 1995a) and can be achieved through supplementary feeding. The host plane of nutrition is suggested to be the most crucial element influencing host immunity development and maintenance, and the epidemiological differences between the various GINs, especially in lambs (Jackson *et al.*, 2009). A high plane of nutrition has long been recognized for its effect in the reduction of susceptibility of sheep to GI parasites and their depressing effect on growth (Bown *et al.*, 1991a, 1991b; Gibson, 1963).

Given that GINS generally induce protein deficiency through their direct effect on host tissue (Bown *et al.*, 1991a, 1991b; Sykes and Coop, 2001), it has been proposed that protein supplementation

increases the rate of acquisition of immunity and resistance to re-infection (Coop and Holmes, 1996; Coop *et al.*, 1995; Holmes, 1993; Sutherland and Scott, 2010). For young or naive animals such as lambs, improved nutrition through protein supplementation would result in more nutrients being available for faster development of the immune response, and also enhance their resilience in the face of larval challenge. In immunologically mature animals, protein supplementation would enhance their expression of immunity against parasites; commonly referred to as resistance (Sutherland and Scott, 2010; Sykes and Coop, 2001). Protein supplementation studies on naive lambs have observed reduced level of parasitism during the course of an infection, which was associated with enhanced resilience; adding support to the positive effect of protein supplementation on resilience in young animals (Houdijk *et al.*, 2001; Van Houtert *et al.*, 1995a; Van Houtert *et al.*, 1995b).

Further evidence supporting the ability of improved protein nutrition to mitigate impacts of infection is given in the study by Bown *et al.*, (1991b). Lambs infected with 3000 L₃ of *Trichostrongylus colubriformis* larvae and supplemented with 50 g of sodium caseinate per day (protein supplement) had slightly higher body weights than their infected and un-supplemented counterparts and also the uninfected control counterparts (Figure 2.4). The protein supplemented lambs were able to reduce their worm burdens and faecal egg count by 50% compared with lambs on saline or glucose infused controls. Faecal egg output and intestinal worm count of protein supplemented lambs observed at slaughter were reduced at 12 weeks but not at 6 weeks of treatment, suggesting that infusion of sodium caseinate may have permitted the host to limit the size of infection through the development of an effective immune response to infection, rather than by changing its natural resistance (Bown *et al.*, 1991b). Similar results were observed in a previous study (Bown *et al.*, 1986). In another study, Coop *et al.*, (1995) observed lower worm establishment which was associated with a 3-fold increase in mast-cell protease activity in the abomasal tissue of sheep infected with *Teladorsagia circumcincta* when they were supplemented with sodium caseinate compared with those that were not exposed to the parasite. Their findings supports the idea that supplementation of protein to naive lambs has the impact of accelerating development of immunity against GIN parasites. A supplementation study by Donaldson *et al.*, (1998) on peri-parturient ewes also provided clear evidence that changes in resistance to nematode parasites experienced by ewes around parturition can be influenced by protein nutrition.

In addition to protein supplementation, it has also been established that some trace elements, including P, Cu, iron, zinc, molybdenum and cobalt, have some influence in the host-parasite relationship (Coop and Holmes, 1996). A low or deficient mineral diet will have a negative effect on the host animal by increasing the opportunity for nematode establishment. In such studies, supplementation of different minerals will have some influence on the host-parasite relationship. A supplementation study by Coop and Field (1973), showed that increasing the P content of the diet

(from 1.88 to 2.75 P/kg) increased the LWG of lambs receiving 2500 *Trichostrongylus vitrinus* L₃/day. The lambs also had lower FEC and total worm burdens (1240 vs. 10950 worms) compared with the lambs receiving low P diets (below 1.88 P/kg). This finding suggested that a diet deficient in P may impair the development of resistance to continuous infection (Coop and Holmes, 1996), consequently resulting in lower levels of production.

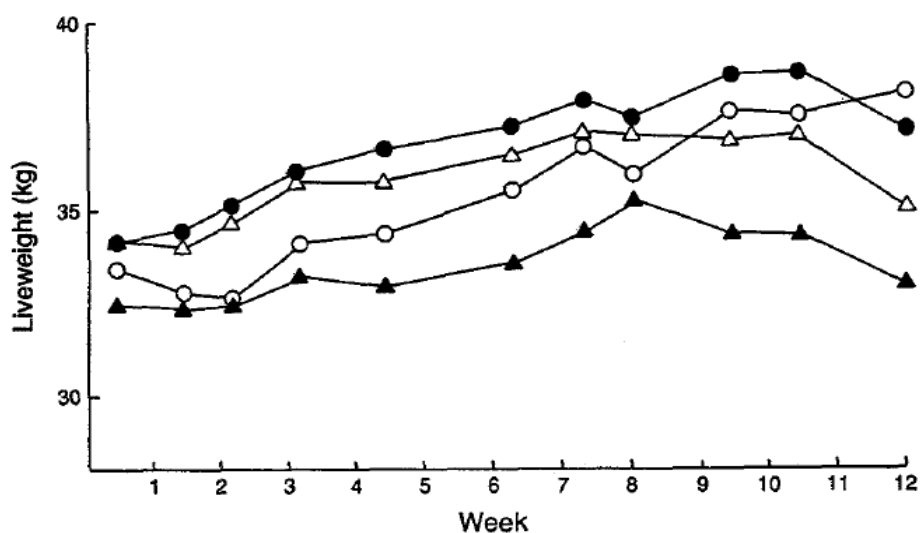


Figure 2.4: Mean body weight (in kg) of lambs infected daily with 3000 *T. colubriformis* larvae (SI, ▲), of lambs similarly infected but given abomasal infusion of protein (PI, ●), or energy (EI, △) and of their uninfected controls (C, ○) over 12 weeks (Source: Bown *et al.*, 1991b).

Bank *et al.*, (1990b), observed a reduction in the establishment of *Haemonchus contortus* and *Teladorsagia circumcincta* by 96% and 56%, respectively, when COWP was administered five days prior to infection of lambs. Their finding suggests that Cu has anti-microbial properties. Molybdenum supplementation to sheep trickle-infected with *Trichostrongylus vitrinus* and *Haemonchus contortus*, reduced their worm populations by 23% and 78%, respectively, with higher number of intraepithelial mast cells occurring in the latter group of lambs (Suttle *et al.*, 1992). This result is suggestive towards a role of molybdenum in development of host immunity. Cobalt supplementation was also reported to have an effect on the host-parasite relationship. Ferguson *et al.*, (1989), observed increased FEC and pepsinogen levels in cobalt deficient lambs infected with *Teladorsagia circumcincta*, suggesting that cobalt plays a role in the mitigation of parasitic burden. Despite mineral elements having some influence in the host-parasite relationship, there is little work done in this area. Therefore, further studies need to be carried out in order to determine how these minerals influence the host-parasite relationship and how they can be used in mitigating effects of GIN infections.

2.5.5 Use of bioactive forages

Bioactive forages are plants that contain compounds that are active against parasites; collectively known as plant secondary metabolites (PSM) (Athanasiadou and Kyriazakis, 2004; Hoste *et al.*, 2006;

Jackson *et al.*, 2009). PSM found in plant parts and extracts of bioactive forages have been used as a control agent for parasites over many centuries and there is evidence of their anti-parasitic effects (Githiori *et al.*, 2005). Table 2.2 contains some of the temperate bioactive forages and their main PSM. These forages are known to have both positive and negative effects associated with the PSM, which vary with species of forage, parasite type and the host animal. The mechanism by which these forages control gastrointestinal parasites in parasitized animals is not clear, but, was suggested and hypothesized to be either from a direct anthelmintic-like effect on the L₃ larvae or adult parasites or an indirect effect as a consequence of nutritional effects upon immunity or resilience of the host animal (Athanasiadou *et al.*, 2005; Hoste *et al.*, 2006; Jackson *et al.*, 2009; Tzamaloukas *et al.*, 2005).

Table 2.2: Concentration of secondary plant compounds in temperate forage species with pastoral value for New Zealand farming systems (Extracted from Ramírez-Restrepo and Barry, 2005).

Forage	Total condensed tannins content (g/kg DM)	Other known plant secondary compounds
Grasses		
<i>Lolium perenne</i> (perennial ryegrass)	1.8	Endophyte alkaloids 12-30 mg/kg DM
Legumes		
<i>Lotus corniculatus</i> (birdsfoot trefoil)	47	
<i>Lotus pedunculatus</i> (big trefoil)	77	
<i>Hedysarum coronarium</i> (sulla)		
Spring	84	
Autumn	51	
<i>Trifolium repens</i> (white clover)		
Normal	3.1	Cynaogenic glycosides
High CT selection	6.7	
<i>Trifolium pratense</i> (red clover)	1.7	Iso-flavones 7-14 g/kg DM
<i>Medigo sativa</i> (Lucerne)	0.5	Counmestrol 0 - 100 mg/kg DM
Herbs		
<i>Cichorium intybus</i> (chicory)	4.2	Sesquiterpene lactones 3.6 g/kg DM
<i>Plantago lanceolata</i> (plantain)	14	Iridoid glycosides Catapol 8 g/kg DM Aucubin 22 g/kg DM

2.5.5.1 Condensed tannins

Bioactive forages are usually leguminous in nature; hence, a common PSM is condensed tannins (CT). CTs are plant phenols that form pH reversible bonds with forage protein, which protects protein from being broken down in the rumen, but allows digestion to occur in the abomasum; thereby increasing

the metabolisable protein supply to the animal (Mueller Harvey and McAllan, 1992; Niezen *et al.*, 1993; Ramírez-Restrepo and Barry, 2005; Waghorn *et al.*, 1987). This effect has been considered to be responsible for the enhanced immunological response of the parasitized host towards GINs (Coop and Kyriazakis, 1999) and agrees with previous observations of benefits of improving the protein supply to the young lambs. CTs consumed in average levels have been associated indirectly with increased weight gain, wool growth, milk secretion (Barry and McNabb, 1999; Butter *et al.*, 2000, 2001) and decreased detrimental effects of gastrointestinal parasitism (Aerts *et al.*, 1999). The effective dosage varies with forage species. Results from studies using housed, artificially infected sheep has suggested 35g of CT per kg DM to be the minimum active concentration against GINs (Athanasidou *et al.*, 2001a; Athanasidou *et al.*, 2000a). Several other studies investigating the indirect effect of CT against GIN parasitism obtained inconsistent results suggesting the need for further studies to be carried out to determine effect of this PSM (Athanasidou *et al.*, 2000a, 2000b, 2001b, 2001c; Athanasidou *et al.*, 2005; Niezen *et al.*, 2002a; Tzamaloukas *et al.*, 2005). Nevertheless, the positive results obtained in experimental studies suggests that feeding of plants containing CT might help reduce the effect of parasitism by increasing post-ruminal availability of dietary protein, consequently playing a role in mitigating the establishment or persistence of GINs (Niezen *et al.*, 1993).

2.5.5.2 Evidence of anti-parasitic effect of PSM in bioactive forages

Evidence of anti-parasitic effect of PSM-rich forages against GINs is mainly provided through *in vitro* assays (Athanasidou *et al.*, 2005). *In vitro* studies using CT extract, reduced the number and survival ability (Athanasidou *et al.*, 2001c), and motility and migration of L₃ larvae in sheep (Lorimer *et al.*, 1996). These results confirm that PSMs have an anti-parasitic effect. *In vivo* supplementation studies have also provided some evidence (Athanasidou and Kyriazakis, 2004). For example, Athanasidou *et al.*, (2000a) observed a 50% reduction in egg nematode (parasite worm burden) excreted by sheep supplemented with tannin-rich forage than in sheep offered tannin-free food. Results from grazing studies, on the other hand, are inconsistent. Marley *et al.*, (2003) observed a 56% reduction in total helminth intensity associated with a 50% reduction in total adult worm burdens in sheep grazed on birdsfoot trefoil compared with sheep on grass/clover while other studies using the same forage did not observe any effects (Niezen *et al.*, 1998a; Niezen *et al.*, 1998b). Some factors suggested for the variable effect of birdsfoot trefoil and the other PSM-rich forages in the mentioned grazing studies were the concentration and structure of the PSM in the forages (Athanasidou and Kyriazakis, 2004; Hoste *et al.*, 2006), initial parasitic challenge at start of study, cultivar differences in relation to the forage (Marley *et al.*, 2003) and difference in other nutritional characteristics of the forage.

Although results from *in vitro* and *in vivo* supplementation studies provide evidence of anti-parasitic effect of PSM-rich forages, results from *in vivo* grazing trials show inconsistent effects. This is attributed to the different conditions in which these studies are carried out. In the two former studies, most parameters in the study are controlled, whereas in grazing trials this is not always possible, suggesting that conditions of the grazing trial may affect the bioavailability of the active compounds (Athanasidou and Kyriazakis, 2004; Athanasidou *et al.*, 2005).

2.5.5.3 Consequences of consumption of PSM-rich forages

Besides having anti-parasitic properties, bioactive forages also have anti-nutritional properties which are associated with the PSM present in the forage. The anti-nutritional consequences of consuming PSM-rich forages known in studies on herbivores are shown in Table 2.3. It is evident that the anti-parasitic effect (positive) of these forages must outweigh the anti-nutritional consequence (negative), in order for them to have a role in GIN parasite control in the parasitized host (Table 2.4; consequence (C)). The success of this is dependent on the strength of the PSM on the performance of the parasitized host (Athanasidou and Kyriazakis, 2004).

Table 2.3: Negative effect of some plant secondary metabolites on the host animal.

PSM	Negative effect on host	References
Condensed tannins	Reduced feed intake, reduced digestibility, impaired rumen metabolism, mucosal toxicity consequently leading to reduction in nutrient absorption.	(Barry and McNabb, 1999; Butter <i>et al.</i> , 2000; Dawson <i>et al.</i> , 1999; Min <i>et al.</i> , 2003; Reed, 1995)
Saponins	Reduced feed intake, growth impairment, haemolytic action and bloat in ruminants.	(Milgate and Roberts, 1995)
Alkaloids	Lesions in the nervous system	(Conn, 1979)
Glycosides		
Terpenoids		

Table 2.4 shows three possible consequences of consuming bioactive forages. These consequences vary with type of feeding or grazing management. In supplementation studies, net cost (A) and neither net cost nor benefit (B) were observed, whereas, in grazing studies, all three consequences were observed. Supplementation studies have observed lower performance in sheep on PSM diets compared with that of their PSM-free counterparts (Athanasidou *et al.*, 2000a, 2000b, 2001b, 2001c), which as described by Table 2.4, is a net cost (A). In the study by Athanasidou *et al.*, (2000a), *Trichostrongylus colubriformis* infected sheep given access to food containing CT reduced their level of parasitism but at the same time reduced their body weight by 20% when compared with their counterparts fed on tannin-free food. In another study, by the same authors 40 g/kg food intake of CT extract reduced *T. colubriformis* burdens but didn't provide any advantage in animal performance

(Athanasidou *et al.*, 2001b). This indicates that the parasitized herbivores obtained neither cost nor benefit from PSM consumption (consequence B), which is a result of the anti-nutritional effects cancelling out the anti-parasitic effect. No literature on supplementation studies have been found to support consequence (C), which is improved performance of PSM-fed animals compared with that of PSM-free herbivores. However, in contrast to supplementation studies, results from grazing studies on PSM-rich forages showed reduced level of parasitism and improved performance of parasitized host (Hoskin *et al.*, 1999; Marley *et al.*, 2003; Niezen *et al.*, 1995).

Table 2.4: The consequences of PSM consumption on the performance of parasitized herbivores in relation to the performance of non-parasitized herbivores not given access to PSM* (Source: Athanasidou and Kyriazakis, 2004).

Performance of non-parasitized herbivores (NPH; arbitrary units)	Performance of parasitized herbivores (PH)		Consequences of PSM consumption on parasitized herbivores
	- PSM (% NPH)	+PSM (% NPH)	
100	80	60	Net cost (A)
100	80	60	Neither cost nor benefit (B)
100	80	81-100	Net benefit (C)

*Assumption: Consumption of PSM will lead to a reduction in parasitism.

(A), when the performance of the herbivores given PSM is lower than that of their PSM-free counterparts, a net cost is expected from PSM consumption; (B), when the performance of PSM and PSM-free herbivores is similar, then parasitized herbivores obtain neither cost nor benefit from PSM consumption; and (C), when performance of PSM fed herbivores is improved compared with that of PSM-free herbivores, then the parasitized herbivores obtain a net benefit from PSM consumption.

2.5.5.4 Strategies for use of PSM-rich forages

Bioactive forages can be used as a control for GIN parasitism in two ways; either short or long term. Short-term strategies would see parasitized animals given PSM-rich forages for short periods of time in order to reduce their level of parasitism before returning to conventional grazing (Athanasidou and Kyriazakis, 2004; Thamsborg *et al.*, 1999), whereas, in long-term strategies, parasitized animals will be given access to PSM-rich forages throughout most of the grazing season (Athanasidou and Kyriazakis, 2004; Thamsborg *et al.*, 1999).

