Relationship between gastrointestinal (GI) nematode infection and immune response in dairy cows

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Dhrubajit Saikia

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Intensive pasture grazing systems commonly seen in New Zealand dairy farms provide favourable conditions for the development of gastrointestinal (GI) parasitism. The objectives of this study were to seek associations between levels of serum or milk antibody to gastrointestinal nematode parasites and faecal egg counts (FEC), milk yield, infective L3 larvae ingested from pasture and worm burden. This study was performed on the Lincoln University Research dairy Farm (LURDF) from October, 2011 to June, 2012. In Experiment A, a group of lactating dairy cows grazing pasture at a high stocking rate (HSE, n=31) and fed supplements were compared with a low stocking rate group (LSE, n=30) without supplements, both of which grazed a combination of new and established (old) pastures. Monthly measurements were performed for faecal egg counts (FEC), pasture L3 larvae level, daily milk yield and anti-*Ostertagia* antibody level (OD) in pooled milk or serum. In Experiment B, 60 lactating dairy cows were treated with anthelmintic and randomly allocated to one of three groups i.e. GI, GII & GIII. In GI (n=20), cows were not artificially infected (controls). In GII (n=20), cows were artificially infected twice with infective larvae (oral dose of 50×10^3 L3/cow) which was twice truncated 10 days after infection with an anthelmintic treatment. In GIII (n=20), cows were artificially infected once with L3 (oral dose of 50×10^3 L3/cow) without truncation. Individual faecal egg counts and anti-*Ostertagia* antibody levels (OD) in pooled serum were measured. At the end of the trials, 17 cull cows (6-Exp. A; 11-Exp. B) were slaughtered and worm burdens were quantified.

A significant positive relationship was observed between daily milk yield and serum OD (but not milk OD) in both the HSE ($r=0.909$; $p=0.002$) and the LSE ($r=0.908$; $p=0.002$) groups in

Experiment A. This is likely to be due to a low level of infective larval ingestion leading to a decline in immunity over the lactation period rather than a direct link. The mean worm burden was not found to be significantly different (p= 0.492) between the HSE and LSE groups. The correlation between anti-*Ostertagia* antibody levels (OD) in serum and worm burdens in the slaughtered animals was negative (r= -0.805, p=0.054). There were no significant differences in the milk OD (p=0.126) or serum OD (p=0.500) between the HSE and LSE groups. There was no significant difference (p=0.26) between the HSE and LSE groups with regard to FEC, but there was a trend (p=0.059) for a greater percentage of younger (cluster of 2–4 years) cows to have positive FEC. There was a positive correlation (r=0.868, p=0.056) between FEC and pasture L3 levels in the HSE group. Pasture L3 levels showed a significant difference in the old versus new pasture (p=0.009) and a trend for higher levels (p=0.092) in the HSE versus the LSE groups. In Exp. B, there was a positive relationship between the percentage of cows with positive FEC and anti- *Ostertagia* antibody levels (OD) in serum (r=0.559, p=0.030). The mean worm burden did not differ significantly (p=0.370) among the treatment groups (GI, GII & GIII). Anti-*Ostertagia* antibody levels (OD) in serum did not differ between groups, and worm burdens in the slaughtered animals did not correlate with antibody titre in serum (r= -0.331, p=0.320).

In summary, there is evidence that levels of parasite antibody in the serum and percentage of positive FEC may be related. The difference in stocking rate appears to have no obvious impact on gastrointestinal worm infections in adult dairy cows. However, the low numbers of animals in this study prevent any association with worm burden and hence there is a need for this research to be repeated in a larger study for validation.

Keywords: Gastrointestinal (GI), nematode, parasite, worm, helminth, anthelmintics, faecal egg counts (FEC), *Ostertagia*, free-living stage, resistance, immunity, antigen, antibody, hypobiosis, worm burden, optical density (OD), refugia, eprinomectin.

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List of Abbreviations

^{o}C	Degree Celsius
^{o}F	Degree Fahrenheit
µ	Micron
µl	Microlitre
Ab	Antibody
ABTS	2, 2'-azino-bis-3-ethylbenzothiazoline-sulfonic acid
AR	Anthelmintic resistance
BCS	Body condition scoring
BTM	Bulk-tank milk
BZ	Benzimidazoles
CP	Crude protein
CT	Condensed tannins
Dip	Diploid
DM	Dry matter
EL4	Early fourth stage larvae
ELISA	Enzyme linked immunosorbent assay
EPG	Eggs per gram
FEC	Faecal egg counts
FECRT	Faecal egg count reduction test
g	gram
× *g*	Times gravity
GI	Gastrointestinal
HCl	Hydrochloric acid
hr	Hour
HRP	Horseradish peroxidase
HSE	High stock efficient
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL4	Interleukin 4
I/M	Intramuscular
IZ	Imidazothiazoles

L	Litre
L1	First stage larvae
L2	Second stage larvae
L3	Third stage larvae
LSE	Low stock efficient
LURDF	Lincoln University Research Dairy Farm
LV	Levamisole
MJ ME	Mega joules metabolisable energy
mg	Milligrams
ml	Millilitre
ML	Macrocyclic lactones
mm	Millimetre
nm	Nanometre
NZ	New Zealand
O_2	Oxygen
OD	Optical density
ODR	Optical density ratio
PBST	Phosphate buffer solution with Tween-20
PSM	Plant secondary metabolite
rpm	Rotations per minute
SI	Small intestine
Tet	Tetraploid
Th 1	T helper 1
Th 2	T helper 2
TST	Targeted selective treatment
TT	Targeted treatment
VFI	Voluntary feed intake
w/v	Weight per volume

Chapter 1
Introduction

Gastrointestinal (GI) nematode infections are believed to be a major constraint to cattle health and production on pasture grazing systems although there is scanty data from New Zealand cattle, especially since the 1990s (Bisset, 1994). A considerable number of nematode parasites of ruminants have been recorded in New Zealand (Brunsdon, 1964) and of these, three major helminths, *Ostertagia ostertagi*, *Trichostrongylus axei* and *Cooperia oncophora* have deleterious effects on first-season grazing (FSG) calves and, to a lesser extent, older cows (Bisset & Marshall, 1987). There is compelling evidence from Europe and Canada that GI nematode infections have negative effects on milk production from dairy cows (Charlier et al., 2009; Sanchez et al., 2004a). Forbes et al. (2004) reported a drop in milk yield in untreated, naturally infected dairy cows when compared with treated controls. Compared with Europe where animals are housed, the climatic variability and different management practices exhibited in both the North and the South Islands of New Zealand are likely to alter the exposure levels to these nematode parasites (Bisset, 1994).

Efficient grazing management can influence the dynamics and survival of the free-living stages of nematode parasites. Conserving pastures for production of supplementary feed (hay, silage), maintaining a good grazing pattern, i.e. moving stock to paddocks before the appearance of infective third-stage larvae (L3) in significant numbers and avoiding winter grazing of the adult herd on pastures previously grazed by young stock may contribute to effective control of nematode infection in adult cattle (Charlier et al., 2011a). Alternatively, maintaining a pool of anthelmintic susceptible worms (free-living stages) on pasture i.e. a population in 'refugia', as an effective tool to delay the development of resistance and extend the efficacy of anthelmintics, is important in this regard (Pomroy, 2006) but may allow the build-up of infective L3 larvae to levels that are dangerous to certain classes of livestock. In addition, a wide range of anthelmintics have been used to treat GI nematode infection in cattle, including the benzimidazoles (BZ), levamisole (LV) and macrocyclic lactones (ML) and, recently, interest in deworming adult dairy cows has increased considerably. The macrocyclic lactones (avermectins/milbemycins) are not only very potent against all parasitic stages (including arrested larvae) but also have a long-lasting efficacy following administration.

Eprinomectin, a member of macrocyclic lactones has no withholding period for milk and is increasingly promoted for use in lactating dairy cows in New Zealand. However, blanket treatment of adult dairy herds with anthelmintics cannot be recommended because not only may this lead to anthelmintic resistance (AR), as a larger proportion of parasite population will be exposed to anthelmintics, but large variations in treatment response occur between different herds, as observed in previous studies (Barger, 1993; Charlier et al., 2007b; Kloosterman et al., 1996).

To optimise anthelmintic treatment, there is a need to identify only those animals with parasite burdens that are likely to benefit from receiving anthelmintic in order to maximise productivity and minimise the development of drug resistance that is a likely sequel to whole herd treatment. In adult cattle, diagnostic tools such as faecal egg counts (FEC) and blood pepsinogen levels appeared to be poor indicators of adult worm burdens particularly in older immunocompetent cattle (Vercruysse & Claerebout, 2001). However, in combination with anthelmintic history and grazing management information, these can be quite useful to monitor the level of exposure to GI nematode parasites (Charlier et al., 2011b). Recently, *Ostertagia ostertagi* antibody levels, measured by ELISA, in individual milk, bulk tank milk (BTM) or individual sera were found to be negatively correlated with milk yield (Charlier et al., 2005a; Forbes et al., 2008). Further studies indicated significant relationships between anti-*Ostertagia* BTM antibody levels and several management practices such as anthelmintic treatment and level of exposure to pasture associated with GI-nematodes (Charlier et al., 2007a; Nødtvedt et al., 2002). These findings have encouraged the measurement of bulk-tank milk (BTM) antibody levels in New Zealand dairy herds. However, no link has yet been shown between antibody levels and parasite burdens of adult cattle grazed on pasture throughout the year, such as in New Zealand. Therefore, to provide information on the immunity to nematode infection in adult dairy cows under New Zealand conditions, a trial was set up to identify antibody responses against GI nematodes with regard to exposure to infective third-stage larvae (L3) and parasite burdens.

In this research, we examine the hypothesis that worm burden or the level of challenge with infective third-stage larvae (L3) is associated with serum or milk antibody levels, faecal egg counts (FEC) and milk yield and attempt to identify the influence of high or low stocking density grazing on gastrointestinal worm infections in dairy cows.

The literature review begins by emphasising the epidemiology of gastrointestinal (GI) worm infection in New Zealand and some other temperate regions. It then summarises the pathophysiology of cattle following a GI nematode infection, the development of immunity and the scenario of anthelmintic resistance in New Zealand cattle. The body of this thesis illustrates how the study was conducted, reports the results from the collected data analysis and then discusses the results with reference to the findings of other related studies. A conclusion chapter highlights the findings of this study, discusses any weaknesses and suggests further scope for future research.