In the short-term strategy, PSM-rich forages would act as a 'de-worming' paddock. Hence, the adverse effects of consumption on parasitized host, as mentioned above, will be short term. For example, a two week grazing study by Tzamaloukas *et al.*, (2005), using chicory, lotus, sulla and grass/clover as control, observed a 68% reduction in *Teladorsagia circumcincta* adult worm burdens in parasitized lambs grazing chicory compared with 44, 37 and 19% reduction in animals in the grass/clover, sulla and lotus treatment groups, respectively (white columns in Figure 2.5). When compared with the grass/clover control group, the number of incoming infective larvae were similar

amongst all forages (black columns in Figure 2.5), suggesting a lack of effect of the forages on the establishment of the incoming larvae. The reduction in adult worm burden indicates that potential short term penalties on animal performance by consumption of chicory could lead to long-term benefits, such as lower level of parasitism in the grazing environment; hence, lower parasite exposure and greater protection from parasites in subsequent grazing seasons (Athanasidou and Kyriazakis, 2004). The results obtained for sulla and lotus treatments were in contrast with those of another grazing study, where reductions in *Teladorsagia circumcincta* worm burden was observed when parasitized sheep were fed lotus for a period of five weeks (Niezen *et al.*, 1998b), and sulla and lucerne for a period of six weeks (Niezen *et al.*, 1994; Niezen *et al.*, 2002b). One of the reasons suggested for the lack of anti-parasitic effect of these forages on adult worm burden was due to the short period of exposure of only two weeks (Tzamaloukas *et al.*, 2005).

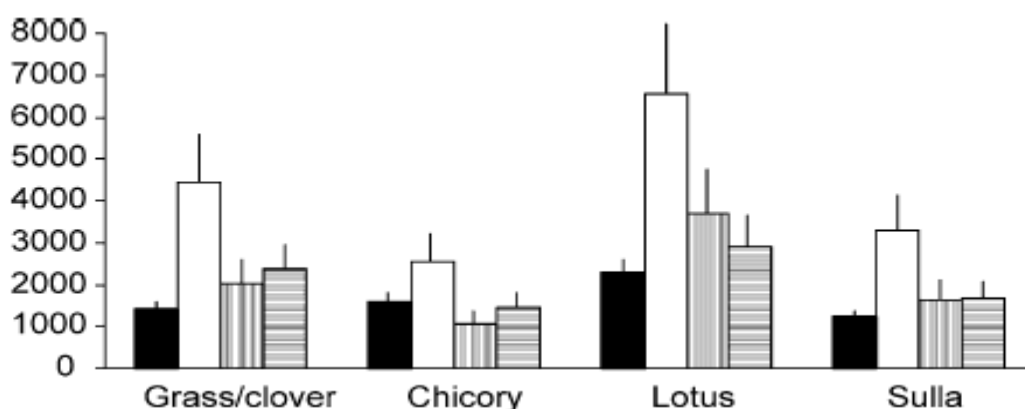


Figure 2.5: Back transformed mean of immature (■), total adult (□), male adult (▨) and female adult (▩) worm burdens recovered from the gastrointestinal tract of lambs infected with two single doses of 8000 L₃ of *Teladorsagia circumcincta* on days 1 and 28 of the experiment and grazing one of the grass/clover, chicory, lotus and sulla sward. The vertical bars indicate the confidence intervals (95%) (Source: Tzamaloukas *et al.*, 2005).

Long term strategies could only be of advantage and benefit the parasitized host if the short term strategies were effective against the parasites. Due to their anti-nutrition properties, long-term negative effects of these forages were reported to be more severe than the short term ones and are usually associated with irreversible post-ingestive consequences (Reed, 1995; Silanikove *et al.*, 2001). Therefore, careful considerations must be made before using long-term strategy.

The adoption and use of bioactive forages such as chicory by livestock farmers will depend on their agronomy under grazing, as well as their nutritive and feeding value (Ramírez-Restrepo and Barry, 2005). Despite having some parasitological benefits, it is simply impractical for farmers to have large areas of their farm dedicated to growing bioactive forages. However, only a small proportion of the farm can be planted as a de-worming paddock, where only animals that would benefit from the

treatment is selected to graze on, then chicory may provide a useful tool in reducing the reliance on anthelmintics.

2.5.6 Chicory

Chicory (*Cichorium intybus*) is a perennial herb, which has a high nutritive value, rapid rate of rumen degradation and high voluntary intake (Niezen *et al.*, 1994). Its mineral content is higher than that of ryegrass (Barry, 1998) and it contains secondary compounds such as sesquiterpene lactones and CTs that are known to have anti-parasitic properties (Molan *et al.*, 2000; Molan *et al.*, 2003). The concentration of CT in chicory varies seasonally, but is usually low around 4.2 g/kg DM. Chicory also contains other PSM including caffeic acid derivatives, flavonoids and coumarins (Hoste *et al.*, 2006); however, no studies have been done to test their effect against GIN infections. Chicory has been demonstrated to have anti-parasitic effect on the abomasal, but does not appear to be as effective against intestinal parasites of sheep in both mixed and mono-specific infections (Athanasidou *et al.*, 2005; Athanasidou *et al.*, 2007; Heckendorn *et al.*, 2007; Hoskin *et al.*, 1999; Hoskin *et al.*, 2003; Marley *et al.*, 2003; Miller *et al.*, 2011; Niezen *et al.*, 1994; Scales *et al.*, 1995; Thamsborg *et al.*, 2003; Tzamaloukas *et al.*, 2005). The ensuing effect of the anti-parasitic effect of chicory were observations of superior growth rates in lambs fed chicory compared with that of other forages, suggesting increased resilience to parasitism. According to Athanasidou *et al.*, (2005), there is no published evidence of a direct or indirect effect of chicory against intestinal nematodes in parasitized sheep.

A short-term grazing study by Tzamaloukas *et al.*, (2005), reported chicory fed *Teladorsagia circumcincta* infected lambs to have a 42% lower total number of adult, consisting of 50% lower male adult worm burden compared with those fed with ryegrass/white clover for two weeks. The concentration of CT used in this study was low, suggesting that the anti-parasitic effect was due to sesquiterpene lactones (Molan *et al.*, 2003); with the other factor being chicory's tall morphology (Moss and Vlassoff, 1993). There is limited information on the anti-parasitic effects of sesquiterpene lactones in chicory in but *in vitro* studies have observed anti-parasitic effects of this PSM on the abomasal nematodes of sheep (Molan *et al.*, 2003). Another short term grazing study using four PSM-rich forages (lotus, sulla, sainfoin, chicory) observed no evidence of a direct anthelmintic effect of the four forages towards an establishing or an established *Trichostrongylus colubriformis* infection; however, trends in reduction of abomasal adult worm burdens were observed in the chicory fed animals (Athanasidou *et al.*, 2005). Heckendorn *et al.*, (2007), observed a 90-96% reduction in egg production capacity of adult female worms of *Haemonchus contortus*, according to the mean FEC per gram of dry faeces and total daily egg output values, and a tendency for a reduction of 15% in worm burdens after two weeks of feeding parasitized sheep with chicory. In another study, low FEC was observed in a study where *Teladorsagia circumcincta* infected ewes grazed chicory compared with

grass/clover (Kidane *et al.*, 2010). In a medium term grazing trial by Marley *et al.*, (2003), lambs fed chicory had 40% lower adult abomasal worm burdens compared to their ryegrass and white clover counterparts after 35 days. There was, however, no effect of chicory on adult small intestine worm burden as indicated by the helminth intensity per gram organ weight of lambs.

Results from studies showing improvement of growth rates of grazing animals and reductions in abomasal worm burdens, suggest that it may be possible to obtain either a parasitological benefit or improved resilience through short-term grazing of chicory. According to Ramirez-Restrepo and Barry, (2005), use of chicory for the mitigation of the effects of gastrointestinal nematode infections is gaining acceptance in the animal farming community. Despite the increasing acceptance, there are several factors that constrain the adoption of chicory into farming systems. These constraints are related to their relatively weak agronomical qualities, which include poor persistence, delicate grazing management and poor matching of growth to animal demand due to its relatively high thermal requirement, and subsequent slow growth in spring when the animals are lactating. Chicory has no means of vegetative propagation under grazing; therefore plant density declines with time. In order for stands of chicory to last longer, careful pasture and animal management is required. According to Ramirez-Restrepo and Barry, (2005), chicory stands can last for 4-6 years in New Zealand conditions, with careful management such as not grazing during wet winter weather.

2.5.7 Plantain

Plantain (*Plantago lanceolata*) is an herb species which is widely distributed in grasslands throughout the temperate areas (Stewart, 1996). It is highly palatable when the leaves are young, but as the plant matures, the leaves, including the stem becomes unpalatable (Ivins, 1952). Plantain contains high levels of calcium, magnesium, phosphorus, zinc, copper and cobalt which are similar to those of perennial ryegrass/white clover pasture combinations (Forbes and Gelman, 1981; Tiley *et al.*, 1990; Wilman and Riley, 1993). It also contains two nematicidal agents; namely aucubin (Ishiguro *et al.*, 1982) and phenylpropanoid glycoside verbascoside (Fajer *et al.*, 1992). These agents were observed to have antimicrobial effects (Andary *et al.*, 1982; Ishiguro *et al.*, 1982). Concentrations of aucubin are very high in plantain, usually up to 3% of DM, but vary depending on genotype, soil fertility (Adler *et al.*, 1995) and other factors such as increasing leaf age (Stewart, 1996). The concentration of phenylpropanoid glycoside verbascoside is also high, up to 9% of DM (Fajer *et al.*, 1992). These compounds could have a negative effect on parasitism, therefore should be understudied.

According to Stewart, (1996), there has been much interest in the anthelmintic properties of plantain. However, field trials up to 1996 have failed to achieve results in favour of the anthelmintic properties (Knight *et al.*, 1996; Robertson *et al.*, 1995). For example, plantain among other bioactive forages, was used in a grazing trial to test its anthelmintic effectiveness on production in parasitized

lambs (Niezen *et al.*, 1998b). Lambs in the plantain treatment group had low intake, resulting in weight loss, high number of FEC and total faecal intensity compared to those grazed on grass/clover. The low intake was suggested to be associated with the low palatability of the herbage as it matured.

2.6 Integrated control of gastrointestinal nematodes

Integrated pest control is based on the rational use of a combination of different control measures available, be it chemical or non-chemical, with the farm management system in order to achieve effective parasite control according to economic thresholds (Kahn and Woodgate, 2012) and reduce the reliance on anthelmintics (Thamsborg *et al.*, 1999). Integrated pest management strategies require detailed knowledge of the ecology of the parasite concerned and the application of ecological principles (Kahn and Woodgate, 2012). The main aim of most GIN integrated control systems for ruminants is to minimize larval challenge on pasture to susceptible stock; this is because of the understanding that larval challenge is responsible for the development and severity of GIN infections (Nicol and Everest, 1997). According to Thamsborg *et al.*, (1999), the combination of two or more less effective control methods can reduce infection levels substantially and reach the appropriate control desired. Integrated parasite control varies with livestock species, farm type and the management system of the farm. According to Waller, (1999), integrated approaches to the control of nematode parasites are the only way to ensure the sustainability of parasite control in the future. Some examples of integrated control include the different types of grazing management; combination of two effective anthelmintics and then releasing animals to low infective pasture which is likely to result in greater selection for resistance to both anthelmintics (Barnes *et al.*, 1995; Leathwick *et al.*, 2009; Waller *et al.*, 1990) and use of FEC to determine when treatment is necessary, thereby reducing the frequency of drenching to applications at epidemiologically determined critical times (Anderson, 1990; Woodgate and Besier, 2010).

Integrated control should start at the most vulnerable and sensitive class of stock, which are the naive lambs, pre- and post-weaning. This could be done in several ways. Ewes are known to be the major source of pasture contamination of L₃ infective larvae before and after lambing (Vlassoff and McKenna, 1994; Vlassoff *et al.*, 2001), thus control should be administered to ewes to reduce the post-parturient rise in larval challenge. This can be achieved by use of three strategies; reduction in ewe contamination, utilisation of low-contamination pasture and control or prevention of infection in lambs. Control options available for reduction in ewe contamination are strategic drenching applied pre- and post-lambing or through slow release anthelmintics administered pre-weaning (Nicol and Everest, 1997). Another option is to maintain the ewe body condition by ensuring that a higher plane of protein nutrition is maintained during pregnancy, as this will assist in minimising the extent of breakdown in ewe immunity, consequently resulting in maintenance of resistance

(Donaldson, 1997). The options available for minimising larval challenge through utilization of low-contamination pasture are via newly replanted pastures, mixed grazing, that is pre-grazing of sheep pasture with cattle as they do not share the same nematode parasites with sheep, and pre-grazing of hoggets' which are considered to be the non-infective sheep (Nicol and Everest, 1997). The only option available for minimising larval challenge through control or prevention of infections in lambs is to monitor lamb FEC and strategically apply anthelmintic treatments post-weaning to prevent increase in parasitic challenge.

Integration of anthelmintic treatment and grazing management can be beneficial in reducing larval challenge on pasture. With the increasing prevalence of AR, it is now accepted that a feature of sustainable strategic anthelmintic usage is to allow a proportion of the worm population to remain in refugia (Besier, 2008; Jackson *et al.*, 2009; Kenyon *et al.*, 2009; Leathwick *et al.*, 2009). The previous practice of drenching and moving parasitized animals to clean pasture has been known to select strongly for AR (Waghorn *et al.*, 2009). Therefore, researchers and veterinarians have advised against use of this system for full flock treatment, but could be integrated with grazing management. One of these methods is to leave the treated animals in the original infected pastures for a few days to ingest susceptible parasites before moving to the clean pasture to reduce development of AR (Abbott *et al.*, 2009). Another method is to move the parasitized animals to a clean pasture and allow them to graze and infect the pasture for a few days before administering anthelmintic (Molento, 2009). Alternatively, leaving 10% of the flock untreated while the rest of the flock is treated at epidemiological significant times is another approach. This approach has shown good control of parasites, and the dilution effect offered by the 10% untreated animals, resulted in less selection for AR (Besier, 2001; Waghorn *et al.*, 2008). Another method is through TST, where the animals that will benefit most from the treatment are selected based on production, parasitological or pathophysiological indicators and treated (Kenyon *et al.*, 2009).

2.7 General summary

In organic farming systems, preventive use of anthelmintics is not allowed; thus integrated methods are likely to be the best control options (Thamsborg *et al.*, 1999). Organic sheep farming systems are environmentally conscious, low input systems that limit or restrict use of synthetic chemicals such as fertilizers and pesticides. According to the New Zealand Meat Industry (New Zealand Meat Industry, 2012), New Zealand has growing organic sheep and beef industries, operating under standards by private sector accreditation organisations such as Bio-Gro. There is increasing interest among sheep and beef farmers towards low-chemical use systems or organic supply systems (Dalton, 2009), with some farmers already converting to organic farming specialisation from their conventional systems (Mackay *et al.*, 2001). Despite the increasing interest in organic farming systems, there are

constraints limiting the growth of this sector. One factor is a general lack of information among livestock producers of the practicalities of converting from conventional farming to a low-chemical or organic supply system (Mackay *et al.*, 2001; Morris and Mackay, 2002). The major constraint is the limitation of production with respect to animal health, from the inability of the system to effectively control disease challenge from GI parasites (Mackay *et al.*, 1998; Mackay *et al.*, 2001). In organic systems, anthelmintics are not the main method of control of gastrointestinal nematodes. Instead, organic farmers rely on non-chemical options for the mitigation of gastrointestinal nematodes, which include, grazing management, use of genetically resilient sheep breeds that can keep producing under parasitic challenge and the use of bioactive forages (Mackay *et al.*, 2001). These options are not as effective as an anthelmintic drench on parasitized animals, but nevertheless offer a mode of reducing the parasitic challenge faced by the animal (Mackay *et al.*, 2001).

Bioactive forages, mainly tannin containing plants have a potential role as a component of an integrated approach towards control of GIN parasitism (Hoste *et al.*, 2006). Some bioactive herbs like chicory and plantain have the potential to be used in integrated nematode control systems. They can be used as a hospital or de-worming paddock in TST regimes, where the animal that will benefit most from the treatment is selected and grazed in these paddocks for short periods of time. Several short-term grazing studies with the use of chicory have observed reductions in adult worm burdens and subsequent improvement in performance, suggesting that chicory could be used in organic farming systems to alleviate the lambs from their worm burdens, thus assisting them to mitigate the effects of infection and improve performance.

Chapter 3

Materials and Methods

3.1 Experimental design, pastures and animals

The experiment was performed at the Biological Husbandry Unit (BHU) of Lincoln University, from December 2012 to April 2013, which lasted for fifteen weeks. All procedures were carried out under authorisation from the Lincoln University Animal Ethics Committee, approval number 505A. Two irrigated certified organic grazing blocks within the BHU were used; one block had a size of 2 Ha and the other of 1.6 Ha. The 2 Ha block had been previously seeded solely in 2012 with *Teladorsagia circumcincta* larvae (TCIRC) and the 1.6 Ha with *Trichostrongylus colubriformis* larvae (TCOL). These areas were used in a previous study (Lundberg, 2012) and have been grazed with pregnant/lactating hoggets in the spring of 2012 to ensure continuation of the seeded infection. Each block consisted of a ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) pasture paddock that took up almost half of each block, a hospital area that took up approximately one-third of each block and a quarantine area that took up the remainder. The pasture areas were divided into four paddocks using temporary electric fences, to allow control of pastures. Approximately half of each of the hospital paddocks was strip-sown with pure swards of chicory, plantain and red clover in autumn, and again in spring of 2012 and allowed to establish during the winter and summer months, respectively. Figure 3.1 depicts the layout of the experimental pastures. The pasture areas were rotationally grazed, while the hospital paddocks were set stocked. All experimental pastures were irrigated on an as-needed basis throughout the summer with spray irrigation and were mown in early February to maintain forage quality.