Chapter 2
Literature review

2.1 Epidemiology of gastrointestinal (GI) nematode parasites

A large number of nematode species which can infect pasture-based ruminants are prevalent in the relatively moist and mild climate of New Zealand. *Ostertagia ostertagi* has been regarded as the most important GI parasite of cattle both in New Zealand and throughout the world. However, under New Zealand climatic conditions mixed infections with GI nematodes commonly occur in cattle and these generally include *Ostertagia ostertagi*, *Cooperia oncophora* and *Trichostrongylus axei*. Sporadic infections with other GI nematodes occur but are of secondary importance. The gastrointestinal nematodes of minor importance include other species of *Ostertagia*, *Trichostrongylus*, *Cooperia*, *Oesophagostomum radiatum* and *Nematodirus helvetianus* (Bisset, 1994; Charleston, 1997).

Thorough investigations into the epidemiology of bovine GI nematode infections in temperate regions were initially conducted in Weybridge, England by Michel (1968); Michel and Ben (1969) and Glasgow, Scotland by Armour (1980). In Australia, Barger (1979) initiated a trial on the use of anthelmintic treatment for an improved milk yield from dairy cows. In the USA, Barger and Gibbs (1981) started investigating the effects of GI parasitism on dairy milk production. Dimander et al. (2003) has documented the impact of GI parasitism on the cattle industry under Swedish climatic conditions. The relative importance of parasite genera differs with host age due to acquired immunity. Generally, the infection levels of GI nematodes drop with ageing cattle but a number of studies in the USA, the UK, the Netherlands, Belgium and Germany have shown that the prevalence of GI nematode infection in pastured adult cattle can be 80−100% as cattle get continual exposure to L3 larvae on pasture during grazing (Charlier et al., 2011a).

In New Zealand, cattle production is based on year-round pasture grazing with some supplementary feeding during winter. Therefore, previous pasture larval contamination (overwintered L3) contributes to the occurrence of gastrointestinal parasitism in cattle. The majority of nematode parasites causing parasitic gastroenteritis fall within the superfamilies *Trichostrongyloidea* and *Strongyloidea* (Bisset, 1994; Pomroy, 1997) and are shown below in Table 2.1.

Table 2.1 Helminth parasites recorded from cattle in NZ (taken from Bisset, 1994)

Parasite	**Class**	**Predilection Site**	**Normal host species**
Moniezia expansa? /benedeni?	Cestode	small intestine	sheep/cattle
Bunostomum phlebotomum	Nematode	small intestine	cattle
Capillaria bovis	Nematode	small intestine	cattle
Chabertia ovina	Nematode	colon	sheep/cattle
Cooperia curticei	Nematode	small intestine	sheep
Cooperia oncophora	Nematode	small intestine	cattle
Cooperia surnabada	Nematode	small intestine	cattle
Cooperia punctata	Nematode	small intestine	cattle
Dictyocaulus viviparus	Nematode	lungs	cattle
Haemonchus contortus	Nematode	abomasum	sheep
Nematodirus filicollis	Nematode	small intestine	sheep
Nematodirus helvetianus	Nematode	small intestine	cattle
Nematodirus spathiger	Nematode	small intestine	sheep
Oesophagostomum radiatum	Nematode	colon	cattle
Oesophagostomum venulosum	Nematode	colon	sheep
Ostertagia leptospicularis	Nematode	abomasum	deer
Ostertagia kolchida	Nematode	abomasum	deer
Ostertagia ostertagi	Nematode	abomasum	cattle
Ostertagia lyrata	Nematode	abomasum	cattle
Strongyloides papillosus	Nematode	small intestine	sheep/cattle
Teladorsagia circumcincta	Nematode	abomasum	sheep
Teladorsagia trifurcata	Nematode	abomasum	sheep
Trichostrongylus axei	Nematode	abomasum	sheep/cattle
Trichostrongylus colubriformis	Nematode	small intestine	sheep
Trichostrongylus longispicularis	Nematode	small intestine	cattle
Trichostrongylus vitrinus	Nematode	small intestine	sheep
Trichuris ovis	Nematode	caecum/colon	sheep/cattle
Trichuris discolor	Nematode	caecum/colon	cattle
Fasciola hepatica	Trematode	liver	sheep/cattle
Calicophoron calicophorum	Trematode	rumen	sheep/cattle

2.1.1 Life Cycle

The nematode parasites prevalent in New Zealand cattle have a direct life cycle that does not require any intermediate host (Bisset, 1994). The direct life cycle has two distinct stages which include a parasitic phase within the host and a free-living phase outside the host. Under New Zealand climatic conditions, it has been documented that more than 90% of the total parasite population exists in the life cycle phase outside the host at any time (Familton & McAnulty, 1996). Adult worms dwell in the gastro-intestinal tract of ruminants and the mature females produce eggs which are excreted in faeces. The eggs hatch within the dung pat into first-stage larvae (L1), which develop to second-stage larvae (L2), and then into infective third-stage larvae (L3). However, there are some exceptions; for example, *Nematodirus* and *Trichuris spp.* contain infective L3 stage within the egg (Bisset, 1995). The first and second-stage larvae (L1 and L2) feed on bacteria, grow and develop. The outer cuticle (external sheath) of third-stage larvae (L3) prevents them from feeding but provides protection from adverse environmental conditions such as desiccation. The L3 have to rely on stored energy reserves for survival. Larval activity and, thus, consumption of stored energy, gets significantly reduced at low temperatures which prolong the survival of L3 larvae in the external environment (Familton & McAnulty, 1997).

Before resuming the next phase of their life cycle, L3 have to be transferred from the dung pat to herbage and so become accessible to the grazing ruminants. This process is known as 'translation' (Rose, 1961).The double-membranous infective L3 are ingested by cattle during grazing. In the GI tract, L3 shed the extra sheath (exsheathment) and invade the GI mucosa (e.g. *Haemonchus* and *Trichostrongylus*) or penetrate the gastric glands (e.g. *Ostertagia*). Within the host in a few days, the L3 larvae transform into L4 and in about two weeks they develop into either a male or female adult nematode. The period between ingestion of infective L3 larvae by the susceptible host and first egg production by adult parasites is known as the 'prepatent period' (Hansen & Perry, 1994). Generally, the Trichostrongyles start producing eggs after 3-4 weeks of infection but under certain conditions, the development of *Ostertagia ostertagi* and *Cooperia oncophora* may be inhibited at the early fourth-stage (EL4), up to six months. This phenomenon of hypobiosis has been thoroughly described by Armour, et al., (1973). Under New Zealand climatic conditions, *Cooperia oncophora* tends to have a much higher propensity for hypobiosis than *Ostertagia ostertagi* (Bisset & Marshall, 1987). The life cycle of GI nematodes of ruminants is shown in Figure 2.1.

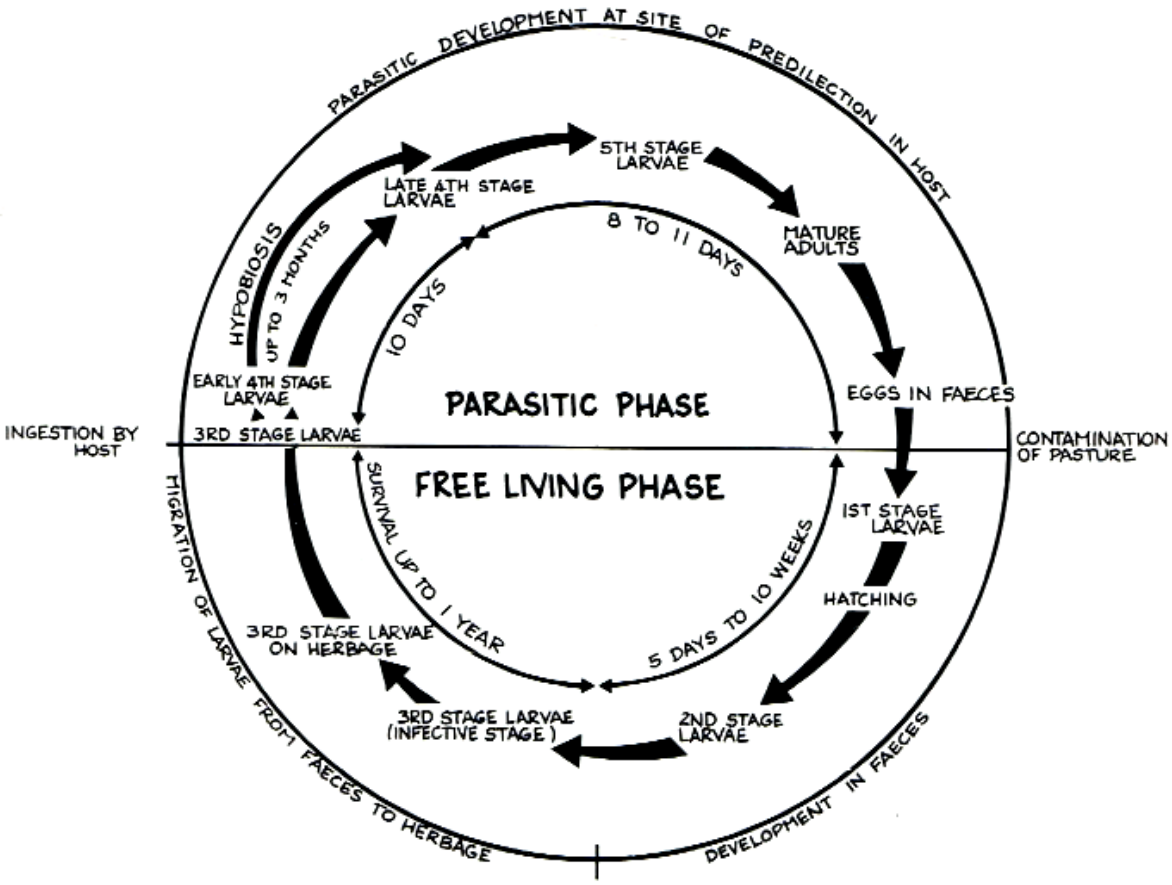

Figure 2.1 Direct life cycle of Trichostrongyle nematodes of ruminants (adapted from Brunsdon, 1982).

2.1.2 Factors contributing to bovine nematodiasis in New Zealand

Major factors contributing to the outcome of bovine nematodiasis are the seasonal dynamics of the free-living stages of GI nematodes on pasture, persistent contamination rates and the development of acquired immunity by the host. Seasonal dynamics refers to the time-dependent development and loss rates in the free-living stages of nematode larvae. In New Zealand, spring-born calves typically pick up infection from pasture where infective third-stage larvae (L3) have overwintered. Larval load on pasture over summer and autumn depends on climatic variations and pasture management. In the North Island of New Zealand, infective L3 populations on pasture grazed by young calves (<1 year) mainly consist of *Cooperia oncophora, Ostertagia ostertagi* and *Trichostrongylus axei*, of which *C. oncophora* are numerically dominant, followed by *Ostertagia spp.* and *T. axei* (Bisset & Marshall, 1987, Fig 2.2). However, in yearling (or older cattle) *Trichostrongylus axei* produces a higher proportion of nematode eggs being shed compared to the others and although faecal egg counts (FEC) in this age group are generally low (i.e. <100 eggs per gram), *T. axei* larvae on pasture sometimes may increase substantially after grazing by yearling cattle (Bisset, 1995).

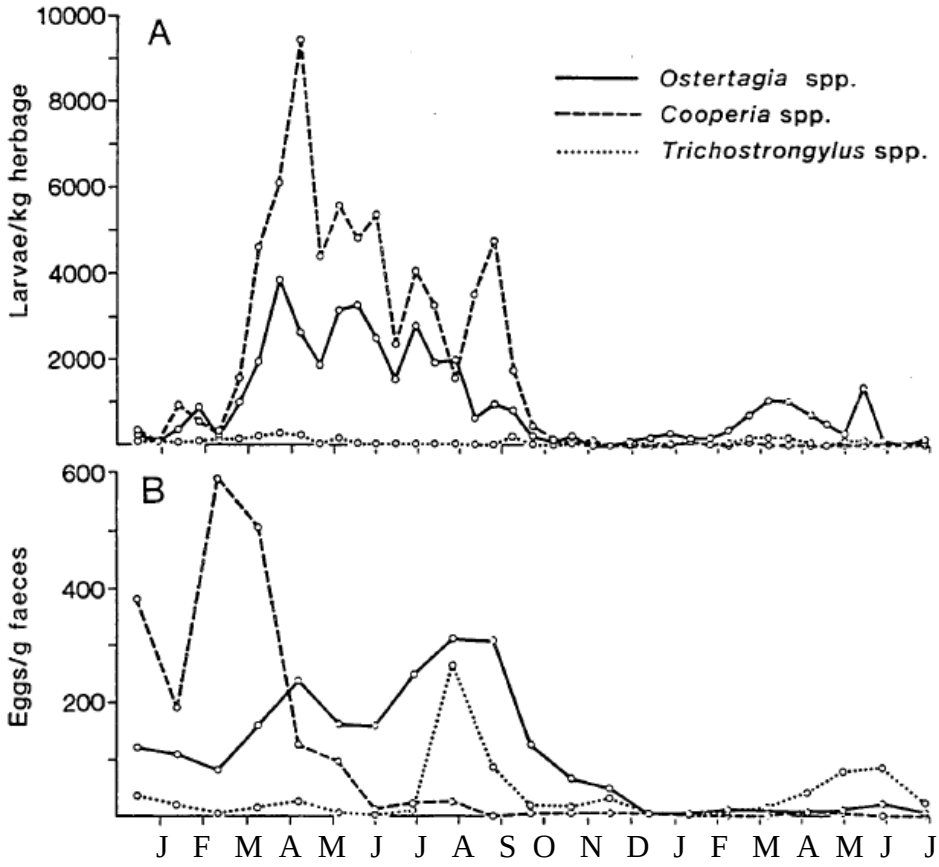

Figure 2.2 Typical species composition of infective third-stage larvae (L3) on pasture and mean worm egg output in faeces of young calves maintained in the Animal Research Centre's Kaitoke farm, Wallaceville, New Zealand (adapted from Bisset & Marshall, 1987).

Unlike sheep, there is no well-defined periparturient rise of worm egg output in cattle faeces that could amplify pasture larval population in late spring or early summer. In New Zealand pastures grazed by young calves, two major seasonal peaks in numbers of infective L3 larvae present on pasture have been observed (Brunsdon & Adam, 1975). The first peak occurs in early summer contributed by the calves initially infected with over-wintered L3 larvae. In early summer, these infective L3 larvae re-infect the calves and give rise to second generation worms in larger numbers that produce more eggs leading to second major peak of infective L3 larvae present on pasture in April/June (autumn/winter peak). At this age, the calves may show some resistance resulting in low worm egg output in faeces even though adult worm burdens may be high. The developing host resistance may cause reduced worm fecundity which leads to low faecal egg output. The low faecal egg output in association with winter weather reduces larval development and pasture larval counts drop to remarkably low levels in late winter which are further diluted by the spring growth of grass (Charleston, 1997). In another study, the observed summer peak of nematode L3 larvae on pasture grazed by young

calves was between 300−4500 L3/kg fresh herbage and the autumn/winter peak mounted as high as 12000 L3/kg fresh herbage (Vlassoff, 1975).

The larval availability on pasture grazed only by adult cows is generally low but adult cows may encounter a relatively high degree of larval challenge under certain circumstances where farmers winter-graze dairy herds in the 'run-off' previously grazed by replacement stock or calves during the lactating season (Bisset, 1994). Although such events rarely cause any clinical parasitic gastroenteritis in adult cattle, administration of an anthelmintic during winter may be worthwhile in intensive dairy production (Bisset, 1995).

2.1.3 Influence of environmental factors on the development of the free-living stages of nematode parasites

The preferred favourable conditions for the development and survival of nematode parasites differ from species to species and from season to season. The three major factors influencing egg hatch, development and the survival of free-living stages of nematode larvae are oxygen (O_2), moisture and temperature (Familton & McAnulty, 1997). A thin water film provides adequate levels of oxygen and moisture to favour the development of larvae. The faecal mass is an ideal environment for egg hatching and larval development and, the presence of bacteria provides food for the microbivorous L1 and L2 larvae. Ambient temperature plays a vital role in egg hatching and survival of pre-parasitic larval stages of nematodes. Generally, optimal development of pre-parasitic larvae occurs between 15^{o} C to 30^{o} C; however development occurs at variable rates between 4^{o} C to 35^{o} C. In the case of *Haemonchus, Trichostrongylus*, *Ostertagia* and *Chabertia* the eggs hatch and develop into infective L3 larvae very rapidly at mean monthly ambient temperatures between 15^{o} C to 24^{o} C. For some nematode parasites such as *Ostertagia* and *Trichostrongylus*, egg hatching and larval development occurs even in winter conditions (5.8^{o} C–7.6^{o} C) as has been observed in Canterbury, New Zealand (Familton & McAnulty, 1997). At temperatures ranging from 5^{o} C to 10^{o} C, some nematode larvae become inactive and can survive several months (Pomroy, 1997).

Only a small proportion of the eggs (1-17%) passed in faeces attain the infective L3 stage, depending on existing environmental conditions (Vlassoff, 1982). However, a higher proportion of cattle nematode larvae (L3) may develop compared to sheep nematode larvae (L3) because of the larger and soft protective faecal mass in cattle (Familton & McAnulty, 1997). The distribution of pasture L3 larvae tends to be highly clumped around faeces,

declining from the centre outwards, horizontally, and from the roots to top of the sward, vertically (Vlassoff, 1982). Larval migration from faeces onto herbage can either be active or passive. An available water film that is dependent on dew, rain or irrigation aids in active larval migration. The migration of L3 from faeces has been found to occur in waves, coinciding with the presence of water. Larvae are usually not found any further than 90 cm away, horizontally, from the faecal mass (Grønvold & Høgh-Schmidt, 1989). Vertical movement of infective L3 occurs up the plant material but the majority of larvae are found in the lower 2 cm of the plant. The presence of L3 larvae in the upper sward component may increase with high humidity, frequent rainfall and mild temperatures (Vlassoff, 1982). Passive migration of L3 away from faeces is caused by the splash effect of rain drops and this may be a vital factor in the transport of *Cooperia* and *Ostertagia* larvae. Some relocation may be performed by insects, earthworms, birds or fungi although earthworms may play an important role in the destruction of L3 larvae (Stromberg, 1997). Larvae die in adverse climatic conditions such as desiccation and they can be removed from the pasture by grazing. If larvae are not specific to the animal species or if the animal has a high degree of natural immunity they may not establish within the host; such animals may be used as 'vacuum cleaners' for the removal of pasture larval contamination (Waller, 2006).

2.1.4 Influence of pasture types on gastrointestinal (GI) parasitism in ruminants

There have been a number of studies in New Zealand that showed the effects of pasture species on GI parasitism in sheep (Knight et al., 1996; Niezen et al., 2002). Pastures modify the microclimate, which may directly affect the survival and development of nematode larvae. Larval migration may also be impacted by different forage morphology and composition and, thereby, reduce parasite infections in grazing ruminants (Niezen et al., 1998). Chicory (*Cichorium intybus*) retards the vertical migration of infective larvae compared to grass and therefore, lambs grazing on chicory have been shown to have a much lower GI parasite burden (Marley et al., 2006). Larval survival on chicory has been found to be lower than rye grass. Larval survival is also lower in legumes such as lucerne (*Medicago sativa*), but lucerne favours the upward movement of existing infective L3 larvae, which can bunch up in the higher components of the sward and may, subsequently, boost GI parasitism in livestock (Niezen, 1995).