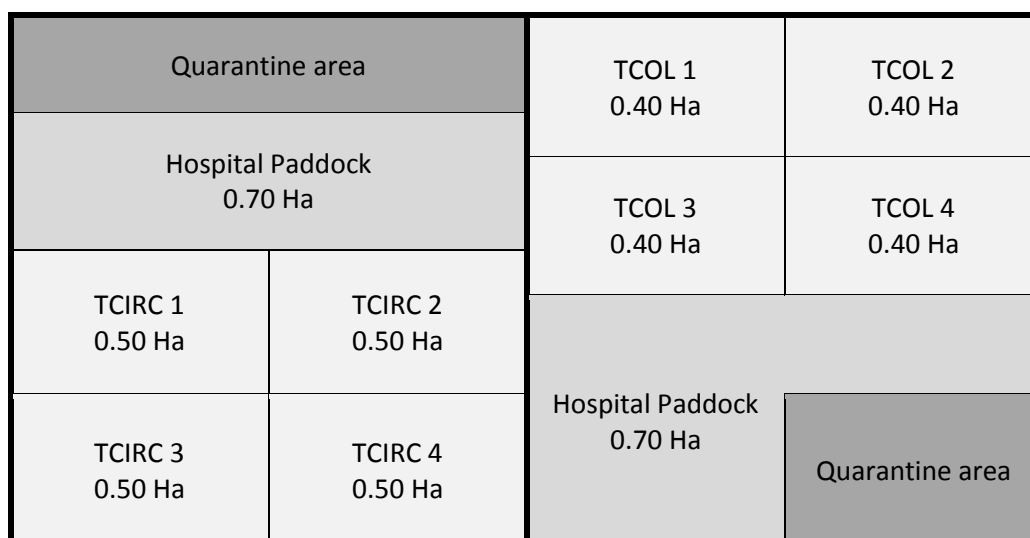


Figure 3.1: Schematic diagram of the trial site at the Lincoln BHU organic farm.

A total of sixty four Suffolk crossed lambs with a mean LW of 25.60 ± 0.29 kg, sourced from the Ashley Dene Farm, Lincoln University, were weaned at three months of age (December 2012) and transported to the experimental area where they were all orally treated with a triple combination anthelmintic of abamectin (1 g/L), albendazole (25 g/L) and levamisole hydrochloride (40 g/L) at 1ml per 5 kg of LW (Trio Sheep, Ravensdown Animal Health, Christchurch), jetted with fly repellent (Click, Novartis Animal Health), weighed and contained within the quarantine areas for 48 hours prior to being released to graze pasture.

3.2 Treatments and measurements

3.2.1 Treatments

Following weighing at Day 0, the lambs were fitted with electronic ear tags and allocated hierarchically by LW into one of eight groups (n=8). Four groups were randomly assigned to graze each of the experimentally infected pastures (TCIRC or TCOL). Within each of the four groups exposed to each parasite species, one group (n=8) (TCIRC-D and TCOL-D) was randomly selected to receive a neo-suppressive anthelmintic treatment regime (active compounds described above) on a monthly basis to act as a positive control group for the potential productivity achievable on each of the pastures; hence called the drenched group. The remaining three groups from each parasite infected pasture were combined to form one mob of 24 animals (TCIRC-TST and TCOL-TST) which were subjected to a TST regime (described below). The lambs were introduced into their respective parasite infected pasture paddocks (TCIRC or TCOL) as one mob and allowed an adjustment period of one week; after which they were weighed and faecal sampled to assess the efficacy of treatment at Day 0. Then on a fortnightly basis (Day 23, 37, 51, 65, 79, 93), LW change of each lamb was assessed, with poor performing individuals in the TST regime groups identified at each time of weighing (described below) and put to graze the hospital paddocks for a duration of fifteen weeks.

3.2.2 System used in identification of poor performing individuals

Identification of poor performing individuals in the TST regime groups was based on their ability to achieve pre-determined growth rates calculated using the Happy Factor system of Greer *et al.*, (2009). In this system, the production efficiency (Happy Factor) of each individual animal is calculated after taking into account the level of herbage on offer, climate, growth and relative maturity of the lambs. The Happy Factor (HF) model is based on an estimate of the animal's efficiency of gross energy utilization and is obtained by 1 less the maximum inefficiency (Greer *et al.*, 2009). The inefficiency is calculated by first determining the potential metabolisable energy intake (MEI) of the animal, which is the product of maximum dry matter intake and pasture quality, assuming that the animal is non-parasitized. Using this information, the metabolisable energy available for growth is

calculated by MEI less the maintenance requirement. Then, the deposition of net energy is estimated from the cost of growth multiplied by the LWG. From these calculations, the potential energy that was available for growth, but was not deposited on the carcass would have been lost as heat, is used as a measure of inefficiency. This measure of inefficiency also includes the energy not consumed as a consequence of parasites influencing voluntary feed intake. Hence, efficiency of gross energy utilization is calculated as 1 less the inefficiency. For Coopworth lambs in a Canterbury environment, the efficiency of gross energy utilization value of 0.74 was previously determined as the optimum treatment threshold (Greer *et al.*, 2010).

3.2.3 Target LW

The target LW of each animal in this study was then calculated assuming a HF of 0.74, together with the mean temperature which were taken from the NIWA Christchurch website, herbage mass (described below) and animal's previous LW on the day prior to each fortnightly weight recording. These weights were uploaded onto a Tru-test head unit, where at the time of weight recording (Days 23, 37, 51, 65, 79 and 93), animals that failed to reach their target LW were removed and put to graze on the hospital paddocks for a period of four weeks before returning to the infected pastures. The four week period of grazing in the hospital paddock was selected based on observations from a previous study in which over 80% of the animals returned to normal growth after spending four weeks grazing a low infectivity pasture (Lundberg, 2012). Drenched and TST animals that achieved their target LW, at each time of weighing, were returned to graze pastures for the next two week grazing period.

3.3 Pasture measurements

3.3.1 Herbage mass

Pre- and post-grazing herbage mass were assessed on a weekly basis for all the paddocks that have been grazed or were about to be grazed using a FILIP's rising plate meter, with measurements taken every 10 paces in a transverse 'W' pattern down the field. The rising plate meter was calibrated using forage cuts' from each of the hospital and pasture paddocks taken from a 0.2 m² quadrant. The samples were oven dried at 70°C for 48 hours or until dried and the dry matter (DM) was determined. Pasture mass from the cuts were estimated by multiplying the DM by the area of the respective paddock, from which linear calibration equations were obtained, where y = pasture mass (kg DM/ha) and x = plate meter reading of height per click. Pasture mass throughout the trial was estimated using these equations and the pasture height per click of the rising plate meter for every reading and expressed as kg DM/ha. The herbage mass used in the calculation of the target LW of each animal were measured on Days 22, 36, 50, 64, 78 and 92 of the trial.

3.3.2 Hospital paddock botanical composition

Using the dried pasture sample collected for pasture larval determination, pasture dissection of the hospital paddocks was performed on a fortnightly basis (Days 22, 36, 50, 64, 78 and 92) in order to determine the composition of bioactive forages in the hospital paddock. A subsample (one third) was sorted into individual species of grass, clover, chicory, plantain and others (weeds and dead matter), weighed and the respective proportions calculated and expressed in percentage for each paddock.

3.3.3 Herbage contamination

Pasture samples for the determination of larval contamination on each paddock were collected on a fortnightly basis (Days 22, 36, 50, 64, 78 and 92) at each rotation shift, from paddocks that are about to be grazed, at every thirteen paces in a transverse 'W' pattern down the field. These samples were processed and larvae counted using a modified Baermann technique (MAFF, 1986). In this technique, each bagged pasture sample was filled with cold and lukewarm water at a ratio of 1:10 and washed in a specially designed grass washing machine operated at 230 revolutions per minute for 3 minutes. Using a strainer, the solution was collected in a 4 litre beaker and the pasture oven dried at 70°C for 48 hours to determine the DM content. The collected solution was refrigerated for 48 hours to allow larvae to settle to the bottom of the beaker, siphoned to remove excess water, then transferred into a 200 ml measuring cylinder and refrigerated for another 12 hours to allow larvae to settle to the bottom of the beaker again. The content of the measuring cylinder was siphoned down to the 40ml level, swirled to mix contents and poured onto a 150 millimetre Whatman No. 1 filter paper, resting on newspaper at room temperature for three to four hours to allow the water to dry out, leaving the larvae trapped on the filter paper. The filter paper was then inverted onto a Baermann filter funnel filled with water, to allow larvae to migrate out of the filter paper and settle at the bottom of the funnel. After 48 hours, a sample of 50ml was collected from the base of the collection tube. From this sample, a subsample of 2ml was pipetted into a microscopic counting chamber with iodine added to it, and parasitic larvae counted under the microscope at 100x magnification. Parasitic larvae were morphologically differentiated from the free-living larvae through features such as presence of sheath, middle section stain retention, completely enclosed anterior or posterior orifices and presence of sheath tail. Two readings or counts were performed for each sample and expressed as L₃/ kg of DM.

3.4 Animal measurements

3.4.1 Faecal Egg Count (FEC)

Faecal samples were collected from the rectum of all lambs on a fortnightly basis (Days 23, 37, 51, 65, 79 and 93) to determine the concentration of nematode eggs in the faeces. Determination of FEC

was performed within two days after collection, using a modified McMaster technique (MAFF, 1986). In this technique, a sample of 1.7 grams of faeces was weighed into a glass jar; with 10 ml of water added and refrigerated at 4°C overnight to soften the faeces. Then, 20ml of saturated sodium chloride solution was added to each sample to act as a floatation medium, and each sample was homogenized to ensure uniform distribution of eggs. A subsample was pipetted into a microscopic counting chamber, rested for a few minutes to allow the eggs to float to the surface, and the eggs counted by microscopic examination at 20x magnification. Each egg counted was equivalent to 100 eggs per gram (epg) of faeces.

3.4.2 Live weight change

LW change in each lamb was monitored through the use of electronic scales fitted with a Tru-Test head unit and an electronic tag reader on a fortnightly basis, that is, on Days 23, 37, 51, 65, 79 and 93 of the trial.

3.5 Data Analysis

For the agronomic measurements, no statistical analysis was performed, as they only aimed to describe the experimental plots and no replicates in each time were available. All experimental data were analysed using Genstat statistical software (15th Edition SP1, version 15.1.0.8821, VSN International Limited). To determine performance of lambs at time of selection and admittance for each parasite specie group, live weight, live weight gain and FEC data were analysed using one-way analysis of variance (ANOVA) with distribution according to drench treatment (drench, hospital, pasture or recovered) and draft days as factors. Repeated Measures and one-way ANOVA was used to analyse change in efficiency (happy factor), live weight gain and FEC data to determine the effect of hospitalisation on parasitized lambs for each time of weighing and selection, at the time of admittance, during the first and second two-week hospital grazing periods and during the first and second two week grazing periods after the hospitalised animals returned to pasture, with drench treatment (drench, hospital) and time as factors for each parasite species. Response in terms of happy factor to hospital and drench treatment graphs were plotted using excel and one-way ANOVA was used to determine effect of treatment on performance with drench treatment (hospital and drench) as factors at two and four weeks after treatment. The effect of treatment was declared significant at $P < 0.05$. Faecal egg count data were log transformed ($\log(x+1)$) prior to analysis in order to stabilise the variance. For all data, arithmetic means with standard error of the means (SEM) are reported.

Chapter 4

Results

There were no mortalities throughout the trial. Salvage anthelmintic treatment was administered to one lamb from the TCOL TST group on Day 59, as it lost 8.5 kg (from 29.0 to 20.5 kg) in a period of two weeks. This animal was then returned to graze with its cohorts for the remainder of the trial. In early January, mild flystrike (myiasis) was detected on three lambs on consecutive days, which were immediately crutched and treated with Tea tree oil before returning to graze with their cohorts. Consequently, following the third case of flystrike, all lambs were immediately jetted on Day 37 of the trial as previously described, after which time there were no further incidences of fly strike. Data for the salvage treated and for animals treated for flystrike were included in the statistical analysis.

Anthelmintic treatment administered to TCOL drenched animals on Day 37 was not successful due to a faulty drenching gun, with lambs still losing weight (from mean of 0.07 on Day 37 to 0.04 kg/day on Day 51) and displaying elevated faecal egg count (mean of 963 epg) two weeks after treatment. These lambs were re-drenched at the next opportunity on Day 59, after which time anthelmintic treatment resumed at monthly intervals.

4.1 Herbage measurements

4.1.1 Herbage Mass

Herbage mass for each grazing area at each sampling time is given in Figure 4.1. Pasture mass was greater for TCIRC than TCOL pasture paddock on Day 23 (1672 and 1250 kg DM/ha, respectively), which declined to 1501 and 1176 kg DM/ha, respectively by Day 37. Pasture mass then increased to peak on Day 79 at 3228 and 2274 kg DM/ha for TCIRC and TCOL, respectively, before declining by Day 93, which coincided with the change in grazing management as described below. Herbage mass for the TCOL hospital paddock was slightly greater than that of the TCIRC hospital paddocks throughout the trial, with mass generally decreasing after the introduction of animals into each paddock on Day 23 to Day 93 of the trial (2061 to 1041 vs. 2017 to 898 kg DM/ha for TCOL and TCIRC, respectively).

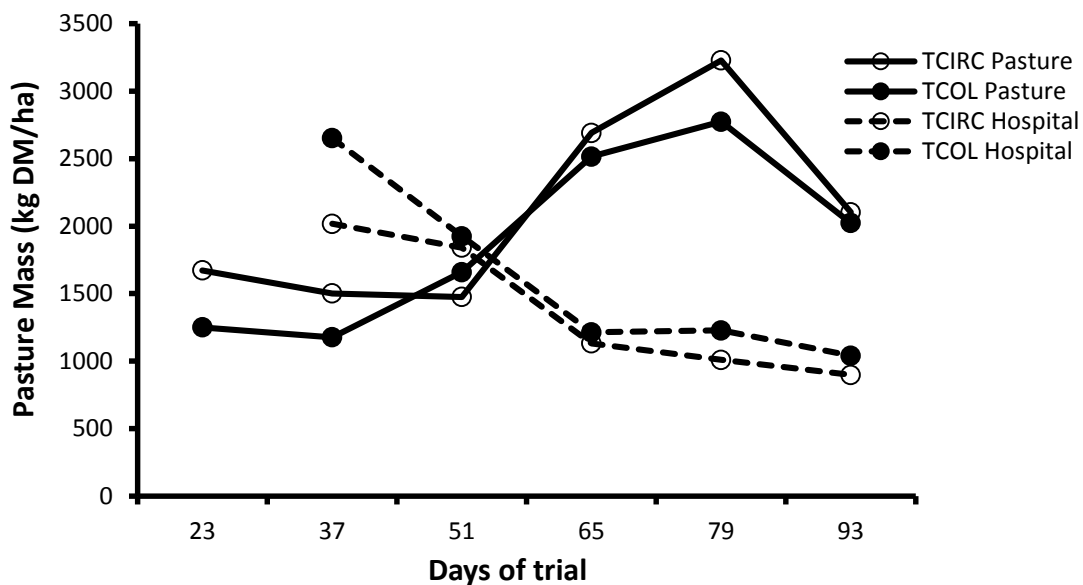


Figure 4.1: Pasture mass (kg DM/ha) for TCIRC (—○—) and TCOL (—●—) infected pasture paddocks and TCIRC (---○---) and TCOL (---●---) hospital paddocks throughout the trial.

Due to sufficient pasture mass, animals grazing the main pasture paddock were set stocked on Day 1 to Day 37 of the trial, after which the paddocks were divided into four areas and weekly rotational grazing was employed from Day 38 to 64. From Day 65, pasture growth exceeded demand; consequently, two of the four areas were left out of rotation and a fortnightly rotational regime was employed until the conclusion of the study. Animals on the hospital paddocks were set-stocked until Day 62 at which point, due to declining herbage levels, areas of approximately one-third of the total hospital areas were spelled to allow regrowth. Irrigation was employed in all paddocks from mid-January until the conclusion of the study.

4.1.2 Herbage composition of the hospital paddocks

Herbage composition of the TCIRC and TCOL hospital paddocks from dissection is given in Figures 4.2 and 4.3, respectively. Overall, the change in the herbage composition was similar for both hospital areas. On Day 23, chicory contributed 24 and 25% of DM, which declined during the trial to 8 and 6% of DM by Day 79, in the TCIRC and TCOL hospital paddocks, respectively. Clover was 29 and 32% of DM on Day 23, and gradually declined to 8% of DM by Day 65 for the TCIRC hospital paddock and 5% by Day 79 for the TCOL hospital paddock. Plantain was 14 and 17% of DM on Day 23, declined by Day 37 to 6 and 3%, and then gradually increased to 28 and 24% of DM by Day 93 for the TCIRC and TCOL hospital paddock, respectively. The proportion of grass was 19 and 15% of DM on Day 23, which gradually increased to 25 and 28% of DM by Day 93 for TCIRC and TCOL hospital paddocks, respectively. The proportion of weed and dead matter was 9 and 8% of DM on Day 23 and increased to 25% of DM by Day 65 and 31% of DM by Day 79 for TCIRC and TCOL hospital paddocks, respectively; after which proportion on both paddocks slightly declined. At all times, animals in the hospital paddock had access to clover, plantain and chicory.

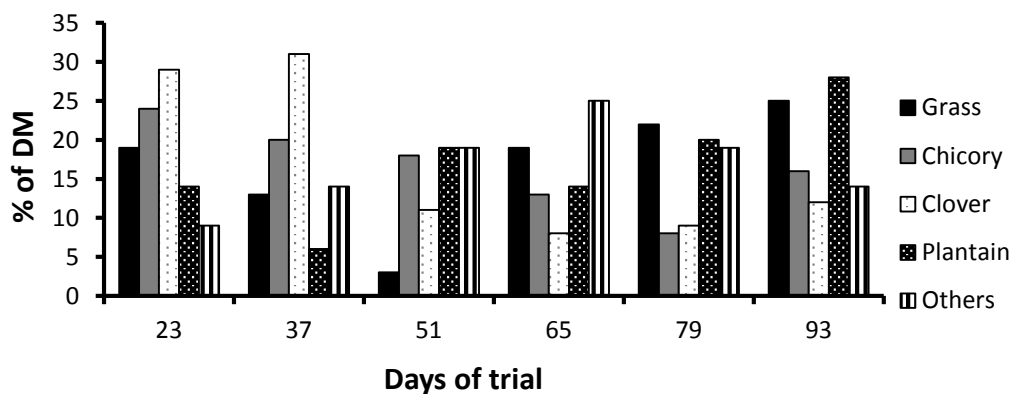


Figure 4.2: Percentage distribution of grass (■), chicory (■), clover (□), plantain (■) and others (▨) in the TCIRC hospital paddock at each dissection throughout the trial. Note: Others category contains weeds and dead matter.

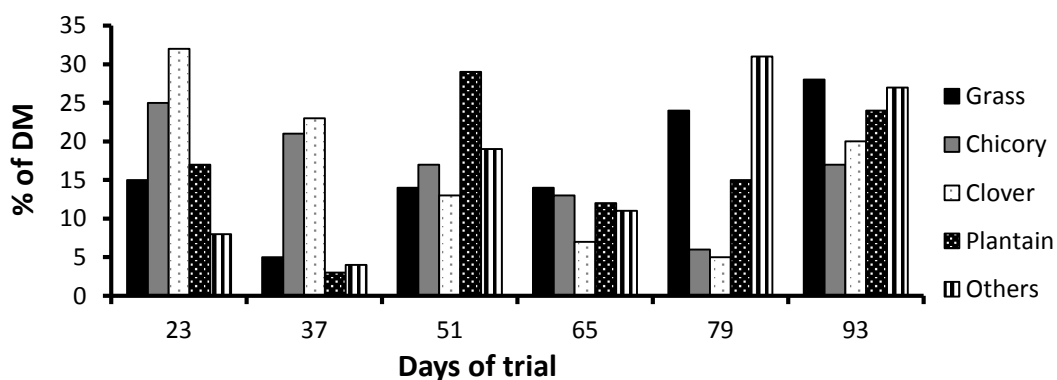


Figure 4.3: Percentage distribution of grass (■), chicory (■), clover (□), plantain (■) and others (▨) in the TCOL hospital paddock at each dissection throughout the trial. Note: Others category contains weeds and dead matter.

4.1.3 L₃ parasitic larvae contamination

The number of L₃ larvae recovered per kg DM for pastures infected with TCIRC and TCOL and their respective hospital paddocks is given in Figure 4.4. Pasture contamination increased in all paddocks after Day 23. Contamination in the TCIRC hospital paddock increased from 197 L₃ per kg DM on Day 23 to 10241 L₃ per kg DM by Day 37, declined to 248 L₃ per kg DM by Day 51, then increased to 16832 L₃ per kg DM by Day 65 and declined below 10000 L₃ per kg DM on Day 79 to 93. Contamination in the TCOL hospital paddock was 0 L₃ per kg DM on Day 23, increased to 3000 L₃ per kg DM by Day 65, then declined to 1724 L₃ per kg DM by Day 93. Level of infective larvae in the TCIRC pasture paddock was 1408 L₃ per kg DM on Day 23, then increased and declined throughout the trial, whereas contamination in the TCOL pasture paddock increased from 0 L₃ per kg DM on Day 23 to 7692 L₃ per kg DM by Day 65, then declined below 1000 L₃ per kg DM.

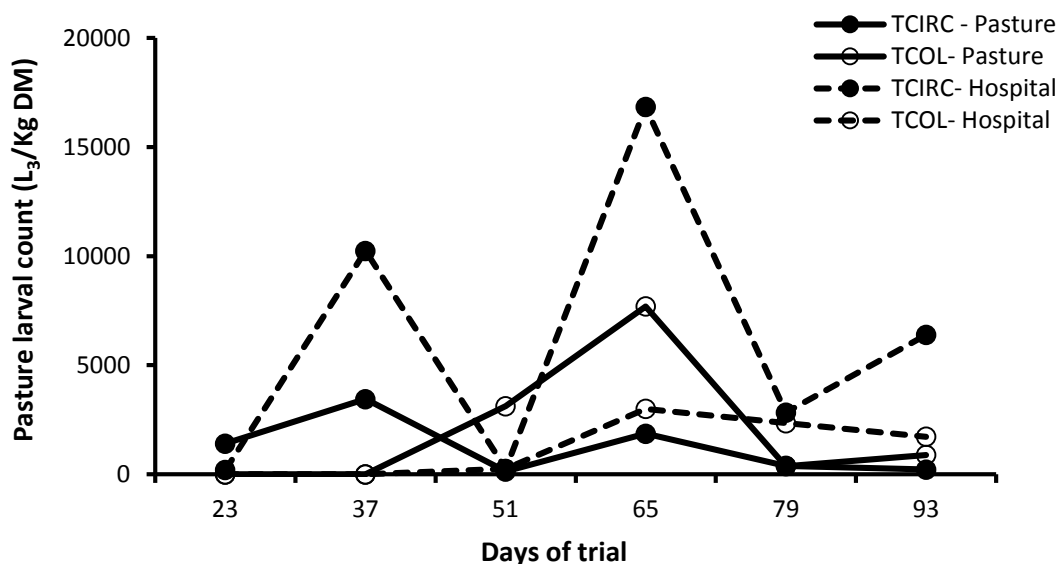


Figure 4.4: The number of parasitic L₃ larvae (L₃ per kg DM/ha) recovered from the TCIRC (—●—) and TCOL (—○—) infected pasture paddocks, and the TCIRC (---●---) and TCOL (---○---) hospital paddocks throughout the trial.

4.2 Overall animal measurements

4.2.1 Faecal Egg Count

Arithmetic mean FEC for each treatment group is given in Figure 4.5. Overall, there were interactions between parasite species and drench treatment ($p=0.039$), parasite species and sampling time ($p<0.001$) and drench treatment and sampling time ($p<0.001$). These were a reflection of the greater mean FEC observed in animals subjected to TST than drenched regime from Day 51 onwards (range 1531 epg to 2247 vs. 7 to 538 epg, respectively), in addition to the greater FEC observed in TCIRC-TST lambs compared with TCOL-TST lambs from Day 79 onwards (range 1463 epg to 2425 vs. 835 epg to 1071 epg, respectively). Mean FEC of TCIRC-D animals remained lower than 50 epg at all times, while the FEC of TCOL-D animals increased to 1000 epg by Day 51, after which point, they remained at less than 50 epg.

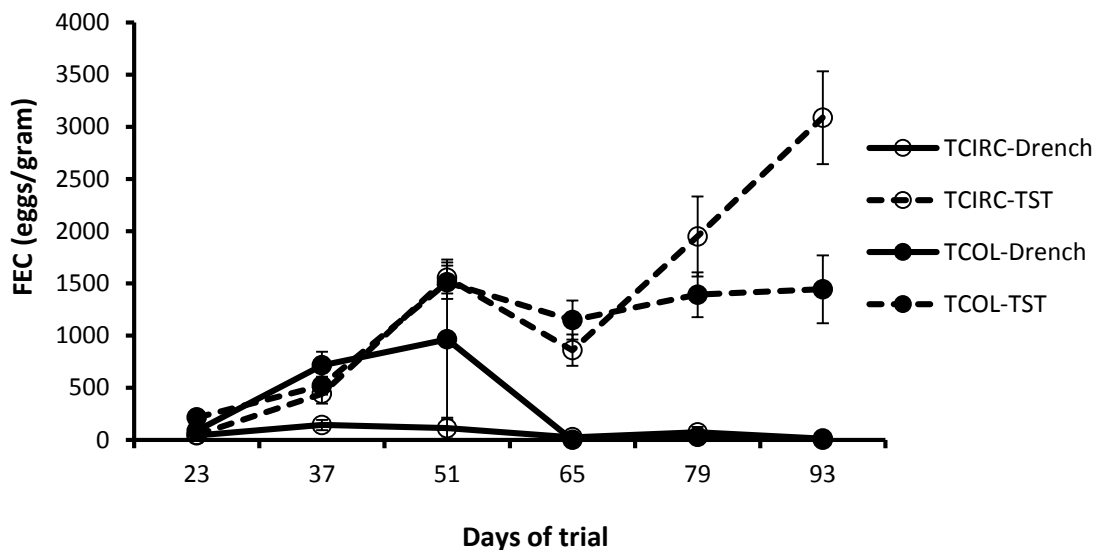


Figure 4.5: Arithmetic mean FEC (epg) for animals grazing pastures infected with either TCIRC (—○—) or TCOL (—●—) that were drenched every four weeks, and animals grazing TCIRC (---○---) or TCOL (---●---) infected pastures that were not drenched, but exposed to a TST regime in which poor performing individuals were placed onto respective hospital paddocks for four weeks before returned to pasture. Bars represent the SEM.

4.2.2 Live weight (LW) and live weight gain (LWG)

Mean LW of lambs for each treatment group is given in Figure 4.6. Overall, there were interactions between parasite species (TCIRC or TCOL) and sampling time ($P < 0.002$), and treatment (Drenched or TST) and sampling time ($P < 0.001$). These reflected an increase in LW in all groups with time, which was similar for the drenched groups, irrespective of parasite species, and greater for drenched compared with TST animals from Day 65 to 93 for TCOL than TCIRC, respectively.

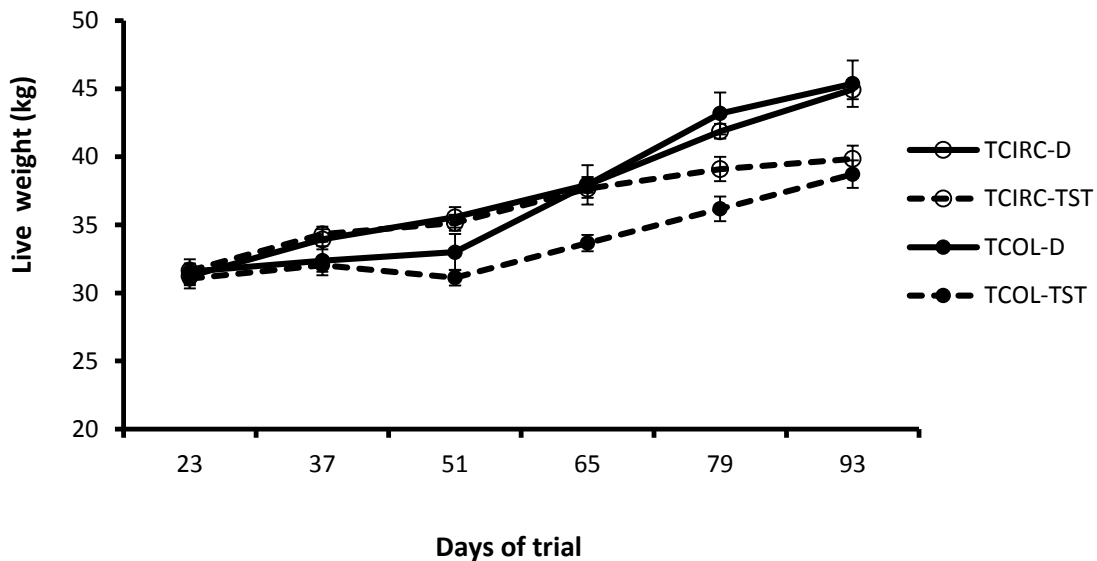


Figure 4.6: Mean LW (kg) for animals grazing pastures infected with either TCIRC (—○—) or TCOL (—●—) that were drenched every four weeks, and animals grazing TCIRC (---○---) or TCOL (---●---) infected pastures that were not drenched, but exposed to a TST regime in which poor performing individuals were placed onto respective hospital paddocks for four weeks before returned to pasture. Bars represent the SEM.

Cumulative mean LWG during the trial period for all treatment group was 15.29 ± 0.67 kg, and was influenced by the drench treatment regime (Drenched or TST) ($p = 0.000$), but not parasite species ($p = 0.459$). Animals subjected to a four weekly anthelmintic drenching regime in both parasite infected pasture treatments (TCIRC-D or TCOL-D) gained 19.97 ± 1.63 and 21.96 ± 1.27 kg compared with their TST counterparts which gained 13.47 ± 1.06 and 13.32 ± 0.65 kg, respectively.

4.2.3 Number of animals reaching slaughter weight

From the LW measurements, the number of lambs that reached slaughter weight of 38 kg during the trial is given in Table 4.1. Overall, all drenched animals reached 38 kg live weight by Day 79 and 93 for TCIRC and TCOL infected pastures, respectively. By comparison, 19 out of 24 and 14 out of 24 TST animals grazing the TCIRC and TCOL pasture, respectively, reached slaughter weight by Day 93.

Table 4.1: Number of lambs that reached slaughter weight of 38 kg, grazing either TCIRC or TCOL infected pasture, that were either exposed to a four weekly anthelmintic treatment regime (drenched) or a TST regime (TST) in which poor performing individuals were placed into respective hospital paddocks for four weeks before returned to pasture during the trial.

Days of trial	Day 37	Day 51	Day 65	Day 79	Day 93
<i>Teladorsagia circumcincta</i> (TCIRC) infective pasture					
Drenched (n=8)	0	0	5	8	8
TST (n=24)	4	6	11	16	19
<i>Trichostrongylus colubriformis</i> (TCOL) infective pasture					
Drenched (n=8)	1	1	4	7	8
TST (n=24)	1	2	3	11	14

4.2.4 Distribution of lambs at each time of selection

The distribution of lambs into TCIRC and TCOL hospital and pasture paddocks according to pre-set weight gains at each selection time is given in Table 4.2. A total of seven selections were made (from 21st Dec, 2012 to 13th Mar, 2013), where animals subjected to the TST regime were placed into the hospital paddock. All lambs reached their pre-set target weight in the first selection (21 Dec). For both TCOL and TCIRC TST animals, all lambs were drafted into the hospital paddock by Day 37, except for one lamb in the TCIRC group, which was drafted in on Day 51.

Table 4.2: Distribution of lambs grazing either TCIRC or TCOL infected pasture and subjected to a TST regime where lambs that failed to meet their target pre-set weight gains were placed respective hospital paddocks (-H), while lambs that reached their target weight gain were placed back on pasture (-P) at each selection time. The numbers in brackets indicate the number of lambs that were in the same paddock for the previous two week grazing period.

Dates	Day of trial	TCIRC-P (n)	TCIRC-H (n)	TCOL-P (n)	TCOL-H (n)
21-Dec	9	24 (24)	0 (0)	24 (24)	0 (0)
4-Jan	23	17 (17)	7 (0)	21 (21)	3 (0)
16-Jan	37	10 (10)	14 (7)	10 (10)	14 (3)
30-Jan	51	8 (1)	16 (7)	3 (0)	21 (11)
13-Feb	65	11 (4)	13 (9)	13 (2)	11 (10)
27-Feb	79	15 (6)	9 (4)	14 (4)	10 (1)
13-Mar	93	19 (15)	5 (5)	15 (14)	9 (9)

The number of animals that were re-admitted into either TCIRC or TCOL hospital paddocks after being returned to respective infected pasture for two weeks or four weeks is shown in Figure 4.7. Overall, sixteen lambs from the TCIRC-TST group and fourteen lambs from the TCOL-TST group were admitted for one four-week period. Eight TCIRC-TST and ten TCOL-TST lambs were admitted for two four-week intervals. No lambs were admitted more than twice. Of those admitted twice, 75% from the TCIRC group and 90% from the TCOL group were re-admitted after two weeks grazing back out at pasture, while 25% and 10% were re-admitted after one month on respective infected pasture paddocks.