Some plants contain certain compounds that have anthelmintic properties. Plants containing condensed tannins (CTs) have beneficial effects on the resilience and resistance of parasitised

ruminants (Niezen et al., 1994). Condensed tannin-containing plants such as sulla (*Hedysarium coronarium*), Maku Lotus (*Lotus pedunculatus*) and Goldie Lotus (*Lotus corniculatus)* have been shown to reduce faecal egg counts (FEC) and/or worm burdens and thereby significantly enhance lamb performance while grazing on pastures mixed with these forages (Niezen et al., 1998). This may be due to direct anthelmintic activity, as shown by Molan et al.(2000a; 2000b), where tannins extracted from plants such as sulla (*Hedysarum coronarium*) and large birdsfoot trefoil (*Lotus pedunculatus*) inhibited the development of *Trichostrongylus colubriformis* eggs to the larvae stage and, thereby, reduced pasture contamination with infective L3 larvae or an indirect effect from an enhanced immune response due to the presence of tannins in the forages, which favoured an increased flow of by-passed proteins towards the abomasum (Coop & Kyriazakis, 1999).

2.2 Pathophysiology of cattle following a GI nematode infection

After ingestion by a susceptible host, the L3 larvae migrate to their predilected site (abomasum for *Ostertagia ostertagi* and *Trichostrongylus axei*, and small intestine for *Cooperia oncophora*). The L3 larvae invade the GI mucosa or the glands in the abomasal wall within a few hours of ingestion by the host and tend to stay there for 2-3 weeks (Hansen & Perry, 1994). The infective third-stage larvae (L3) of *Ostertagia* invade the gastric glands of the abomasum where the morphology of parietal cells is altered in heavy infections. As L3 larvae invade and young adults emerge from the gastric glands, the secreting cells lining the gland (parietal and zymogen cells) are damaged and replaced by immature cells with weak secretory activity and lacking in intact junctions (McKellar, 1993). Elevated abomasal pH is a characteristic feature of *Ostertagia* infection in both cattle and sheep (Anderson et al., 1965). Abomasal pH elevates from about pH 2 to as high as pH 7 due to the interference of HCl secretion by parietal cells in the gastric mucosa that subsequently prevents the conversion of pepsinogen to pepsin, denaturation of proteins and this favours bacterial proliferation. There is an increased permeability of the abomasal mucosa as a result of the proliferation of undifferentiated cells and disintegration of cell junctions, which eventually leads to an elevation of blood pepsinogen levels (hyperpepsinogenaemia) and loss of plasma proteins into the lumen of the abomasum (Armour, 1970). An altered abomasal pH seems to stimulate gastrin secretions in sheep and cattle infected with *Ostertagia spp.* (Fox et al., 2006) and that stimulates the abomasal secretion of HCl and pepsinogen and inhibits gastrointestinal motility (Hoste, 2001). In contrast, McKellar et al. (1987) indicated that hypergastrinaemia in the absence of an altered abomasal pH in cattle was due to physical or chemical stimuli from *Ostertagia ostertagi*. Clinical symptoms and abomasal pathology from infections with *T. axei*

are quite similar to that of *O. ostertagi* while pure infections with *C. oncophora* exhibit milder pathogenicity with only minor intestinal damage. Clinically, the symptoms of GI nematode infections are loss of appetite and diarrhoea resulting in weight loss and dehydration (Bisset, 1994). The following figure (Figure 2.3) outlines the pathogenesis of *Ostertagia ostertagi* infection in cattle.

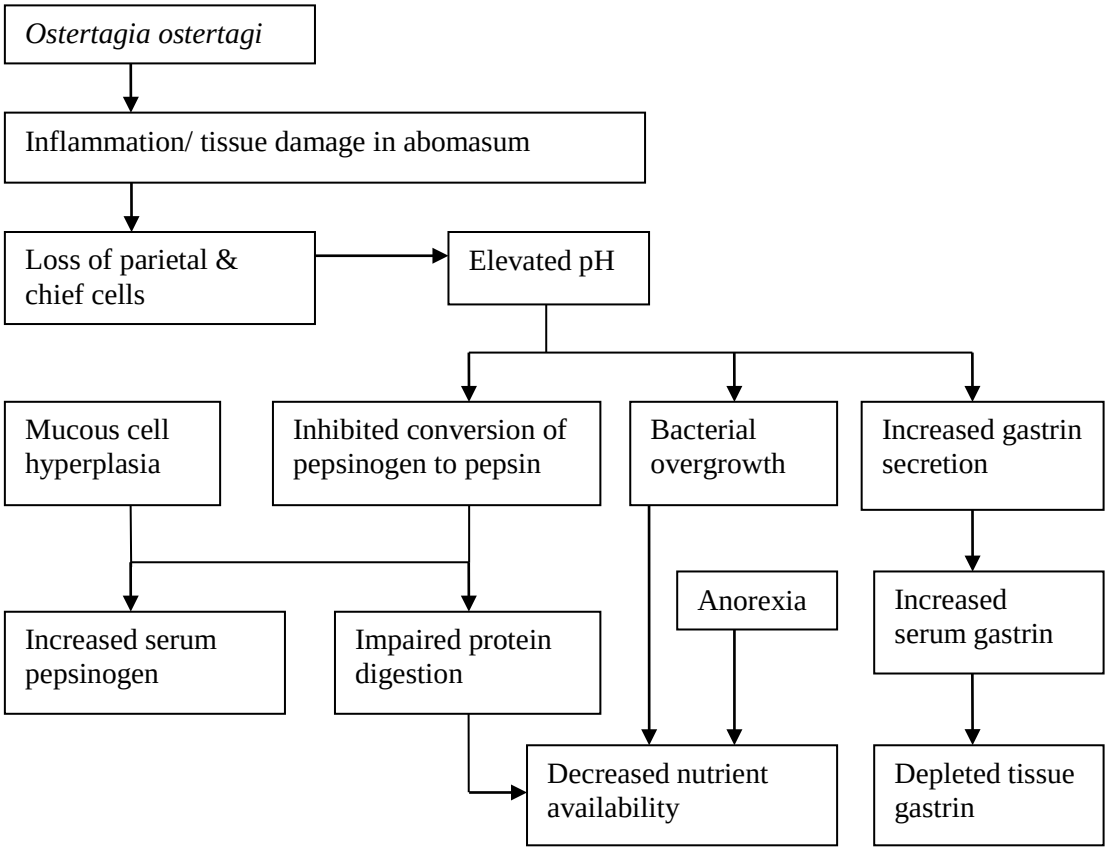

Figure 2.3 *Ostertagia ostertagi* causes abomasal inflammation and tissue damage (loss of parietal and chief cells) resulting in a series of morphological, physiological and biochemical changes (adapted from Saverwyns, 2008).

Type I ostertagiasis is predominant in weaned calves in New Zealand and in first-season grazing (FSG) calves (in autumn/early winter) that display the typical symptoms of parasitic gastritis, such as marked weight loss and diarrhoea. Type II ostertagiasis mainly affects yearlings with rare involvement of adult cattle (Bisset, 1994). Under New Zealand climatic conditions, cattle generally accumulate arrested *Ostertagia* larvae through late autumn and winter. Type II ostertagiasis is subdivided into ‘acute’ and ‘chronic’ forms. In ‘acute’ cases, there is a rapid development of the inhibited larvae over a very short time resulting in significant weight loss and profuse diarrhoea (Brunsdon & Adam, 1980). Usually these inhibited larvae degenerate over spring and summer without the manifestation of any clinical signs (Charleston, 1997) but less commonly they may develop, emerge from the abomasal

mucosa and cause ‘chronic’ Type II ostertagiasis; this is sporadic or rare in New Zealand (Gill & Mason, 1989; Wedderburn, 1970).

A common feature of a parasitised animal with GI nematodes is the marked reduction in voluntary feed intake (VFI) and this is regarded as a major factor in the pathogenesis of such infections. The degree of reduction in VFI depends on the infection load and nematode species (Holmes, 1986). Reductions of about 20% in VFI have been reported in cattle infected with *T. axei* (Ross et al., 1969) and *O. ostertagi* (Entrocasso et al., 1986). The causes of VFI reduction are unclear but several studies suggest that changes in abomasal pH, GI motility and digesta flow, altered bacterial population, altered plasma concentration of various hormones or immune response may be linked with changes in VFI of parasitised ruminants (Coop & Holmes, 1996; Holmes, 1986). Another distinct feature of GI nematodiasis is the substantial loss of endogenous protein into the alimentary tract. This protein loss is represented by plasma and red cells, sloughed epithelial cells and mucus (Holmes, 1986; Holmes, 1993). Some of the leaked protein into the lumen of the alimentary tract gets reabsorbed depending on the lesions present on the proximal or distal part of the tract (Coop & Holmes, 1996). Despite this re-absorption, protein losses are high. In sheep, as a result, there is a partitioning in protein synthesis away from productive channels such as wool, bone, meat and milk yield towards the liver and repair of the alimentary tract (Poppi et al., 1990).

2.3 Immunity to GI nematodes in cattle

The mammalian immune system can be divided into two functional divisions known as innate and adaptive immunity. Innate immunity is the first line of defence against pathogens and includes physical barriers such as the skin and biochemical components such as lysozyme that can prevent the establishment of infections and destroy infectious agents. Innate immunity is non-specific in nature and immunity does not improve by repeated infection. In contrast, adaptive immunity is specific in nature and provides prolonged protection against the infective agent after repeated exposure (Tizard, 2004). Adaptive immunity is divided into humoral (antibody-mediated) and cellular (cell-mediated) immunity. Both mechanisms are closely interrelated in eliciting immune response to worm infections (Gasbarre et al., 2001).

Gastrointestinal nematodes are, in general, highly host specific and responsible for chronic subclinical infections with a high morbidity. The complexity of the life cycle and the presence of a wide range of antigens in different developmental stages mean that a very complicated immune response is elicited from the host (McFarlane, 1997). Host immunity has a crucial

influence on the establishment of ingested L3 larvae, size of worm burden, egg production by adult worms and worm-induced production losses (Bisset, 1994; Greer, 2008). The bovine immune response to GI nematodes varies and greatly depends on a number of factors such as exposure levels (infective L3 larvae on pasture), worm species, climatic conditions, management practices, control measures (anthelmintics) as well as nutrition, genetic make-up, age, gender and hormonal status (Vercruysse & Claerebout, 1997) but data are scarce on these factors that may influence GI parasites in cattle (Gasbarre et al., 1993). In sheep, genetic components play a vital role in the difference in resistance to GI nematode infections between sheep breeds or even within a breed (Windon, 1996). Factors such as age (Gibson & Parfitt, 1972; Kambara et al., 1993), gender (Barger, 1993) and, nutritional (van Houtert & Sykes, 1996) and hormonal (Donaldson et al., 1997; McKellar, 1993) status can also influence the development of immunity against GI nematodes. Schmidt et al. (1998) did not identify differences in the diversity of nematode genera in calves from different Aberdeen Angus sires and stated that resistant cattle can be resistant across a wide array of nematode species. In contrast, Gasbarre et al. (2001) found that immunity to two parasites (*Ostertagia* and *Cooperia*) was not the same in individuals within cattle herds. This implies that different mechanisms operate in developing resistance to different parasite species.