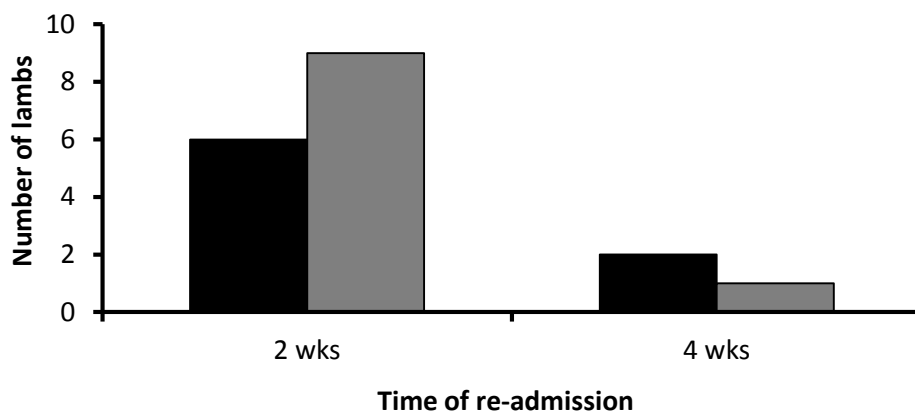


Figure 4.7: Number of lambs subjected to a TST regime and grazing either TCIRC (■) or TCOL (■) infected pastures that were re-admitted into the respective hospital paddocks at either two or four weeks after being returned to respective pastures.

4.3 Performance attributes at time of selection

Mean LW, LWG and FEC of lambs at each weighing and selection time, according draft are given in Tables 4.3 and 4.4, for the TCIRC and TCOL groups, respectively. For animals grazing TCIRC infected pastures, those placed into the hospital paddock were lighter than their TST counterparts that remained on pasture on Day 37 only ($P=0.008$), while those TST animals that remained on pasture and those that returned to pasture after four weeks in the hospital paddock had a similar LW to their drenched counterparts at all times ($P>0.05$). Mean LWG was lower in TST animals placed into the hospital paddock than their TST counterparts that remained on pasture on Days 23, 65 and 79 ($P<0.05$ for all). In comparison, mean LWG of TST animals that remained on pasture were similar to that of the drenched animals, except on Days 23 and 93 ($P<0.05$). TST animals that returned to pasture after four weeks in the hospital paddock (TST-Recovered) only differed from their drenched counterparts in LWG on Day 79 ($P<0.05$). There was no difference in mean FEC between TST animals that were either placed in the hospital paddock, remained on pasture or returned to pasture after hospitalisation at any time ($P<0.05$ for all times), whereas mean FEC in drenched animals were consistently lower than all TST animals from Day 65 ($P<0.05$).

Table 4.3: Mean LW (kg), LWG (kg/day) and FEC (epg) of lambs grazing TCIRC infected pastures at each weighing and selection time according draft; drenched or TST animals that remained on pasture (TST-Pasture), placed into the hospital paddock (TST-Hospital) or returned to pasture from hospital paddock (TST-Recovered).

Day	Drenched	TST-Pasture	TST-Hospital	TST-Recovered	P-values
<i>Mean live weight (kg)</i>					
23	31.25 ± 0.75	32.18 ± 0.65	30.37 ± 1.11		0.315
37	33.94 ± 0.57 (AB)	36.40 ± 0.81 (B)	32.82 ± 0.69 (A)		0.008
51	35.56 ± 0.51	42.0 ± 3.78	34.59 ± 0.95	35.43 ± 1.32	0.312
65	37.94 ± 0.70	38.38 ± 2.04	38.85 ± 1.13	35.07 ± 1.54	0.277
79	41.88 ± 0.77	40.08 ± 1.85	37.72 ± 1.51	39.83 ± 1.51	0.328
93	44.94 ± 1.79 (B)	40.80 ± 1.30 (AB)	35.80 ± 2.25 (A)	41.38 ± 2.51 (AB)	0.031
<i>Mean live weight gain (kg/day)</i>					
23	0.16 ± 0.02 (A)	0.26 ± 0.01 (B)	0.11 ± 0.01 (A)		<0.001
37	0.22 ± 0.03	0.30 ± 0.04	0.17 ± 0.04		0.111
51	0.12 ± 0.01	0.32 ± 0.14	0.02 ± 0.03	0.12 ± 0.04	0.079
65	0.17 ± 0.04 (AB)	0.30 ± 0.07 (B)	0.10 ± 0.04 (A)	0.26 ± 0.05 (B)	0.031
79	0.28 ± 0.05 (B)	0.27 ± 0.05 (B)	0.01 ± 0.04 (A)	0.08 ± 0.04 (A)	<0.001
93	0.22 ± 0.04 (B)	0.06 ± 0.03 (A)	0.00 ± 0.05 (A)	0.09 ± 0.06 (AB)	0.008
<i>Arithmetic mean faecal egg count (eggs per gram)</i>					
23	43 ± 30	50 ± 18	43 ± 20		0.911
37	143 ± 53	340 ± 142	514 ± 126		0.229
51	113 ± 99 (A)	900 ± 0 (AB)	1507 ± 169 (B)	1743 ± 342 (B)	<0.001
65	25 ± 16 (A)	425 ± 138 (B)	789 ± 109 (B)	1229 ± 441 (B)	<0.001
79	71 ± 57 (A)	3140 ± 1404 (B)	1777 ± 460 (B)	1217 ± 255 (B)	<0.001
93	13 ± 13 (A)	3417 ± 715 (B)	2900 ± 787 (B)	2250 ± 484 (B)	<0.001

A B & C - letters showing Tukey separation of means ($P<0.05$)

For animals grazing the TCOL infected pastures, those placed into the hospital paddock had a similar mean LW to their TST counterparts that remained on pasture at all times ($P>0.05$), while those TST animals that remained on pasture had a similar LW to their drenched counterparts at all times ($P<0.05$). In addition, those TST animals that returned to pasture from the hospital paddock had similar mean LW to their drenched counterparts at all times except on Day 93, where they were lighter ($P<0.05$). Mean LWG was lower in TST animals placed into the hospital paddock than their TST counterparts that remained on pasture on Days 23 and 37 only ($P<0.05$ for both). In comparison, mean LWG of the TST animals that remained on pasture was similar to their drenched counterparts throughout the trial ($P>0.05$ for all times), while mean LWG of TST animals that returned to pasture from the hospital paddock was lower than their drenched counterparts on Day 65 only ($P<0.05$). There was no difference in mean FEC amongst TST animals that were either placed into the hospital paddock, remained on pasture or returned to pasture from the hospital paddock at any time ($P<0.05$ for all times), whereas mean FEC in drenched animals were consistently lower than all TST animals from Day 65 ($P<0.05$).

Table 4.4: Mean LW (kg), LWG (kg/day) and FEC (epg) for lambs grazing TCOL infected pastures at each weighing and selection time according to draft; drench or TST animals that remained on pasture (TST-Pasture), placed into the hospital paddock (TST-Hospital) or returned to pasture from hospital paddock (TST-Recovered).

Day	Drenched	TST-Pasture	TST-Hospital	TST-Recovered	P-values
<i>Mean live weight (kg)</i>					
23	31.58 ± 1.10	31.27 ± 0.68	29.47 ± 1.80		0.597
37	32.38 ± 1.12	33.55 ± 1.0	30.96 ± 0.84		0.155
51	33.00 ± 1.58		31.50 ± 1.0	28.50 ± 2.58	0.341
65	37.94 ± 1.84	34.75 ± 3.68	33.55 ± 1.57	33.59 ± 1.57	0.217
79	43.19 ± 1.66 (B)	35.50 ± 2.34 (AB)	34.35 ± 1.48 (A)	38.30 ± 1.48 (AB)	0.004
93	45.38 ± 1.63 (C)	39.86 ± 1.23 (ABC)	37.56 ± 1.54 (AB)	33.50 ± 4.62 (A)	0.006
<i>Mean live weight gain (kg/day)</i>					
23	0.22 ± 0.03 (AB)	0.23 ± 0.02 (B)	0.07 ± 0.06 (A)		0.043
37	0.07 ± 0.02 (AB)	0.17 ± 0.04 (B)	0.02 ± 0.03 (A)		0.014
51	0.04 ± 0.06		-0.06 ± 0.04	-0.08 ± 0.10	0.385
65	0.35 ± 0.03 (B)	0.27 ± 0.08 (AB)	0.15 ± 0.03 (A)	0.20 ± 0.03 (A)	0.004
79	0.38 ± 0.03 (B)	0.25 ± 0.06 (AB)	0.08 ± 0.04 (A)	0.26 ± 0.04 (B)	<0.001
93	0.16 ± 0.05	0.17 ± 0.03	0.16 ± 0.04	0.54	0.069
<i>Arithmetic mean faecal egg count (eggs per gram)</i>					
23	88 ± 35	224 ± 68	167 ± 88		0.847
37	714 ± 139	600 ± 176	447 ± 84		0.576
51	963 ± 765 (A)		1490 ± 184 (B)	1633 ± 448 (B)	<0.001
65	0 (A)	500 ± 300 (B)	1191 ± 340 (B)	1222 ± 238 (B)	<0.001
79	29 ± 19 (A)	2025 ± 340 (B)	1044 ± 348 (B)	1463 ± 421 (B)	<0.001
93	0 (A)	2008 ± 549 (B)	744 ± 140 (B)	400 (B)	<0.001

A B & C - letters showing Tukey separation of means ($P<0.05$)

4.4 Performance attributes of hospitalized animals

Mean HF, LWG (kg/day) and FEC (epg) between drenched and hospitalised animals grazing TCIRC infected pastures at all weighing, selection and admittance times, during hospitalization (1st and 2nd two weeks of hospitalisation) and up to one month after the hospitalised animals returned to pasture (1st and 2nd two weeks after return to pasture) is given in Table 4.5. Overall, mean HF for the drenched lambs throughout the trial was 0.73 ± 0.02 ranging from 0.71 – 0.76. In comparison mean HF value for the hospitalised lambs ranged from 0.50 ± 0.04 - 0.65 ± 0.04 . Mean HF was different between the drenched and hospitalised lambs at the time of admittance, in the first two week grazing period of hospitalisation and in the second two week grazing period after the hospitalised lambs returned to pasture ($P < 0.05$). Mean LWG was greater for the drenched compared with the hospitalised animals at the time of admittance (0.19 ± 0.02 vs. 0.05 ± 0.02 kg/day), in the first two week grazing period of hospitalization (0.20 ± 0.02 vs. 0.10 ± 0.04 kg/day) and in the second two week grazing period after the hospitalised animals returned to pasture (0.18 ± 0.03 vs. -0.01 ± 0.03 kg/day). Mean FEC was lower for the drenched than the hospitalised animals at the time of admittance, during both hospitalisation periods, including the two periods after the hospitalised animals returned to pasture ($P < 0.05$).

Table 4.5: Mean HF, LWG (kg/day) and FEC (epg) between drenched and TST-hospitalised lambs grazing TCIRC infected pastures for all weighing, selection and admittance times, at 1st and 2nd two week hospital grazing periods and at 1st and 2nd two week grazing periods after the hospitalised lambs returned to pasture, irrespective of when the hospitalised lambs were admitted.

Time	Drenched	TST-Hospital	P-value
<i>Mean happy factor</i>			
Time of admittance	0.75 ± 0.02	0.57 ± 0.03	<0.001
1 st two weeks of hospitalisation	0.76 ± 0.04	0.65 ± 0.04	0.042
2 nd two weeks of hospitalisation	0.71 ± 0.03	0.65 ± 0.03	0.166
1 st two weeks after return to pasture	0.73 ± 0.03	0.65 ± 0.04	0.163
2 nd two weeks after return to pasture	0.73 ± 0.04	0.50 ± 0.04	<0.001
<i>Mean live weight gain (kg/day)</i>			
Time of admittance	0.19 ± 0.02	0.05 ± 0.02	<0.001
1 st two weeks of hospitalisation	0.20 ± 0.02	0.10 ± 0.04	0.008
2 nd two weeks of hospitalisation	0.16 ± 0.02	0.11 ± 0.03	0.133
1 st two weeks after return to pasture	0.18 ± 0.02	0.12 ± 0.03	0.127
2 nd two weeks after return to pasture	0.18 ± 0.03	-0.01 ± 0.03	<0.001
<i>Mean faecal egg count (eggs per gram)</i>			
Time of admittance	78 ± 26	890 ± 196	<0.001
1 st two weeks of hospitalisation	71 ± 26	1370 ± 195	<0.001
2 nd two weeks of hospitalisation	55 ± 24	1793 ± 237	<0.001
1 st two weeks after return to pasture	40 ± 16	2254 ± 403	<0.001
2 nd two weeks after return to pasture	45 ± 21	2856 ± 529	<0.001

Mean HF, LWG (kg/day) and FEC (epg) between the drenched and hospitalised animals grazing the TCIRC infected pasture at the time of admittance, in the first and second two week hospital grazing periods (14 and 28 days) and in the first and second two week grazing periods (42 and 56 days) after the hospitalised animals returned to pasture for each time of selection is provided in Table 4.6. For HF, there was a three-way interaction effect of Drench treatment x Day of selection x Time on mean HF ($P=0.015$). Mean HF values were greater for the drenched than hospitalised animals which were admitted on Day 37 at the time of admittance and in the second two week grazing period after these animals returned to pasture; for animals admitted on Day 51 during the second two week hospital grazing period, and in the first and second two week grazing period after these animals returned to pasture and for animals admitted on Day 79 at the time of admittance ($P<0.05$ for all these times). Other than that, mean HF was similar between the drenched and the hospitalised lambs ($P>0.05$).

For LWG, there was a three-way interaction effect of Drench treatment x Day of selection x Time ($P=0.003$). Mean LWG was greater in the drenched than hospitalised animals which were admitted on Day 23 in the second two week grazing period after these animals returned to pasture; for animals admitted on Day 37 at the time of admittance and in the second two week grazing period after they returned to pasture; for animals admitted on Day 51 in the second two week hospital grazing period and in the first and second two week grazing periods after they returned to pasture; for animals admitted on Day 65 in the first two week hospital grazing period and for animals admitted on Day 79 at the time of admittance and in the first two week hospital grazing period ($P<0.05$ for all these times). Apart from these times, mean LWG was similar between the drenched and the hospitalised animals ($P>0.05$).

For FEC, there was a three-way interaction effect of Drench treatment x Day of selection x Time ($P=0.029$). Mean FEC was lower in the drenched than hospitalised animals at all times ($P>0.05$), except for animals admitted on Days 23 and 37 at the time of admittance, where FEC was similar ($P<0.05$).

Table 4.6: Mean HF, LWG (kg/day) and FEC (epg) between the drench and hospitalized lambs grazing the TCIRC infected pasture at time of admittance (0 days), at the 1st (14 days) and 2nd (28 days) two week hospital grazing periods and at the 1st (42 days) and 2nd (56 days) two week grazing period after the hospitalised animals returned to pasture for each weighing and selection time (Day 23-79).