There is a characteristic sequence of events in the development of bovine immunity to GI nematodes such as *Ostertagia ostertagi* that leads to a decline in the fecundity of worms, a stunting of worm growth, followed by arrested development, expulsion of adult worms and, finally, the built up of resistance to re-infection (Claerebout & Vercruysse, 2000; Vercruysse & Claerebout, 1997). In cattle, three to four weeks following an experimental infection with *Ostertagia ostertagi* (Canals & Gasbarre, 1990) and about eight weeks after exposure to L3 contaminated pastures (Gasbarre et al., 1993) parasite-naive calves exhibited a significant elevation of anti-*Ostertagia* antibodies in the peripheral circulation. These antibodies were detectable with the use of a wide range of nematode antigens and included all major immunoglobulin isotypes (IgG, IgM, IgA, and IgE). The *Ostertagia ostertagi* parasite also induces an immediate hypersensitivity (Type I hypersensitivity) response in the abomasal mucosa with a sharp rise of IgE, leukotrienes and prostaglandins in abomasal tissue or lymph, particularly in Type I ostertagiasis (Baker & Gershwin, 1993). Canals et al. (1997) found a massive enlargement in regional lymph nodes draining the abomasum and in a period of about five weeks following infection the weight of these lymph nodes may attain 20-30 times that of normal. In gastrointestinal nematode infections, dramatic alterations occur in tissues surrounding the parasite including mucosal mast cell hyperplasia, globular leucocytosis,

marked eosinophilia and extensive mucus secretion (Balic et al., 2000). *Ostertagia* infections in ruminants seem to be efficient stimulators of lymphocyte subpopulations but poor inducers of effector cell populations which indicate that *Ostertagia* species have evolved measures to suppress the protective immune mechanisms of the host (De Marez et al., 1997).

Pathogens tend to preferentially stimulate one of two types of cells [T helper 1 (Th1) or T helper 2 (Th2)] with mutually antagonistic immune responses due to the stimulation of different subsets of T-helper lymphocytes (Mosmann & Coffman, 1989). This leads to the secretion of a wide range of cytokines and each cytokine has highly specific effects (stimulation or inhibition) on different types of cells. Typically GI nematode infections in some mammals elicit a very strong Th2-like response which is characterised by increased levels of the cytokine Interleukin 4 (IL4), increased levels of IgG1 and IgE antibodies, and the presence of numerous mast cells (Svetic et al., 1993). In contrast to the murine immune response which is regulated by T helper 2 (Th2) lymphocytes and leads to mast cell-mediated or goblet cell-mediated expulsion of adult worms, immune responses in cattle against *O. ostertagi* neither exhibit a typical Th2 cytokine profile nor result in the rapid expulsion of adult worms (Gasbarre et al., 2001). As there is not a clear explanation for protective immunity against GI nematodes, measuring acquired immunity in cattle to nematode parasites by immunological assays may need additional parasitological parameters such as faecal egg counts (FEC), faecal larval cultures and pasture larval counts to give meaningful epidemiological data, as has been proposed by Claerebout and Vercruysse (2000).

An enzyme-linked immunosorbent assay (ELISA) has been used in numerous studies to measure anti- *Ostertagia ostertagi* antibody levels in serum or milk since Keus et al. (1981) conducted an experiment in calves that quantified the levels of serum immunoglobulin G (IgG). The presence of IgG antibodies, indicating exposure to GI nematodes and antibody levels in dairy heifers at the end of the first grazing season, was positively correlated with different levels of exposure to infective L3 larvae (Ploeger et al., 1990). The predominant immunoglobulin involved in the humoral immune response to GI nematodes is IgG. The increased optical density ratio (ODR) in adult cattle is thought to reflect acquired immunity due to repeated exposure to pasture larval challenges (Sanchez et al., 2004b). *Ostertagia ostertagi* antibodies may cross-react with other GI nematodes such as *Cooperia spp*, but this does not hinder the assay's usefulness if an experiment is targeted to evaluate the overall GI nematode infection (Keus et al., 1981).

In a number of studies related to dairy cows, anti-*Ostertagia* antibodies have been detected in individual and bulk tank milk (BTM) samples (Charlier et al., 2005a; Sanchez et al., 2002). Sampling milk is relatively an inexpensive method of monitoring. In most studies, an indirect ELISA is employed for measuring milk antibodies against *Ostertagia ostertagi* and the results are expressed as optical density ratios (ODR) which can provide the most repeatable results (Sanchez et al., 2002). The test is considered robust in terms of being able to measure antibodies in different sample preparations such as milk stored up to three days at 4^{o} C, or subjected to repeated freezing-thawing cycles, or milk with or without a cream fraction and with the addition of preservatives (Charlier et al., 2005b). Several factors, such as lactation stage, age, the presence of mastitis and serum antibody levels may play crucial roles in determining the milk antibody titre of which serum antibody titre is the most influential factor (Charlier et al., 2009). The correlation between antibodies measured in serum and milk of individual housed cows was around 0.5 and, hence, correction factors should be taken into account when comparing them (Sanchez et al., 2004b). With respect to productivity, Charlier et al. (2007b) found that milk ODR values were lower in an eprinomectin (Eprinex Pour-On, Merial) treated group when compared with a control group that received the vehicle liquid without the active compound (placebo). The overall effect of this treatment on milk yield over the four months following treatment was estimated at 1.2 kg/cow/day with no effect on milk-protein % and milk-fat %. This study showed that during autumn an anthelmintic treatment (with eprinomectin) on pasture-based dairy cows could lower the anti-*Ostertagia* bulk- tank milk antibody levels, and a higher milk yield. A high ODR is associated with animals that have been exposed to a high larval challenge on pasture and that production losses have likely occurred (Charlier et al., 2011a). However, data are scarce on the link between ODR and milk production from entirely pasture-based dairy cows. Mejia et al. (2011), working with grazing animals in Argentina did not find any significant difference in milk yield during the first five months of lactation between two groups of cows separated by anti- *Ostertagia* ODR, i.e. ODR <0.5 vs. ODR >0.5, when sampled during early lactation.

2.4 Worm control measures for grazing ruminants

Conventional methods of controlling nematode parasites in grazing livestock have included the use of anthelmintics (chemotherapeutic agents), largely due to their efficacy, safety and spectrum of activity, affordability and ease of use. However, intensive or inappropriate use of anthelmintics, particularly, in small ruminants worldwide has led to anthelmintic resistance (AR) to three (benzimidazoles, imidazothiazoles and macrocyclic lactones) major classes of

broad-spectrum anthelmintic drugs (Waller, 2006). Therefore, integrated worm control programmes which combine different control methods (chemical and non-chemical) should be used for a long-term sustainability instead of relying solely on anthelmintics.

2.4.1 Worm control by anthelmintics

Worm control measures by anthelmintics in cattle have been generally targeted at first season grazing (FSG) calves due to their susceptibility and their ability to contaminate the environment, although increasingly anthelmintics are applied to both second-year grazing cattle and adult cattle (Taylor, 2010). Protecting the efficacy of existing anthelmintics is clearly important and the most important determinant of resistance development is frequency of administration (Leathwick, 2004). This can be achieved by using combinations of anthelmintics, alternate drench families and sound drench practices (Scott et al., 2008). Combining two or more anthelmintic classes such as benzimidazoles (BZ), imidazothiazoles (IZ) and macrocyclic lactones (ML) into one product can broaden the spectrum of activity; field data generated in New Zealand indicates that dual combination products such as benzimidazoles (BZ) + levamisole (LV) are still effective on the majority of cattle farms (Scott et al., 2008). However, there is a contradictory argument internationally that combination use will generate multiple resistance in worms more quickly than using each class of anthelmintic sequentially, as one active class may select resistance to another, but there is a lack of evidence for such occurrence. Also, sound drench practices such as avoiding under-dosing and unnecessary treatment of adult animals will prolong the efficacy of the available anthelmintics (Scott et al., 2008). Alternating the anthelmintic class (drench rotation) commonly used in an annual rotation may also minimise the chances of resistance development to any one class of anthelmintics (Sutherland & Scott, 2010) but this may be fallacious, as shown in sheep by Barnes et al., (1995).

Anthelmintic resistance (AR) in populations of nematode parasites of grazing ruminants is a global issue (Besier, 2007). It is notable that anthelmintic resistance (AR) in nematodes, regardless of anthelmintic class has a tendency to develop and scatter rapidly in the countries of the southern hemisphere (Sutherland & Scott, 2010). Recent reports indicated that AR was particularly severe in South America (Mejia et al., 2003). Up until 1991, only six confirmed cases of anthelmintic resistance in cattle nematodes in New Zealand had been reported. These cases were related to developing resistance to benzimidazole anthelmintics, occurring mostly in *Cooperia* and to a lesser extent in *Ostertagia* and *Trichostrongylus.* However, from 1992 to

1995 a further 13 cases of resistance to benzimidazole in cattle had been detected by faecal egg count reduction tests (FECRT) conducted on samples submitted to the Batchelar and Ruakura Animal Health Laboratories including two cases of multiple resistance to ivermectin and benzimidazole in infections with *Cooperia spp.* (McKenna, 1996). Over recent years, anthelmintic resistance in cattle and sheep in New Zealand has dramatically increased from remarkably low to disturbingly high levels; a recent study documented that over 90% of farms surveyed in the North Island had detectable resistance to ivermectin in *Cooperia spp.* present in cattle (Waghorn et al., 2006). Resistance to a triple combination of ivermectin, albendazole and levamisole has already been reported in sheep (Pomroy, 2006). In beef cattle in New Zealand, a major problem is resistance in *Cooperia spp*. where the prevalence of farms containing animals with resistant worms was found in a national survey to be 92% for ivermectin and 76% for albendazole (Pomroy, 2006). Anthelmintic resistance in bovine nematode parasites recently has also become increasingly prevalent in the regions of the northern hemisphere, such as North America (Gasbarre et al., 2009) and Europe (Demeler et al., 2009). Despite the global problem of anthelmintic resistance, the use of anthelmintics will remain a vital element in control of nematode infections in ruminants for the foreseeable future.

2.4.2 Worm control by grazing management

The extent of nematode parasitism acquired by grazing animals is determined by a number of factors such as physiological state of the animals, nutrition and seasonal conditions. Pasture-borne parasite infection in grazing livestock is, to a great extent, influenced by grazing management (Waller, 2006). Grazing management strategies as a control measure were classified by Michel (1985) as: i) preventive- suppressing worm egg output by anthelmintic treatment in the early part of the grazing season, ii) evasive- moving livestock to another pasture before the appearance of the infective L3 larvae in significant numbers resulting from the previous contamination; iii) dilutive- putting older immunocompetent animals with susceptible young stock or different livestock species to dilute pasture larval infestation resulting from their combined faecal output of parasite eggs. Methods of reducing the intake of infective larvae (L3) also include the provision of so called ‘clean’ pasture, for example newly sown or rested for a long period of time (Stromberg & Averbeck, 1999; Sutherland & Scott, 2010). Alternatively, the grazing of pasture with different species of livestock result in removal of the burden of certain parasites that do not parasitise across all grazing species (Sutherland & Scott, 2010). However, this historical approach to minimise pasture L3

ingestion in parallel with high frequency use of anthelmintics has accelerated anthelmintic resistance.

The pasture type may influence the free-living stages of parasite populations. Beneficial effects on host health and productivity under parasite challenge have been observed with the consumption of plant secondary metabolite (PSM)-containing plants when compared with regular forages lacking PSM, such as ryegrass, white clover and lucerne (Hoste et al., 2006; Niezen et al., 1994). Direct anti-parasitic effects of PSM recorded *in vitro* include decreased egg hatching, larval development, migration and viability. Anti-parasitic effects recorded *in vivo* include reduction in fecundity, faecal output of worm eggs and the establishment of ingested infective L3 larvae (Barrau et al., 2005; Hoste et al., 2006; Paolini et al., 2003). However, some studies have not reported beneficial effects from feeding PSM-containing plants to grazing livestock (Paolini et al., 2005; Pomroy & Adlington, 2006) and the most commonly reported effect in feeding trials has been a lower faecal output of worm eggs which indicates a reduction in worm fecundity not worm burden. No evidence available to date suggests that anthelmintics can be replaced by bioactive forages but they have the potential to substantially reduce anthelmintic use (Hoskin, 2006).

2.4.3 Worm control by biological means

Most nematode control measures are targeted at the parasitic stages within the host whereas biological control is directed at the free-living stages of nematode parasites on pasture. Faecal pats have been shown to provide a protective environment with a buffering capacity against extremes of temperature and moisture, which enhances the development and survival of the free-living stages of nematode parasites (Barger et al., 1984). Dung beetles and earthworms are capable of rapid dung removal and, thus, indirectly reduce significant numbers of the free-living stages of nematode larvae from pasture (Waller & Faedo, 1996). Also a number of micro-organisms use the free-living stages of nematode parasites as a food source and these include viruses, bacteria, fungi, protozoa and also predacious mites (Waller & Faedo, 1996). Several studies on the biological control of nematode parasites are associated with the nematode-destroying microfungus, *Duddingtonia flagrans* because of its important attributes such as, the ability to survive gut passage, the ability to grow rapidly in freshly voided faeces and a voracious nematophagous capacity (Larsen, 1999). The commonly used method of deployment of *Duddingtonia flagrans* spore material is by a feed additive and to be successful in achieving the optimal results, the fungal spores need to continuously shed in animal faeces

simultaneously with parasite eggs (Waller, 2003). Biological control has largely remained experimental.

2.4.4 Integrated worm management

The' refugia' concept has been recently identified as a key element in managing anthelmintic resistance in nematode parasites. A population of infective larvae on pasture from a pool of susceptible nematode worms ('refugia') is valuable to dilute resistant nematodes surviving anthelmintic treatment and, hence, reducing selection pressure for the development of anthelmintic resistance (Kenyon et al., 2009; Leathwick et al., 2006). However, in practice adopting refugia strategies may pose the threat of increased risk of parasitism and production losses. Refugia-based worm control strategies will be most effective when integrated with appropriate anthelmintic regimes and other non-chemical management tools (Jackson et al., 2009). Refugia-based approaches include either changes to the timing and frequency of regimens by which all animals in a flock/herd are treated with anthelmintics or the addition of selective treatment strategies by which some animals within the flock/herd are deliberately left untreated when drenching (Besier, 2012). The basis for drenching with a targeted treatment (TT) strategy involves collecting faecal samples for worm egg counts and treating when counts exceed a 'trigger level' associated with gastrointestinal nematode infection (Besier, 2012). Alternatively, targeted selective treatment (TST) is a part flock/herd treatment where the anthelmintic is administered to those individuals identified as likely to benefit (Kenyon et al., 2009; van Wyk, 2001), based on various parameters such as evidence of parasitism and animal production (Kenyon et al., 2009; van Wyk et al., 2006). In most farm situations, TST indicators such as body-condition scoring (BCS) and short-term variation in live weight are the simplest production parameters for selecting individual animals to drench (van Wyk et al., 2006). A refugia strategy is considered successful if there is a substantial reduction in the number of anthelmintic treatments normally given to livestock, with no significant increase in parasitism or parasite-induced production losses, over a protracted period.

In cattle, such measures of parasite infections (e.g. FEC, BCS) are not clearly associated and therefore, alternative diagnostic measures such as immune response (antibody titre) to parasite antigens have recently been studied.

Chapter 3
Materials and Methods

Experimental design overview

The study was conducted over a whole milking season on the Lincoln University Research Dairy Farm (LURDF) located in Lincoln, Canterbury, New Zealand (43° 39' 0" South, 172° 29' 0" East). In Experiment A, 61 lactating cows were randomly allocated to a high stock efficient (HSE) or a low stock efficient (LSE) farmlet and grazed from October, 2011 through to May 2012, during which time data were collected on faecal egg counts (FEC), pasture L3 levels and serum and milk antibody levels against gastrointestinal (GI) nematodes followed by slaughter of cows for worm burden assessment. In Experiment B, 60 lactating cows were artificially challenged later in lactation with infective third-stage nematode larvae (L3) and, FEC and serum antibody levels against GI nematodes measured followed by slaughter of cows for worm burden assessment. Associations were sought between the worm burdens, FEC and serum/milk antibody levels generated against GI nematode antigen.

All experimental procedures, including animal handling, were approved by the Lincoln University Animal Ethics Committee (Project numbers 435 and 463).

Experiment A

3.1 Animals

The experimental cows were sixty-one (N=61) dairy cows of mixed age (2-10 years) and breed (Holstein, Jersey, and Holstein × Jersey crossbred cows), being part of a parallel stock-density study, grazed on the Lincoln University Research Dairy Farm (LURDF). The cows calved between early August and mid-September, 2012. The experimental animals were randomly allocated into two groups that had been corrected for difference in age, breed and calving date. One group (n=31) was grazed on a high stock efficient (HSE) farmlet (5 cows/ha with supplement 1.5 kg crushed barley/cow/day containing 13.85 MJME/Kg DM & 7.22% crude protein) and the other group (n=30) was grazed on a low stock efficient (LSE) farmlet (3.5 cows/ha with no supplement). The average grazing residuals in the HSE and LSE farmlets were 1450 kg DM/ha and 1600 kg DM/ha, respectively. The following table (Table 3.1) shows pasture allocation of the HSE and LSE herds for the 2011-2012 lactation season. The feed values of pasture were measured by Lincoln University Analytical Services.

Table 3.1 Summary of the HSE and LSE herds for the 2011-2012 lactation season

Month	Group	No. of cows	Farm area (ha)	Stocking rate (cows/ha)	Pasture cover (kg DM/ha)	Rotation length (d)	DM %	MJ ME/Kg DM	Crude Protein %DM
Nov	HSE	31	6.5	3.9	2614	17.3	18.3	12.1	18
	LSE	30	7.7	4.8	2481	20.5	18.2	12.3	16
Dec	HSE	31	6.75	5	2317	18	12	11.6	20
	LSE	30	8.25	3.5	2517	22	12.1	11.2	20
Jan	HSE	31	6.75	5	2357	18	18	12	21
	LSE	30	8.25	3.5	2475	20	19.6	12	19
Feb	HSE	31	6.75	5	2411	18	16	-	-
	LSE	30	8.25	3.5	2561	22	15.4	-	-
Mar	HSE	31	6.75	5	2637	27	16.8	12.1	22
	LSE	30	8.25	3.5	2506	22	17.7	11.9	21
Apr	HSE	31	6.75	5	2363	27	15.4	11.8	23
	LSE	30	8.25	3.5	2263	33	15.8	11.7	23
May	HSE	31	6.75	5	2163	40	18.8	12.2	25
	LSE	30	8.25	3.5	2215	44	19.4	12.2	25

3.2 Collection and processing of samples

3.2.1 Blood samples

Blood samples (1 serum tube) were collected into 10 ml vacutainer tubes without anticoagulant from the coccygeal vein of each cow once a month. Following clotting at room temperature and 4^{o} C, the sera were decanted after centrifugation of the blood at 850 × *g* for 10 minutes (Sanchez et al., 2002). The sera were aliquoted into 10 ml storage tubes and stored at −20^{o} C until analysis.

3.2.2 Milk samples

Individual milk samples for ELISA analysis were collected once a month through a complete milking cycle and kept at 4^{o} C before being sealed and stored at −20^{o} C. The whole milk was centrifuged at 16000 × *g* for 5 minutes (Charlier et al., 2005a), the fat was removed and the skim milk harvested, sealed and stored at −20^{o} C. An equal portion of skim milk from all cows within a treatment group and within each sampling time was pooled for analysis and

that represented the bulk tank milk (BTM) sample for each treatment group. All the samples were preserved at −20° C until analysis.