Time Day	0 days		P-value	14 days		P-value	28 days		P-value	42 days		P-value	56 days		P-value
	Dren	Hos		Dren	Hos		Dren	Hos		Dren	Hos		Dren	Hos	
<i>Mean happy factor</i>															
23	0.71±0.03	0.64±0.03	0.131	0.81±0.06	0.90±0.07	0.368	0.64±0.04	0.64±0.04	0.980	0.72±0.06	0.80±0.06	0.400	0.87±0.06	0.69±0.06	0.056
37	0.81±0.06	0.57±0.06	0.012	0.64±0.04	0.44±0.09	0.121	0.72±0.06	0.85±0.06	0.154	0.87±0.06	0.69±0.09	0.153	0.76±0.07	0.50±0.07	0.019
51	0.64±0.04	0.55±0.03	0.088	0.72±0.06	0.72±0.07	0.598	0.87±0.06	0.60±0.07	0.019	0.76±0.07	0.54±0.04	0.001	0.55±0.04	0.35±0.05	0.014
65	0.72±0.06	0.57±0.08	0.146	0.87±0.06	0.64±0.09	0.056	0.76±0.07	0.59±0.06	0.058	0.55±0.04	0.58±0.06	0.597			
79	0.87±0.06	0.48±0.07	0.002	0.76±0.07	0.57±0.09	0.135	0.55±0.04	0.50±0.05	0.506						
<i>Mean live weight gain (kg/day)</i>															
23	0.16±0.03	0.11±0.01	0.129	0.22±0.03	0.28±0.06	0.367	0.12±0.01	0.12±0.01	0.977	0.17±0.05	0.22±0.05	0.452	0.28±0.05	0.12±0.05	0.027
37	0.22±0.03	0.05±0.05	0.010	0.12±0.01	-0.03±0.09	0.115	0.17±0.01	0.26±0.05	0.189	0.28±0.05	0.13±0.08	0.122	0.22±0.03	-0.01±0.06	0.005
51	0.12±0.01	0.05±0.03	0.096	0.17±0.04	0.12±0.06	0.517	0.28±0.05	0.08±0.06	0.018	0.22±0.03	0.06±0.03	<0.001	0.03±0.03	-0.11±0.04	0.011
65	0.17±0.04	0.05±0.05	0.122	0.28±0.05	0.07±0.05	0.019	0.22±0.03	0.09±0.06	0.054	0.03±0.03	0.05±0.03	0.651			
79	0.28±0.05	-0.04±0.04	<0.001	0.22±0.03	0.00±0.08	0.014	0.03±0.03	-0.01±0.03	0.412						
<i>Mean faecal egg count (eggs per gram)</i>															
23	43±30	43±20	0.676	143±53	757±195	0.015	113±99	1743±342	<0.001	25±16	643±193	<0.001	71±57	1370±338	<0.001
37	143±53	271±104	0.761	113±99	1771±201	<0.001	25±16	1229±441	<0.001	71±57	3550±1128	<0.001	13±13	3860±1222	<0.001
51	113±99	1275±246	0.002	25±16	758±101	<0.001	71±57	1217±255	<0.001	13±13	2529±628	<0.001	57±30	3231±836	<0.001
65	25±16	850±284	<0.001	71±57	1050±176	0.003	13±13	2250±484	<0.001	57±30	2650±558	0.002			
79	71±57	2358±744	<0.001	13±13	2900±787	<0.001	57±30	2980±886	<0.001						

Keys: Dren – Drench regime, Hos – Hospital paddock treatment

“0 days” – time of admittance

“14 days” – first two week hospital grazing period

“28 days” – second two week hospital grazing period

“42 days” – first two week grazing period after the hospitalised animals returned to pasture

“56 days” – second two week grazing period after the hospitalised animals returned to pasture

Day 23 – 79 – weighing and selection times

Mean HF, LWG (kg/day) and FEC (epg) between the drenched and hospitalised animals grazing the TCOL infected pasture at all time of weighing, selection and admittance, in the 1st and 2nd two week hospital grazing periods and in the 1st and 2nd two week grazing period after the hospitalised animals returned to pasture is given in Table 4.7. Overall, mean HF value for the drenched lambs for the entire trial was 0.80 ± 0.01 , ranging from 0.78 – 0.84. In comparison, mean HF value for the hospitalised lambs ranged from 0.50 ± 0.04 - 0.73 ± 0.04 throughout the trial. Mean HF was greater for the drenched than hospitalised animals at the time of admittance ($P < 0.001$) and in the first ($P = 0.028$) and second ($P = 0.016$) two week grazing periods after the hospitalised lambs returned to pasture. Mean LWG was greater for the drenched compared with the hospitalised animals, at all times ($P < 0.05$) except in the second two week hospital grazing period, where mean LWG was similar ($P = 2.67$; 0.21 ± 0.03 cf. 0.17 ± 0.03 kg/ day). Mean FEC was lower for the drenched than the hospitalised animals at all times ($P < 0.05$ at all times).

Table 4.7: Mean HF, LWG (kg/day) and FEC (epg) between drenched (Dren) and TST-hospitalised (Hos) lambs grazing TCOL infected pastures for all weighing, selection and admittance times, at 1st and 2nd two week hospital grazing periods and at 1st and 2nd two week grazing periods after the hospitalised lambs returned to pasture, irrespective of when the hospitalised lambs were admitted.

Time	Dren	Hos	P-value
<i>Mean happy factor</i>			
Time of admittance	0.82 ± 0.04	0.50 ± 0.04	<0.001
1 st two weeks of hospitalisation	0.78 ± 0.04	0.66 ± 0.05	0.072
2 nd two weeks of hospitalisation	0.78 ± 0.04	0.73 ± 0.04	0.416
1 st two weeks after return to pasture	0.84 ± 0.04	0.71 ± 0.04	0.028
2 nd two weeks after return to pasture	0.79 ± 0.04	0.62 ± 0.05	0.016
<i>Mean live weight gain(kg/day)</i>			
Time of admittance	0.21 ± 0.03	-0.001 ± 0.03	<0.001
1 st two weeks of hospitalisation	0.20 ± 0.03	0.10 ± 0.03	0.012
2 nd two weeks of hospitalisation	0.21 ± 0.03	0.17 ± 0.03	0.267
1 st two weeks after return to pasture	0.26 ± 0.03	0.16 ± 0.03	0.020
2 nd two weeks after return to pasture	0.22 ± 0.03	0.06 ± 0.03	<0.001
<i>Mean faecal egg count (eggs per gram)</i>			
Time of admittance	358 ± 168	876 ± 150	<0.001
1 st two weeks of hospitalisation	340 ± 169	1174 ± 160	<0.001
2 nd two weeks of hospitalisation	231 ± 161	1211 ± 154	<0.001
1 st two weeks after return to pasture	42 ± 17	1662 ± 339	<0.001
2 nd two weeks after return to pasture	57 ± 22	1081 ± 146	<0.001

Mean HF, LWG (kg/day) and FEC (epg) between the drenched and hospitalised animals grazing TCOL infected pastures at the time of admittance, in the first and second two week hospital grazing periods and in the first and second two week grazing periods after the hospitalised animals returned to pasture for each weighing and selection time is provided in Table 4.8. For HF, there was a three-way interaction effect of Drench treatment x Day of selection x Time ($P < 0.001$). Mean HF was greater in the drenched than hospitalised animals which were admitted on Day 23 at the time of admittance and in the first two week grazing period after these animals returned to pasture; for animals admitted on Day 37 in the second two week hospital grazing period and in the first two week grazing period after these animals returned to pasture; for animals admitted on Day 51 in the first two week hospital grazing period and in the second two week grazing period after these animals returned to pasture; for animals admitted on Day 65 at the time of admittance and in the second two week hospital grazing period and for animals selected on Day 79 at the time of admittance and in the first two week hospital grazing period ($P < 0.05$). Mean HF was similar between the drenched and hospitalised animals at all other times ($P > 0.05$).

For LWG, there was a three-way interaction effect of Drench treatment x Day of selection x Time ($P < 0.001$). Mean LWG was greater in the drenched than hospitalised animals which were admitted on Day 23 at the time of admittance and in the first and second two week grazing periods after these animals returned to pasture; for animals admitted on Day 37 in the second two week hospital grazing period and in the first two week grazing period after these animals returned to pasture; for animals admitted on Day 51 in the first and second two week hospital grazing periods and in the second two week grazing period after these animals returned to pasture; for the animal admitted on Day 65 at the time of admittance and in the second two week hospital grazing period and for animals admitted on Day 79 at the time of admittance ($P < 0.05$ for all these times). Other than that, mean LWG was similar between the drenched and the hospitalised animals ($P > 0.05$).

For FEC, there was a three-way interaction effect of Drench treatment x Day of selection x Time ($P < 0.001$). Mean FEC was lower for the drenched than the hospitalised animals at all times, except for animals admitted on Day 23 at the time of admittance, in the first and second two week hospital grazing periods and in the second two week grazing period after these animals returned to pasture and for animals admitted on Day 37 at the time of admittance ($P > 0.05$ for all these times). Since only one animal was admitted on Day 65, statistical comparisons could not be made at the time of admittance and in the second two week hospital grazing period. Nevertheless, FEC was similar between the drenched lambs and this lamb at all times ($P > 0.05$).

Table 4.8: Mean HF, LWG (kg/day) and FEC (epg) between the drench (Dren) and hospitalized (Hos) lambs grazing the TCOL infected pasture at time of admittance (0 days), at the 1st (14 days) and 2nd (28 days) two week hospital grazing periods and at the 1st (42 days) and 2nd (56 days) two week grazing period after the hospitalised animals returned to pasture for each weighing and selection time (Day 23-79).

Time Day	0 days		P-value	14 days		P-value	28 days		P-value	42 days		P-value	56 days		P-value
	Dren	Hos		Dren	Hos		Dren	Hos		Dren	Hos		Dren	Hos	
<i>Mean happy factor</i>															
23	0.91±0.04	0.61±0.06	0.002	0.68±0.06	0.52±0.16	0.420	0.54±0.11	0.37±0.15	0.327	0.98±0.04	0.71±0.10	0.044	1.00±0.05	0.82±0.07	0.056
37	0.68±0.06	0.52±0.05	0.081	0.54±0.11	0.43±0.07	0.325	0.98±0.04	0.78±0.04	0.002	1.00±0.05	0.66±0.05	<0.001	0.67±0.05	0.76±0.06	0.310
51	0.54±0.11	0.35±0.10	0.198	0.98±0.04	0.79±0.05	0.017	1.00±0.05	0.85±0.05	0.061	0.67±0.05	0.75±0.06	0.435	0.70±0.05	0.41±0.05	0.002
65	0.98±0.04	0.38±0.11	0.001	1.00±0.05	1.00±0.11	0.902	0.67±0.05	1.22±0.18	0.027	0.70±0.05	0.93±0.16	0.219			
79	1.00±0.05	0.60±0.05	<0.001	0.67±0.05	0.83±0.05	0.050	0.70±0.05	0.62±0.05	0.302						
<i>Mean live weight gain (kg/day)</i>															
23	0.22±0.03	0.07±0.03	0.013	0.07±0.05	0.02±0.15	0.789	0.04±0.06	-0.08±0.10	0.321	0.35±0.03	0.14±0.14	0.043	0.38±0.03	0.20±0.05	0.012
37	0.07±0.05	0.02±0.02	0.381	0.04±0.06	-0.04±0.05	0.321	0.35±0.03	0.20±0.03	0.002	0.38±0.03	0.10±0.04	<0.001	0.16±0.05	0.14±0.03	0.760
51	0.04±0.06	-0.09±0.07	0.200	0.35±0.03	0.17±0.04	0.002	0.38±0.03	0.26±0.04	0.043	0.16±0.05	0.21±0.05	0.478	0.14±0.04	-0.06±0.04	0.002
65	0.35±0.03	-0.11	0.001	0.38±0.03	0.29	0.320	0.16±0.05	0.54	0.034	0.14±0.04	0.29	0.273			
79	0.38±0.03	0.05±0.04	<0.001	0.16±0.05	0.16±0.02	0.901	0.14±0.04	0.08±0.03	0.226						
<i>Mean faecal egg count (eggs per gram)</i>															
23	88±35	167±88	0.582	714±139	400±122	0.697	963±765	1633±448	0.085	0.00±0.00	333±240	0.006	29±18	533±318	0.140
37	714±139	452±93	0.277	963±765	1621±247	0.002	0±0	1222±238	<0.001	29±18	1590±326	<0.001	0±0	970±169	<0.001
51	963±765	1360±279	0.005	0±0	1310±352	<0.001	29±18	1463±421	<0.001	0±0	2180±709	<0.001	138±50	1425±258	0.002
65	0±0	0	*	29±19	0	0.626	0±0	400	*	138±50	1200	0.232			
79	29±19	1175±366	<0.001	0±0	744±140	<0.001	137±50	922±224	0.004						

Keys: Dren. – Drench regime, Hos. – Hospital paddock treatment

“0 days” – time of admittance

“14 days” – first two week hospital grazing period

“28 days” – second two week hospital grazing period

“42 days” – first two week grazing period after the hospitalised animals returned to pasture

“56 days” – second two week grazing period after the hospitalised animals returned to pasture

Day 23 – 79 – weighing and selection times

4.5 Response to treatment (% change in HF)

The response to treatment (change in HF values) relative to the pre-treatment HF values for animals either receiving a four weekly anthelmintic treatment or subjected to a TST regime at two and four weeks and grazed on the hospital paddock for all selection times is given in Figures 4.8 and 4.9, for animals grazing the TCIRC and TCOL infected pastures, respectively.

For animals grazing TCIRC infected pastures, the response to treatment was relative to pre-treatment HF values, with drench and TST animals exhibiting a lower happy factor value at the time of selection or treatment decision, demonstrating a negative response (decrease in HF value) to treatment after two weeks and a positive response for drench animals (increase in HF value), but negative response for the TST lambs after four weeks of treatment. Overall, 60% of TST animals admitted into the hospital paddock had a negative response compared with 63% of the drench animals at two weeks after treatment and 64% compared with 66% at four weeks after treatment. The relationship between pre-and post-treatment HF for animals grazing the TCIRC infected pastures, either subjected to a four weekly drench treatment or a TST regime at two and four weeks after treatment are described by the following equations:

TCIRC - Drench: $y = -7.9x^2 - 76x + 58.4$ ($P < 0.001$, $R^2 = 69\%$) – two weeks after treatment

TCIRC-TST: $y = -1.1x^2 - 106.5x + 71.7$ ($P < 0.001$, $R^2 = 51\%$) – two weeks after treatment

TCIRC- Drench: $y = 139.6x^2 - 358x + 187.1$ ($P < 0.001$, $R^2 = 61\%$) – four weeks after treatment

TCIRC- TST: $y = -7.6x^2 - 88.4x + 54.6$ ($P < 0.001$, $R^2 = 47\%$) – four weeks after treatment

The mean response (mean % change in HF) of lambs grazing the TCIRC infected pasture was similar between animals grazing the hospital paddock compared with the drenched animals'; viz, -4.0% cf. -10.0%, respectively, at two weeks ($P = 0.261$) and -12.0% cf. -11.0%, respectively, at four weeks ($P = 0.868$) after treatment.

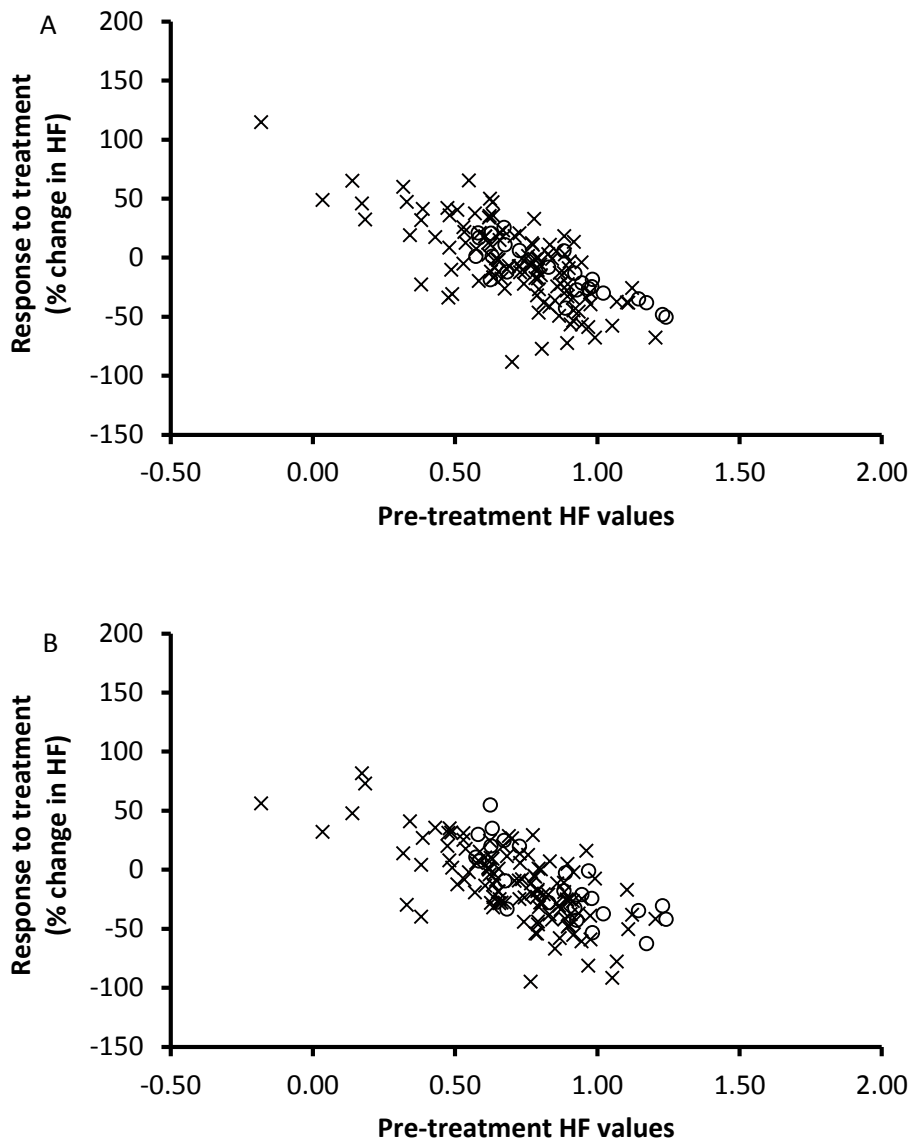


Figure 4.8: Change in HF (%) of animals grazing the TCIRC infected pasture which were either subjected to a four weekly anthelmintic treatment regime (○) or to a TST regime (x) at two (A) and four (B) weeks after treatment relative to the pre-treatment happy factor values for all selection times. Each data point represents an individual animal.

For animals grazing TCOL infected pastures, the response to treatment was relative to pre-treatment happy factor values. Animals subjected to the TST regime exhibiting a lower happy factor values at the time of treatment decision, demonstrated a positive response after two and four weeks of treatment whereas, animals subjected to a four weekly anthelmintic treatment regime demonstrated a positive response to treatment after two weeks but not after four weeks. Overall, 50% of TST animals admitted into the hospital paddock had a negative response compared with 66% of the drench animals at two weeks after treatment and 55% compared with 58% at four weeks after treatment. The relationship between pre-and post-treatment HF for animals grazing the TCOL infected pastures, either subjected to a four weekly drench treatment or a TST regime at two and four weeks after treatment are described by the following equations:

TCOL - drench: $y = 1.0x^2 - 109.7x + 93.4$ ($P < 0.001$, $R^2 = 71\%$) – two weeks after treatment

TCOL- TST: $y = 8.2x^2 - 115.4x + 73.15$ ($P < 0.001$, $R^2 = 54\%$) – two weeks after treatment

TCOL - drench: $y = -48.1x^2 - 91.9x + 113.1$ ($P < 0.001$, $R^2 = 92\%$) – four weeks after treatment

TCOL-TST: $y = 9.2x^2 - 137.1x + 84.97$ ($P < 0.001$, $R^2 = 65\%$) – four weeks after treatment

For animals grazing the TCOL infected pasture, mean response to treatment (mean % change in happy factor) was similar between animals receiving a four weekly anthelmintic treatment regime and animals grazing the TCOL hospital paddock after two weeks ($P=0.945$); viz. -2.0% cf. -2.0%, respectively, and after four weeks ($P=0.762$) viz. -8.0% cf. -5.0%, respectively, of treatment.

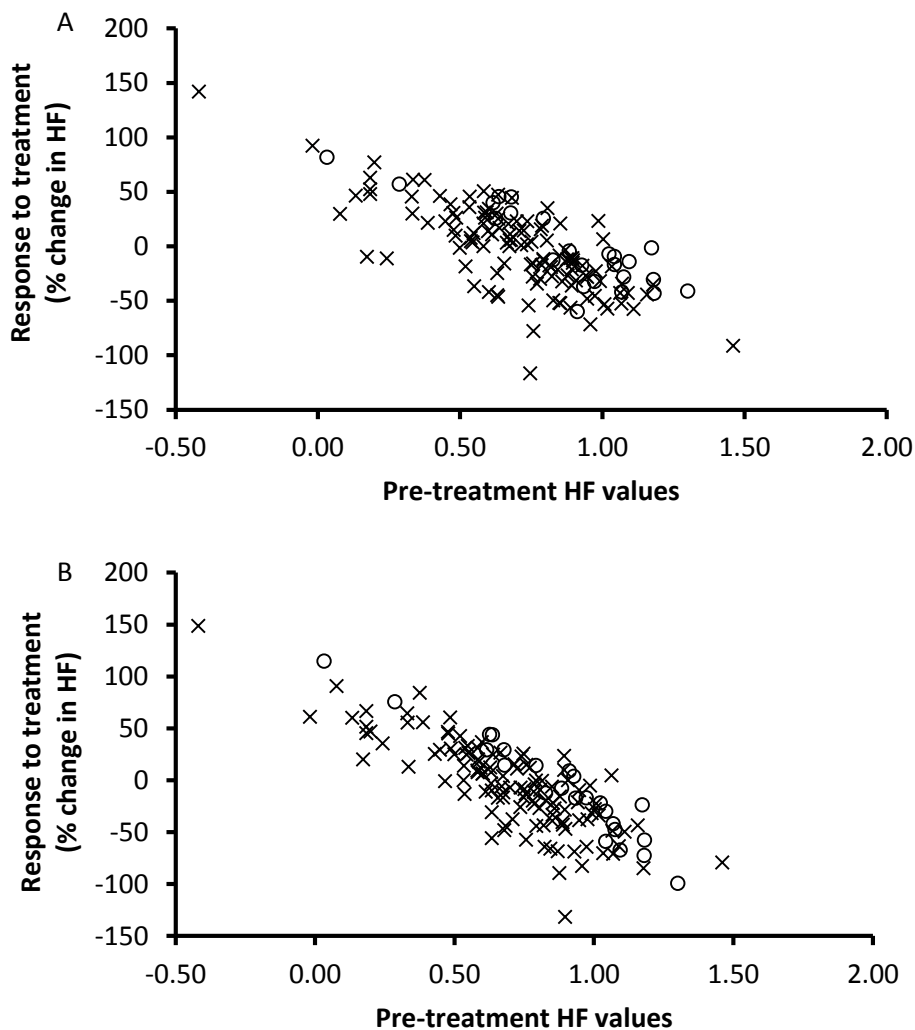


Figure 4.9: Change in happy factor values (%) of animals grazing the TCOL infected pastures which were either subjected to a four weekly anthelmintic treatment regime (o) or to a TST regime (x) at two (A) and four (B) weeks after treatment relative to the pre-treatment HF values for all selection times. Each data point represents an individual animal.

Chapter 5

Discussion

This study was carried out in Lincoln, New Zealand with the aim of evaluating the ability of the TST regime without use of anthelmintic to maintain performance of organic lambs exposed to *Teladorsagia circumcincta* or *Trichostrongylus colubriformis* challenge in a field grazing situation. More specifically, the objective of the study was to determine whether the use of a TST regime with a hospital paddock containing bioactive forages in place of anthelmintic treatment would be able to provide enough of an area of reduced larval challenge and/or a direct anthelmintic effect to mitigate the effects of infection and assist organic parasitized lambs to recover and return to normal performance rates.

5.1 Ability of the TST regime in maintaining lamb performance

Overall, the TST regime, together with a short-term treatment comprised of bioactive forages instead of chemotherapy, was able to maintain reasonable performance in animals exposed to the abomasal, but not intestinal infections in this environment. Typically, the required time for weaned lambs to reach market weight in conventional systems is around 12-14 weeks post-weaning, given that feed requirements are met and parasitism in animals is controlled effectively. In the current study, mean LW of the drenched group challenged with both *T. circumcincta* and *T. colubriformis* was similar (Figure 4.6), with 100% of lambs reaching the target slaughter weight of 38 kg by 11 and 13 weeks post weaning, respectively (Table 4.1). Overall, this indicates that sufficient feed reserves were available for lambs to reach slaughter weights within an acceptable time frame, and more importantly, the drench treatment regime for both parasite species provided an adequate comparison for the potential growth that would be observed if adequate parasite control regimes are employed in this environment. It may have been expected that organic lambs would take longer than 13 weeks to reach market weight than conventional lambs (Niezen *et al.*, 1991). This seemed to be the case in the growth rate of the TST animals challenged with both GIN species; more so in lambs exposed to the intestinal than the abomasal nematode specie (Figure 4.6). Unfortunately, due to ethical reasons, an un-drenched control group was not included in this trial to determine exactly how much of the loss in productivity caused by parasite infection was able to be recovered with the use of the TST regime. Nevertheless, the TST regime with the hospital paddock treatment did enable 80% and 58% of lambs infected with *T. circumcincta* and *T. colubriformis*, respectively, to reach the required slaughter weight of 38 kg by 13 weeks post- weaning. Furthermore, the lack of mortalities, coupled with the relatively few number of lambs re-admitted and that only one lamb in the TCOL-TST group received salvage anthelmintic treatment, provides further evidence in support of the

effectiveness of this regime, in maintaining reasonable performance in lambs infected with *T. circumcinta*, but not *T. colubriformis*.

5.1.1 Reductions in LW under the TST regime and larval challenge

Despite more than half of the lambs reaching marketable weights in an acceptable time frame under an organic environment, production losses, with respect to cumulative LW were evident. When compared with the drench group, cumulative LW was similar between the drench and the TST group in the early part of the trial (Day 0 – 51). However, significant differences were evident in the latter part of the trial (Day 79 and 65-93), which resulted in an overall 32% and 39% reduction in cumulative LWG in the TST animals; viz., 13.47 ± 1.06 cf. 19.97 ± 1.63 kg and 13.32 ± 0.65 cf. 21.96 ± 1.27 kg for the TCIRC and TCOL group, respectively, during the entire trial period (Figure 4.6). Presumably, the observed production losses in the current study reflect the impact of parasitism on the host, which includes reduction in voluntary feed intake (VFI) and nutrient utilisation (Sykes *et al.*, 1988), both of which were not directly measured in this study. Nevertheless, apparent DM intake/day, estimated from the pre-and post-grazing herbage mass in this study indicate a daily DM intake by lambs of 0.88 kg DM/day at the start of the trial, which increased with time to 1.28 kg DM/day at end of the trial (Table 5.1). With this in mind, it is apparent from larvae recovered from herbage in this study that the level of challenge on the pasture and hospital paddock was different for the two GIN species and varied with time.

Table 5.1: Apparent DM intake (kg DM/day) of lambs estimated from the pre- and post-grazing herbage mass and recovered larvae from herbage at each of the two week grazing period, for both the pasture and hospital paddock for *T. circumcinta* (TCIRC-P & TCIRC-H) and *T. colubriformis* (TCOL-P & TCOL-H) throughout the entire trial.

Dates	Day of trial	TCIRC-P	TCIRC-H	TCOL-P	TCOL-H	Average
12-Dec	0	0.88		0.88		0.88
21-Dec	9	0.98		0.96		0.97
4-Jan	23	1.07	1.03	1.05	1.00	1.04
16-Jan	37	1.17	1.10	1.10	1.04	1.10
30-Jan	51	1.18	1.15	1.07	1.06	1.11
13-Feb	65	1.21	1.27	1.17	1.12	1.19
27-Feb	79	1.32	1.24	1.29	1.14	1.24
13-Mar	93	1.36	1.18	1.34	1.23	1.28

The assumed levels of larval intakes for the current study estimated from apparent DM intakes in Table 5.1 is provided in Table 5.2. Lambs that grazed TCIRC pasture paddock could have been exposed to larval intakes ranging between 1071 – 28238 L₃/week, with levels below 4000 L₃/ week observed on three of the six two week grazing periods, whereas those in the hospital paddock could have been exposed to intakes ranging between 1421 – 149639 L₃/ week, with levels greater than 4000 L₃/day observed on four out of six two week grazing periods. Lambs that grazed TCOL pasture

paddock could have been exposed larval intakes ranging between 3360 - 63000 L₃/ week, while those in the hospital paddock could have been exposed to larval intakes ranging between 1848 - 18760 L₃/ week.

Table 5.2: Estimated daily larval intakes of lambs grazing the *T. circumcincta* and *T. colubriformis* infected pasture paddocks (TCIRC-P and TCOL-P) and their respective hospital paddocks (TCIRC-H and TCOL-H) at each of the two week grazing period throughout the trial.

Larval intakes estimated from apparent DM intakes and recovered L ₃					
Dates	Day of trial	TCIRC-P	TCIRC-H	TCOL-P	TCOL-H
21-Dec	9	0	0	443	0
4-Jan	23	1507	203	0	0
16-Jan	37	4034	11265	0	0
30-Jan	51	153	285	3344	264
13-Feb	65	2254	21377	9000	336
27-Feb	79	516	3509	480	2680
13-Mar	93	305	7540	1194	2121
AVERAGE		1253	6311	2066	772
Apparent intake taking into consideration distribution of L ₃ on sward and selective ability of grazing animals					
Dates	Day of trial	TCIRC-P	TCIRC-H	TCOL-P	TCOL-H
21-Dec	9	0	0	89	0
4-Jan	23	301	41	0	0
16-Jan	37	807	2253	0	0
30-Jan	51	31	285	669	53
13-Feb	65	451	4376	1800	76
27-Feb	79	103	702	96	536
13-Mar	93	61	1508	239	424
AVERAGE		251	1309	413	156

These ranges of larval intakes could, however, have been over-estimated. Studies have indicated that distribution of L₃ increases with proximity to the transitional zone between pasture and soil (Sutherland and Scott, 2010). Callinan and Westcott, (1986) observed an eight-fold recovery of L₃ in soil than on herbage, with 71% of these recovered between ground level and 2 cm height of herbage, and was dependent on temperature and humidity (Callinan and Westcott, 1986). In addition, when given the opportunity, grazing sheep are known to select green material in preference to dead material, and leaf in preference to stem (Cosgrove *et al.*, 1999; Hughes *et al.*, 1984). Since, the method of herbage sampling for determination of L₃ used in the current study; viz., the whole pluck method, included the harvest of leaf together with the stem and at times some roots as well, larval intakes in this study could have been lower than previous estimations. Therefore, taking into account the distribution of L₃ on sward structure and the selective ability of grazing animals, and given that the pre- and post-grazing herbage mass in the current study indicated that more than 2 cm height of herbage was on offer in the early part of the trial, it is probable that apparent larval intakes at those times could have been lower (Table 5.2). Furthermore, apparent larval intakes, especially in the

hospital paddocks of both GIN species, could have been higher in the latter part of the trial, as herbage mass fell below 1200 kg DM/Ha. This could have resulted in lambs grazing herbage between ground level to 2 cm in height, hence higher larval intakes in the latter part of the trial (Table 5.2).

5.1.2 Temporal variations in production loss under TST regime

There appeared to be temporal variations in the reduction in cumulative LW in lambs that was dependent on parasite species. When compared with the respective drench group, the reductions in cumulative LW of the TST animals observed from Day 23 to Day 65, were minimal for the TCIRC-TST (1%; viz., 12.18 kg cf. 12.54 kg respectively), but greater for the TCOL-TST group (18%; viz., 8.09 kg cf. 12.59 kg, respectively) (Figure 4.6). The estimated larval intakes indicate that lambs in the TCOL pasture paddock were exposed to a challenge of 90 L₃/ day from Day 9 to 23 of the trial. This was associated with a 7% check in growth rate which wasn't recovered in the subsequent weeks, resulting in the 18% loss on Day 65 of the trial. Results of larvae recovered from herbage from Day 23 to 51 indicate that there was no challenge on both TCOL pasture and hospital paddocks (Figure 4.4 and Table 5.2). However, the reductions in performance and the high and increasing FECs of lambs in both paddocks observed during this period suggests the presence of a considerable level of larval challenge encountered by the lambs. It is possible that samples collected for determination of L₃ did not adequately represent of the level of infection on the respective paddocks. On the other hand, estimated larval intake, ranging from as low as 50 to over 10000 L₃/ day were observed in the TCIRC pasture and hospital paddocks during this period, but reduction cumulative LWG was minimal. This observation provides evidence in support of the theory that at equal or similar rates of infection, intestinal nematodes are more pathogenic than the abomasal nematodes, which concurs with the findings of previous pen studies who observed more severe effects of the intestinal compared with the abomasal nematode on the host (Steel, 1978; Steel *et al.*, 1980; Steel *et al.*, 1982; Symons *et al.*, 1981). Furthermore, this finding also supports the notion that hospitalisation using bioactive forages was more successful on animals infected with the abomasal than intestinal nematode species, and is in agreement with the findings of several other studies who observed a greater improvement in performance of animals challenged with the abomasal, but minimal to no improvement in performance of animals challenged with the intestinal nematode species with the use of similar bioactive forages (Athanasiadou *et al.*, 2005; Heckendorn *et al.*, 2007; Tzamaloukas *et al.*, 2005; Tzamaloukas *et al.*, 2006).

The loss in production was reversed between the two parasite species from Day 65 onwards, with minimal reductions in cumulative LW observed in the TCOL-TST (5%; viz., 5.06 kg cf. 7.44 kg) than TCIRC-TST lambs (12%; viz., 2.19 kg cf. 7.00 kg) when compared with their drenched counterparts (Figure 4.6). The reasons for this are unclear as herbage mass on both hospital paddocks was similar,

but may reflect either the greater contamination observed in the TCIRC hospital paddock, or alternatively due either to the ability of an animal to suppress establishment and/or subsequent development of a worm infection (resistance) or the ability of an animal to maintain a relatively undepressed performance under nematode challenge (resilience) in the TCOL group (Albers *et al.*, 1987; Bisset *et al.*, 2001; Dobson *et al.*, 1992; Van Houtert. *et al.*, 1996). The estimated larval intake in the TCIRC hospital paddock in the latter part of the trial (Table 5.2) was threefold greater than the threshold level of exposure of 12000 – 37000 L₃/week for a reduction in LWG to be evident (Symons *et al.*, 1981), whereas intake level was within that range from Day 65 to 79, but lower than 5000 L₃/week on the last two grazing period (Table 5.2). From these observations, it is possible that the size, together with the variations in larval challenge in the TCIRC pasture and hospital paddock in the latter part of the trial could be partly responsible for the greater check in growth rate observed. In contrast, estimated larval intakes of the TCOL lambs in both the pasture and hospital paddock in the latter part of the trial exceeded the threshold levels of exposure of 950 – 3000 L₃/ week in which a reduction in cumulative LWG of naive lambs exposed to *Teladorsagia circumcincta* was evident (Steel *et al.*, 1980), but the reduction in cumulative LWG was minimal. In addition, mean FEC of the TCOL-TST lambs were greater than that of their drenched counterparts (Figure 4.5). Despite the high FEC, the TCOL-TST lambs were able to maintain productivity in the face of larval challenge, suggesting that they were resilient. This finding is in agreement with findings of several studies in New Zealand which have shown that resilient animals can have a greater productivity, despite exhibiting high FEC (Morris *et al.*, 2005; Morris *et al.*, 1997), although it is not clear why this was evident in the TCOL but not the TCIRC infected animals. It is also possible that the declining herbage mass in the TCIRC hospital paddock during the latter part of the trial (Figure 4.1) may have restricted feed intake, hence growth potential, and/or increased the larval intakes due to the shorter sward height, resulting in a greater reduction in cumulative LWG. In reality, this effect may have been greater as it was noticed that the lambs had an aversion to plantain (Figure 4.2 and 4.3), as such the pasture mass of the hospital paddocks probably have overstated the true availability of herbage to the animals.