3.2.2.1 Use of an indirect enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against *Ostertagia ostertagi* in milk and serum

The *Ostertagia ostertagi*-Ab ELISA Kit (Svanova Biotech AB, Uppsala, Sweden) was used to measure antibodies from serum or milk reactive to the *Ostertagia* antigen. The positive and negative controls (provided by the manufacturer) were used in each ELISA to compare the optical density (OD) of the samples to the controls (the recommended control values: OD of positive control >0.9 and OD of negative control <0.4). The indirect ELISA procedure was performed as described by Charlier *et al* (2005a). Flat bottom, 96-well microplates had been pre-coated with the crude adult *Ostertagia ostertagi* antigen at a concentration of 1 µg/ml in a 0.050 M carbonate-bicarbonate buffer (pH 9.6) and were stored at 4° C overnight. Wells were washed three times with 0.3 ml phosphate buffered saline with 0.05% Tween-20 (PBST) and non-specific binding sites were blocked with the addition of 3% foetal calf serum (200 µl per well) in PBST. The microplates were then incubated at room temperature 18° C–25° C (64° F–77° F) for 1 hour and washed as before. Skim milk or serum samples (100 µl) were added to the wells. Each plate contained a negative and a positive control, which were provided with the kit. After incubation and washing, rabbit anti-bovine IgG coupled to horseradish peroxidase (HRP) was added as a conjugate. The microtitre plates were incubated and then washed again three times. A fifty milligram (50 mg) substrate of 2, 2'-azino-bis-3 ethylbenzothiazoline-sulfonic acid (ABTS tablets, Boehringer Mannheim) was diluted in 50ml of freshly prepared buffer (ABTS buffer, Boehringer Mannheim). The reconstituted substrate was then added and incubated for 30 minutes in the dark at room temperature 18°C–25° C (64° F–77° F) and absorbance read at 405nm in a spectrophotometer. The results were expressed as optical density (OD).

The milk (skim milk) and serum samples collected monthly during the trial were pooled for each month and treatment group; that is eight milk samples (October, 2011 to May, 2012) and eight serum samples (October, 2011 to May, 2012) for each of the HSE and LSE groups. The samples were analysed in duplicate. Sixteen-fold dilutions of pooled milk samples and 512-fold dilutions of pooled serum samples were used for the detection of anti- *Ostertagia* antibodies. These dilutions were used based on an ELISA (Figure 3.1) performed on a dilution set of milk and serum samples.

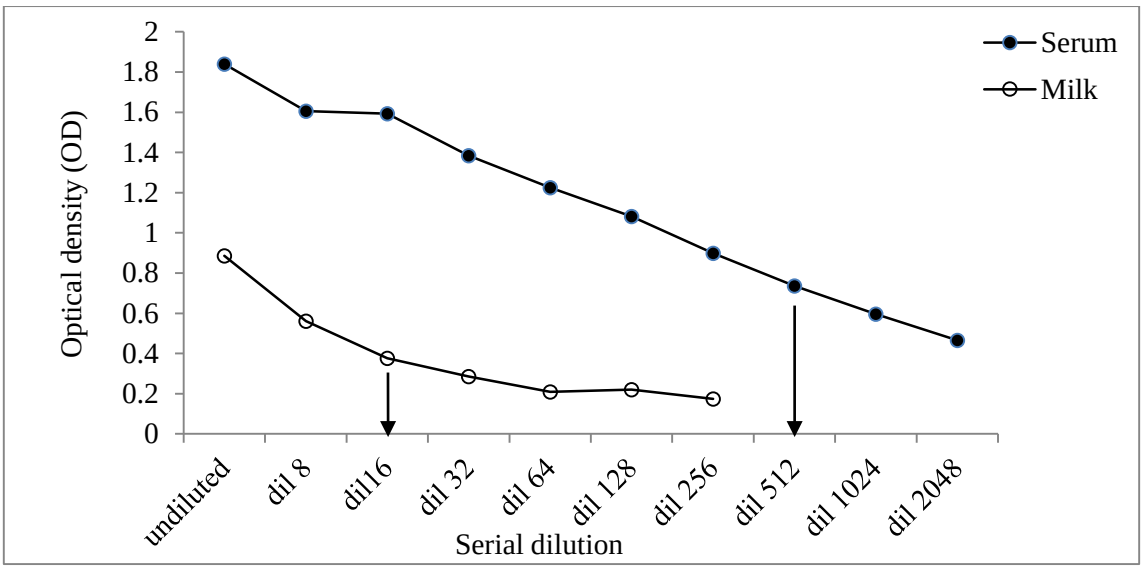

Figure 3.1 ELISA optical density (OD) of milk and serum samples measured at different dilutions (the down arrows indicate the preferred dilutions for milk and serum samples).

In order to 'correct' for the dilution effects of milk yield changing over the course of lactation, a 'corrected milk OD' was calculated for both the HSE and LSE herds based on the milk OD and corrected for milk yield at each sampling time. This was done based on the following calculations: Mean milk OD at first, second... sampling time = a, $a_{1...,}$ respectively; mean milk yield at first, second... sampling time = b, $b_{1...}$, respectively. So, corrected milk OD for first sampling time = a × (b/b). Likewise, corrected milk OD for second sampling time = a_1 × (b_1/b) and so on.

3.2.3 Faecal samples

Faecal samples (approximately 10 g) were collected directly from the rectum of each individual once a month. The samples were transferred to the laboratory and processed for individual faecal egg counts (worm eggs in faeces) using a modified McMaster technique (MAF, 1973) with a minimum detection threshold of 50 eggs per gram (EPG). In brief, faeces (3.4 g) were placed in a glass jar; 8 ml of water added and soaked overnight to soften. Then, 43 ml of saturated NaCl solution was added (final volume 51 ml) and the sample mixed with an electrical stirrer for 25-30 seconds until the faecal pellets were fully broken up and the particles evenly distributed. A clean Pasteur pipette was used to transfer an aliquot to both chambers of a moistened McMaster slide. The slide was allowed to stand for two minutes. Eggs present on the slide floated to the surface of the saturated salt solution where they were counted within the engraved area of both the chambers. The slide was placed under a compound microscope at 10 × 10 magnification and the number of eggs in both chambers

counted, summed up and multiplied by 50 to obtain the concentration of worm eggs per gram (EPG) in that faecal sample. The FEC procedure is shown in the following figure (Figure 3.2).

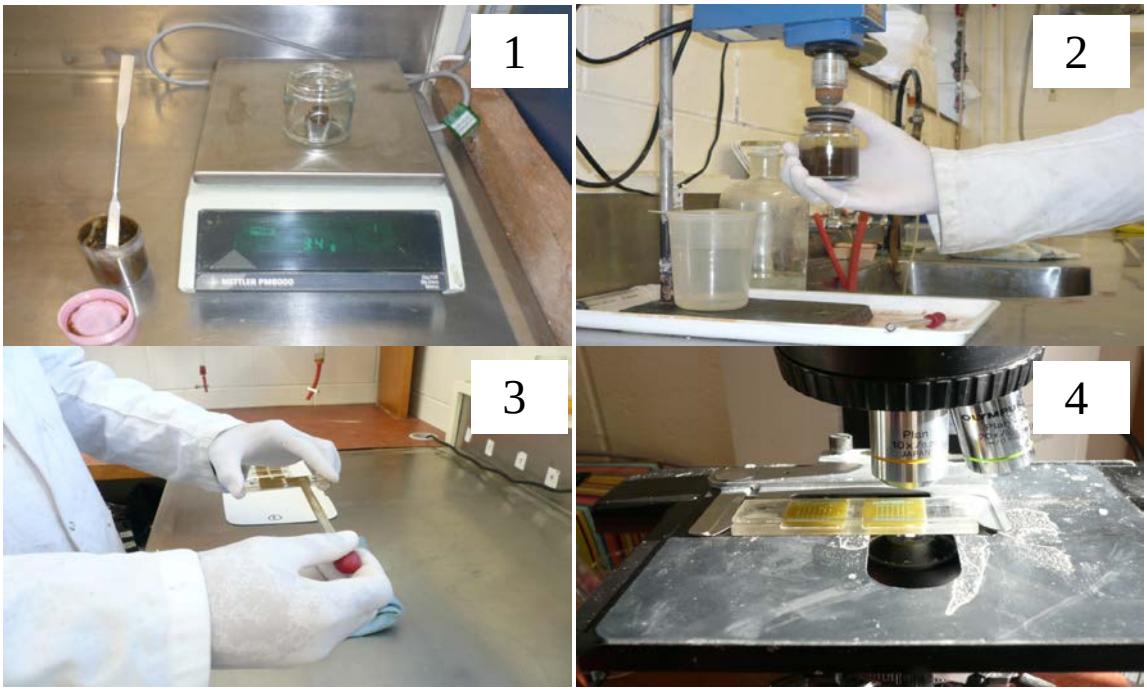

Figure 3.2 (1) Weighing faecal samples (3.4 g). (2) Mixing faecal sample in salt solution with an electrical stirrer. (3) Transferring faecal suspension into the chambers of a McMaster slide. (4) Examining the chambers of a McMaster slide for nematode eggs under a microscope at 10 × 10 magnification.

3.2.4 Pasture samples

Pasture larval counting was carried out each month throughout the milking season on the selected paddocks (two×HSE and two×LSE paddocks) each month on the Lincoln University Research Dairy Farm (LURDF). On the high stock efficient (HSE) and low stock efficient (LSE) farmlets pre-grazing pasture samples were collected (October, 2011-May, 2012) from the same four paddocks (two×HSE and two×LSE) where cows were to graze within the next 2–4 days and after at least sixteen days since the last grazing. Two paddocks, one each in the HSE and LSE had newly planted pastures that were sown 6 months previously and were composed of tetraploid ryegrass with AR37 endophyte (Tet AR37) and white clover (WC). Two paddocks had relatively old pastures that were sown 2.5 years previously and were composed of arrow perennial ryegrass with AR1 endophyte (Arrow AR1) and diploid white clover (Dip WC).