With respect to the estimated larval challenge observed at each two week grazing period during the entire trial, it is apparent that the reduction in cumulative LWG observed in lambs challenged with both the abomasal and intestinal nematode was associated with the level of challenge on herbage and was possibly influenced by distribution of lambs and changes in herbage mass. That being said, it is possible that the losses in production observed in the current study was not only associated with changes in level of nematode challenge, but also to other factors which possibly include the anti-nutritional properties of the bioactive forages, changes in herbage mass and the interaction of individual lambs with these factors. Since the acceptability of any parasite control regime is dependent on the ability of animals to achieve saleable weights, the overall findings that 80% and

58% of lambs infected with TCIRC and TCOL, respectively, reached marketable weights in an acceptable time frame in an organic environment, despite the loss in production, indicate that the TST regime with the use of the hospital paddock was able to maintain reasonable production levels, in terms of cumulative LWG in lambs infected with *T. circumcincta*, but not *T. colubriformis*.

5.2 Performance responses of hospital treatment

5.2.1 Effect of hospital paddock treatment on LWG

The hospital paddock treatment appeared to provide a short-term benefit, in terms of lamb growth. In comparison with the drench group, LWG of the hospitalised lambs in the second two-week hospital grazing period for the TCIRC group (Table 4.5), and in the first and second two-week grazing periods after the hospitalised animals returned to pasture for the TCOL group (Table 4.7) was similar. These observations are in agreement with previous observations from several studies who have reported superior growth rates in lambs fed chicory and red clover (Jagusch *et al.*, 1979; Marley *et al.*, 2003; Marley *et al.*, 2005; Niezen *et al.*, 1994; Scales *et al.*, 1995; Speijers *et al.*, 2000; Tzamaloukas *et al.*, 2005; Tzamaloukas *et al.*, 2006). However, average growth rate of hospitalised animals in this study at the first and second two-week hospital grazing periods (100 – 170 g/day), are lower than growth rates of 200 – 300 g/day observed in the studies mentioned above. The differences in growth rate between those studies and this study is probably due to differences in the nature, setup and objective of the each trial. All the studies mentioned above used monoculture swards, and while some used mono-specific infections, others used natural infections, whereas in this study, a mixed sward of chicory, plantain and red clover, with main paddocks infected with mono-specific infections of *T. circumcincta* and *T. colubriformis* were used.

The benefit of hospitalisation on LWG of lambs observed in this study was partly a reflection of the temporal changes in level of exposure to challenge from main paddock to hospital paddock in the first two week grazing period after admission, and the subsequent two week grazing period in the hospital paddock, as indicated by the larvae recovered from these paddocks (Figure 4.4). The improved performance observed in this study is in agreement with observations of several studies which have pointed out the importance of removing larval challenge in parasitized animals to restore appetite, hence reduce production losses (Coop *et al.*, 1982; Kyriazakis *et al.*, 1996b; Steel *et al.*, 1980; Symons *et al.*, 1981). Another possible factor that could be responsible for this benefit in LWG observed during and after hospitalisation could be related to the quality of the bioactive forages. Although quality of the forages was not measured in this study, literature from previous studies mentioned above indicated that chicory, plantain and red clover have high nutritive values, and it is apparent from pasture dissection measurements that hospitalized animals had access to these three forages at all times while in the hospital paddock, although the contribution of chicory and red clover

declined with time (Figures 4.2 and 4.3). These bioactive forages have a greater mineral content and less fibre than ryegrass, hence are highly digestible (Barry, 1998). Therefore voluntary feed intake (VFI) would be expected to be higher, resulting in improve plane of nutrition, hence improved performance (Barry, 1998). It is also possible that the higher mineral content of the bioactive forages could have improved VFI resulting in the short-term benefits on HF and LWG observed. It was postulated that for naive animals such as lambs, improved nutrition, especially protein nutrition, would make more nutrients become available, thus will enhance their resilience in the face of larval challenge (Sutherland and Scott, 2010; Sykes and Coop, 2001). Although evidence of this is provided by several studies who have indicated that the improved performance was due to the higher dietary protein content and metabolisable protein supply of these forages (Bown *et al.*, 1991b; Coop and Kyriazakis, 1999; Coop *et al.*, 1995; Houdijk *et al.*, 2001; Tzamaloukas *et al.*, 2006; Van Houtert *et al.*, 1995b), the high FEC observed in the current study indicate that hospital treatment did not enhance the immunity of the lambs either through protein or mineral supply. In part, this may have been due to the observed aversion of lambs to consuming plantain which is known to supply condensed tannins which may improve metabolisable protein supply. Therefore, the short-term benefit of hospitalisation was probably associated with the nutritive value of the bioactive forages on VFI and consequently, growth rate.

5.2.2 Re-admitted lambs

For a majority of animals, one four-week period of hospitalisation was sufficient to allow individuals to return to normal growth rate. Of the twenty four TST animals in each parasite species group, eight animals in the TCIRC and ten animals in the TCOL groups were readmitted into the hospital paddock throughout the entire trial (Figure 4.7). In comparison, Lundberg, (2012) observed that four weeks of hospitalisation on these forages was sufficient to enable 80% of lambs to return to normal growth rates. Furthermore, of the lambs that were re-admitted, 80% and 90% in the TCIRC and TCOL group respectively, were readmitted after two weeks on pasture with the remainder (20% and 10%) after four weeks on pasture (Figure 4.7). Due to lack of comparable data, valid conclusions could not be made from this result. Nevertheless, the high proportion of animals re-admitted after two weeks on pasture suggests that effect of hospital treatment, albeit rapidness in improving performance, was reversible. It should be noted that performance of hospitalised animals in this study varied with changes in level of challenge on pasture, herbage mass and stocking rate at each two-week grazing intervals. Alternatively, it is possible that the overall lower number of animals re-admitted in this study may reflect variability within a flock, of either the distribution of the parasite population as GI populations in grazing sheep are highly aggregated and over dispersed with 80% of nematodes found in only 20-30% of the hosts (Sreter *et al.*, 1994) or even individual variability in their response to nematode challenge (Greer *et al.*, 2005). It is also interesting to note that a majority of animals

appeared to respond positively within two weeks after being hospitalised (Figure 4.8 A and 4.9 A). The decision to use the four weeks treatment period in the current study was based on previous observations in which 80% of lambs recovered within four weeks of grazing a low contamination area (Lundberg, 2012). However, it is possible that a lesser area of bioactive herbage is required if hospitalisation time is reduced to two weeks, which would further enhance the weak agronomical qualities of these forages within production systems. However, the ability of such a short time frame of grazing on the re-admittance rates would still require further evaluation.

Despite the improved LWG of the hospitalised to similar levels with those of the drench animals, mean efficiency (HF) did not reach the cut-off efficiency of 0.74 at most times; more so in the hospitalised than drench animals. Furthermore, response to treatment, as judged by the change in HF values, indicate that only 36% of all hospitalised lambs in the TCIRC group responded positively to treatment at four weeks after hospitalization (Figure 4.8B), while 50% and 45% of all hospitalised animals in the TCOL group responded positively at two and four weeks after hospital paddock treatment, respectively (Figure 4.9 A and B). This findings are in contrast with the finding of Lundberg, (2012) where 80% of lambs responded positively to the hospital paddock treatment at four weeks after hospitalisation. The difference in response of lambs to hospitalisation between the two studies could be related to differences in level of challenge on pasture. Alternatively, it is possible that the lack of a positive control in the former study could have overestimated of the response of lambs to the hospital paddock treatment. Overall, these results do suggest that hospitalisation on bioactive forages alone is not sufficient to completely alleviate the impacts of parasitism.

5.2.3 Anti-parasitic benefits of hospital treatment

Hospitalization did not appear to provide any anti-parasitic benefits to the host. Although the interpretation of FEC as an indicator of worm burden is severely limited for *T. circumcincta* infections (Greer and Sykes, 2012) and thus must be interpreted with some caution, FEC in both TST groups and in the hospitalised animals was consistently high. This remained so for all selection times at the time of admittance, during hospitalisation and up to one month after the hospital animals returned to pasture throughout the trial (Tables 4.5 and 4.7). Patent nematode infections were evident from eggs on the faeces of all animals at three weeks after the start of the trial, which is consistent with the pre-patent period in the life cycle of nematodes (Vlassoff *et al.*, 2001; West *et al.*, 2009a). As time progressed, mean FEC increased and remained consistently greater in the hospitalised than drenched animals throughout the trial. In contrast to this study, several studies using natural (grazing studies) or experimental (infection studies) challenges have reported reduced TCIRC burdens in growing lambs that grazed chicory and red clover than lambs grazing conventional forages, resulting in lower

FEC (Heckendorn *et al.*, 2007; Marley *et al.*, 2005; Scales *et al.*, 1995). On the other hand, the findings of this study are in agreement with findings of several other short-term studies using 3-5 month old lambs grazing chicory and red clover swards where there were no reductions in worm burdens, resulting in high FEC that was statistically similar to those animals grazing conventional forages (Athanasiadou *et al.*, 2005; Lundberg, 2012; Marley *et al.*, 2003; Niezen *et al.*, 1994; Tzamaloukas *et al.*, 2005; Tzamaloukas *et al.*, 2006). Although this study was not tailored to measure the direct anthelmintic effect of the bioactive forages on established worm populations or development and establishment of incoming infective larvae ingested while in the hospital paddock, the high FEC observed suggests that hospitalization did not appear to have any direct anthelmintic effect on the established adult worm population or incoming larvae ingested.

5.2.4 Temporal variations in performance of hospital animals

There appeared to be temporal variations performance of hospital animals during hospitalisation which was partly dependent on parasite species. There was a lack of benefit of hospital treatment, in terms of HF and LWG, on TCIRC-hospitalised animals when compared with the drench animals at two weeks after hospitalization (Table 4.5). This finding agrees with the findings of Heckendorn *et al.*, (2007) who observed no change in performance of lambs grazing chicory compared to grasses, and Lundberg (2012) who observed lower performance of parasitized lambs grazing a mixture of chicory, plantain and red clover compared with those grazing ryegrass/white clover pastures. The lack of effect on performance in the current study was probably associated with the temporal variations in performance as observed when data was analysed for each time of weighing and selection during hospitalization (Table 4.6). HF and LWG increased at four out of five selection times, but the increases were smaller than those observed by their drench counterparts, which resulted in greater performance of the drench than hospitalised animals. It is possible that these observations may have reflected the changes in level of exposure to challenge, hence apparent larval intake of lambs on the hospital paddock (Table 5.2) and its subsequent impact on animal performance.

The performance, in terms of LW, of animals selected and admitted into the hospital paddock in the early part of the trial may have been expected to be greater than those subsequently admitted because the hospital areas were expected to provide an area of reduced larval contamination as they have been sown in the preceding year. However, LW of hospitalised lambs in both the TCIRC and TCOL groups did not follow any pattern, but varied with time (Figure 4.6). It is possible that the variations observed in the early part of the trial (Day 23-51) are due to changes in the level of challenge, hence apparent larval intakes on the hospital paddocks (Table 5.2), whereas the declining LW change towards the end of the trial (Day 65-79) is probably due to a combination of high larval

challenge and declining herbage mass (Figure 4.1) or the declining composition of bioactive forages, especially chicory and red clover on the hospital paddocks (Figures 4.2 and 4.3).

There appeared to be a trend towards a lower performance of lambs observed in the first two-week than the second two-week hospital grazing period. Results indicated that animals that stayed on the hospital paddock for a period of 28 days performed slightly better than animals that stayed for a period of 14 days in both parasite species (Tables 4.6 and 4.8). This finding suggests that there was an effect of transition or adaptation to the new forage being consumed, that is, from ryegrass/clover to herb mix of chicory, plantain and red clover. According to Waghorn *et al.*, (2007), feeding the ruminant is really about feeding the rumen microorganisms, and existence of differences in the physical and chemical properties of different forages imply that rumen microflora would need time to adapt to the forage substrate that is being consumed so that maximum digestion can be achieved. In part, the incorporation of clover in the hospital paddock and that fact that, given these were organic pastures, weed control was limited, it was anticipated that transition to the hospital areas would be minimal. However this was not the case.

5.3 Performance of lambs after their return to pasture

The TCIRC lambs were able to maintain their performance at levels similar to post-hospital treatment levels at two weeks after their return to pasture, at which point HF and LWG were similar to that of the drench animals (Table 4.5). This was probably associated with the relieving of the parasitized lambs from high challenge environments, which is supported by counts of larvae recovered from herbage on three out of four selection times in this study (Figure 4.4). This finding is in agreement with the findings of several other authors who observed improved lamb performances coinciding with move from high to low infectivity pastures (Boa *et al.*, 2001; Coop *et al.*, 1982; Kyriazakis *et al.*, 1996a; Kyriazakis *et al.*, 1996b; Steel *et al.*, 1982; Symons *et al.*, 1981). Studies have widely documented that the two major consequences of exposure to high larval challenge on pasture is a depression in appetite and food utilization resulting in reduced performance and that relieving lambs from high challenge pasture resulted in increased appetite hence improved performance (Coop *et al.*, 1982; Steel *et al.*, 1982; Symons *et al.*, 1981; Thamsborg and Agergaard, 2002). The lower performance observed at four weeks after the hospitalised animals returned to pasture for both TCOL and TCIRC lambs probably reflected the temporal variations in the level of challenge (Figure 4.4), hence larval intakes (Table 5.2) and the declining herbage mass in both pasture paddocks (Figure 4.1).

5.4 Predictors of parasitism

The success of any TST regime is dependent on its suitability to adequately identify individuals that are suffering from parasitism, and thus are likely to respond to treatment. In this study, the HF system, which used LWG as an indicator for parasitism (Greer *et al.*, 2009), was used to identify poor performing individuals suffering from parasitism which are likely to respond to treatment. LWG of animals identified as poor performing (hospital) at the time of selection was significantly lower than of those not needing treatment (pasture) on three and two out of six selection times in the TCIRC and TCOL groups, respectively (Tables 4.3 and 4.4). This is not surprising as the HF model is largely based on animal growth. However, this finding is in contrast with the finding of Greer *et al.*, (2010) who observed significantly lower LWG in animals deemed to need treatment than those that met their target weights at all four selection times. In addition, poor performing animals had similar FEC at the time of selection, at all selection times with those animals that were deemed to not need treatment (Tables 4.3 and 4.4). The similar mean FEC between lambs that met their target weight and those that did not in the current study also concurs with the findings of the former study and is suggested to be due to either the poor relationship between FEC and worm burden and/or larval challenge as observed in several previous studies (Steel *et al.*, 1980; Symons *et al.*, 1981) or to variations in the response of individual lambs to GIN infections (Greer *et al.*, 2008). In contrast, the consistently similar LW observed between these two groups of animals in the current study does not agree with the findings of several studies who observed lower LW of those animals deemed to need treatment than those not deemed to need treatment on half of the times of treatment decision (Greer *et al.*, 2010; Leathwick *et al.*, 2006). Overall, this indicates that the ability of the HF model to identify underperforming individuals may not necessarily be those that are lighter or have the highest FEC (Greer *et al.*, 2010). Given that FEC is still used as the primary determinant of parasitism in growing lambs, it remains to be determined what the most appropriate indicator for parasitism is on an individual basis.

Chapter 6

Conclusion

Despite the reduction in cumulative LWG, the use of the TST regime together with a hospital paddock treatment in place of anthelmintic was able to maintain reasonable performance in lambs exposed to and infected with the abomasal nematode (*T. circumcincta*), but not the intestinal nematode (*T. colubriformis*). This resulted in 80% of the TCIRC lambs reaching their required slaughter weight of 38 kg in a reasonable time frame. The loss in production and temporal variations in performance of lambs observed in this study is probably attributed to two main factors; namely the size or level of larval challenge and the changes herbage mass in the hospital areas at each of the grazing periods, and the interactions of these two factors with individual lambs with time. With that in mind, the lack of an un-treated control group in this study limited the ability to quantify how much of the lost productivity due to parasitism was able to be removed through the use of a hospital paddock treatment.

It was apparent that hospitalisation did confer some benefits to lamb performance, but not direct anthelmintic benefits. In part, this presumably reflected the nutritive quality of the bioactive forages and the reduced larval challenge, although the true benefits may be underestimated in this study as the herbage supply in the hospital areas declined to sub-optimal levels which are likely to have influenced food intake.

Performance of lambs, in terms of the efficiency (HF), observed in this study is considerable lower (around 30%) compared with other studies. Despite this finding, LWG of animals deemed to be poor performing and are likely to respond to treatment compared with those deemed to not need treatment was consistently lower at all selection times suggesting LWG to be a possible and potential indicator for parasitism, especially in the temperate regions.

Overall, the implication of this study for the organic farmer is that it is possible to grow lambs to market weight within an acceptable time frame under a TST regime with LWG as an indicator for parasitism and a hospital paddock treatment comprised of bioactive forages. However, the findings of this study should be interpreted with caution, as both abomasal and intestinal nematodes coexist in field grazing situations. Therefore, there is need for further research to be conducted into the possibility of providing organic farmers to a method of control with the use of bioactive forages. In addition, it is possible that the benefits observed in this study may be enhanced if better grazing management of the bioactive forages was adopted to ensure adequate supply at all times.

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