Standard pasture samples were collected in a ‘W’-shaped transect across each paddock in such a manner as to mimic grazing by a cow. A sample of grass was plucked (three to four multiple plucks) every five steps and placed in a plastic bag. Approximately 250 g of fresh herbage per sampling was collected from each paddock. The samples were carefully picked up close to the soil surface in order to avoid collecting only the top portion of plants (shown in Figure 3.3).

Figure 3.3 Collection of pasture samples by walking on the same ‘W’-shaped transect across the paddock.

Pasture L3 larvae were recovered for quantification using the method described by Taylor (1939). The freshly collected pasture samples were weighed and placed in a grass washing machine, 3 L of lukewarm water was poured into the bag and the machine then agitated at 80 rpm for two minutes. The contents were filtered through a coarse mesh (aperture size 1000 μ) into a large 5 L beaker; the grass was rewashed gently with a jet of water and re-filtered. The grass was spread on a tray and dried in an oven at 70^{o} C for 48 hours and the dry herbage weight recorded. The washings were kept at 4^{o} C overnight and sediment collected in a measuring cylinder and again kept overnight at 4^{o} C. The final sediment (approx. 30 ml) was poured onto a 150 mm Whatman filter paper and allowed to dry at room temperature 18^{o} C–25^{o} C until all the surface water had evaporated. When dry, the filter paper was inverted and placed on a Baermann filter funnel. After 36 hours, 100 ml of stem fluid was withdrawn from the Baermann filter funnel and stored in a glass bottle overnight at 4^{o} C. The volume

was then lowered to 20 ml by siphoning off the supernatant and the clean larvae present in two 1 ml sub samples were enumerated.

3.2.5 Daily milk yield

Daily milk yield (morning & afternoon) of individual cows was recorded fortnightly (by means of an Alpro-Milking Computerised System) during the trial.

3.2.6 Worm counts at necropsy

At conclusion of the trial (and lactation), six cull cows (3-HSE & 3-LSE) were slaughtered and examined for worm burdens. At necropsy, each abomasum and small intestine was removed separately from each cow. The contents of each organ of the digestive tract were individually collected and worm burdens assessed using a modification of the technique described by Herlich (1956).

3.2.6.1 Worm burdens - Abomasum

The abomasum was cut open over a container into which the contents were collected. The abomasal tissue was washed with a stream of water and carefully rubbed with fingers to remove any adhered worms. The contents and washings were then transferred to a beaker and made up to a volume of 5 L. The contents of the beaker were then thoroughly mixed and 10×50 ml (500 ml) sub samples withdrawn (dilution factor: 10). Each sub sample was then gently rinsed through a stainless steel sieve (aperture size 38 µ); the retained contents were then made up to180 ml and placed in a labelled container, to which 20 ml of formalin (40% w/v formaldehyde) was added to make a standard volume of 200 ml. From the formalised sample two 10 ml abomasal aliquots were transferred to two Petri dishes (dilution factor: 10) and examined under a microscope. This represented a one hundredth of the original sample; therefore, for each worm count represented 100 ($1 \times 10 \times 10$) worms in the final worm count from the abomasal wash.

After washing, the abomasal tissue was placed upside down in warm (38.5^{o} C) physiological saline in a wide flat tray with a total volume of approximately 7 L and incubated in a heated (25^{o} C) room for 16 hour. The tissue was removed, washed and then discarded. The washings and contents of the tray were then passed through a 38 µ sieve subjected and

washed with a jet of water. The material collected on the sieve was made up to 130 ml and placed in a labelled container, to which 20 ml of formalin (40% w/v formaldehyde) was added to make a standard volume of 150 ml. From the formalised sample two 7.5 ml abomasal aliquots were transferred to two Petri dishes and examined under a microscope. Therefore, collectively a one tenth aliquot (15 ml) of the digested sample was examined (dilution factor: 10). The number of worms counted was then multiplied by the relevant dilution factor to give the total number of worms in the abomasal saline incubation.

3.2.6.2 Worm burdens - Small Intestine (SI)

The washings and contents of the small intestines were collected into a beaker and made up to a volume of two litres (2 L). The contents of the beaker were then thoroughly mixed and four 50 ml sub samples withdrawn (dilution factor: 10). This sub sample was then gently rinsed through a stainless steel sieve (aperture size 38 µ); the retained contents were then placed in a clearly labelled container, to which 20 ml of formalin was added. For convenience, these were later standardised to a total volume of 200 ml. From the formalised sample, two 10 ml small intestinal aliquots were transferred to two Petri dishes (dilution factor: 10) and examined under a microscope. This represented a one hundredth of the original sample, therefore for each worm counted represents 100 ($1 \times 10 \times 10$) worms in the final worm count from the small intestinal wash.

After washing, the intestinal tissue was placed upside down in warm (38.5^{o} C) physiological saline in a wide flat tray with a total volume of approximately 7 L and incubated in a heated (25^{o} C) room for 16 hour. The tissue was removed, washed and then discarded. The washings and contents of the tray were then passed through a 38 µ sieve subjected and washed with a jet of water. The material collected on the sieve was made up to 130 ml and placed in a labelled container, to which 20 ml of formalin (40% w/v formaldehyde) was added to make a standard volume of 150 ml. From the formalised sample two 7.5 ml abomasal aliquots were transferred to two Petri dishes and examined under a microscope. Therefore, collectively a one tenth aliquot (15 ml) of the digested sample was examined (dilution factor: 10). The number of worms counted was then multiplied by the relevant dilution factor to give the total number of worms in the intestinal saline incubation.

Worms were quantified using a dissecting microscope at a magnification of approximately × 12-16 and parasite genera and developmental stages were identified with a compound microscope at a magnification of × 40.

Experiment B

3.3 Animals

Sixty dairy cows (N=60) of mixed age and mixed breed (Holstein, Jersey, and Holstein × Jersey crossbred cows) were provided by the Lincoln University Research Dairy Farm (LURDF) for the experiment. After stratification on the basis of age, the experimental animals were randomly allocated to one of three groups, i.e. Group I (GI, n=20), Group II (GII, n=20) and Group III (GIII, n=20).

During late lactation, cows in all the groups were treated on day -28 with a pour-on formulation of eprinomectin [Eprinex Pour-On for beef and dairy cattle (5mg eprinomectin/ml)] at a dose rate of 1 ml/10kg (22 lb) of live weight. Twenty-eight days later (day 0), cows in GII & GIII were given infective L3 larvae (as a bolus) at a dose rate of 50×10^3 L3 per animal, administered orally. These infective larvae (L3) were a mixture of *Ostertagia ostertagi, Trichostrongylus axei* and *Cooperia oncophora* (at a ratio 1.3**:**1.3**:**7.4) and a very few *Haemonchus spp.* and *Oesophagostomum spp.* Cows in GI were not infected with L3 and kept as controls. Cows in GII were treated again with eprinomectin on day 10 (d10) to truncate the infection, were orally infected again with infective L3 larvae (as a bolus) at a dose rate of 50×10^3 L3 per animal on day 38 (d38) and again treated with eprinomectin 10 days later (d48) to truncate the second artificial infection. The experimental animals in GIII, unlike GII, were not treated with an anthelmintic following the administration of infective L3 larvae on day 0.

3.4 Generation of challenge larvae (L3)

Two young (six-month old) bull calves were sourced from a commercial dairy farm located in Canterbury, New Zealand and rendered parasite free by treatment with Matrix-C (triple combination of Abamectin, Levamislole HCl and Oxfendazole) drench at a dose rate of 1 ml per 20 kg body weight. They were infected orally with infective L3 (as a bolus) at a dose rate of 20×10^3 L3 per calf, simultaneously with an injection of Ilium Depredil 5 ml/calf (40 mg/ml methyl prednisolone acetate) via the I/M route. This was done to suppress immunity and

maximise the development of infective larvae (L3) into adult worms. The calves were kept in an indoor calf pen (approximately 6 m^2) and the indoor pen was located in a single covered and ventilated building near the Johnstone Memorial Laboratory (JML). The pen had two wooden food bins, a water trough and a concrete floor covered in deep litter sawdust; natural lighting was provided by skylights. The water trough was fitted with a float valve such that water was available *ad libitum.* Faeces were removed and the sawdust raked daily; all sawdust in the pen was replaced monthly to prevent build-up of ammonia fumes from calf urine. The health of these calves was monitored as per the guidelines of the Animal Ethics Committee (AEC), Lincoln University. Throughout the period of indoor confinement, the diet consisted of grass silage (11 MJ ME/kg DM, 17% crude protein) plus lucerne hay (9 MJ ME/kg DM, 20% crude protein) for adequate roughage to ensure maintenance of rumen metabolism. Food waste (refusal) was collected each day and new food offered. Freshly voided faeces were collected in a plastic bag, transferred to the parasitology laboratory and cultured using the method described below. The procedure for the culture and extraction of infective nematode larvae from bovine faeces was performed as a modification of the technique described by Pullan and Sewell (1981). This procedure was carried out every day until the required numbers of challenge larvae (infective L3) for the research trial were obtained.

Briefly; i) plastic trays were used in the incubation of faecal cultures. The culture mixtures were usually made up at a ratio of one part of faecal sample to three parts of culture medium (vermiculite). The plastic trays were filled with the mixture and covered with plastic sheets with several tiny holes to facilitate adequate aeration. The cultures were examined and stirred at a regular intervals (every third day) during incubation. Water was sprinkled on them when they appeared dry. All faecal cultures were kept on wooden shelves at a room temperature (24^o C–26^o C) and a relative humidity between 80–85% for a minimum period of ten days; ii) on day 11, the faeces/vermiculite mixture was placed on a combination of a sieve tray (top) and a collection tray (bottom). The infective larvae (L3) were extracted by flooding the faeces/vermiculite mixture with water. The trays were left on the bench overnight and the sieve tray was partially lifted off the collection tray; iii) The fluid in the collection tray was pooled in a bucket and left overnight to allow the infective larvae (L3) to sediment out. Then the supernatant was siphoned off and the sediment collected in a large (5 L) beaker. After a minimum period of four hours, the supernatant from the beaker was discarded and the contents collected in a measuring cylinder and left for a further four-hour period; iv) The sediment drawn off the measuring cylinder was then poured onto a filter paper (Whatman filter paper, size 150 mm) and, when dried, it was inverted and placed on a 2-ply tissue paper

over a Baermann filter funnel. The infective larvae (L3) were allowed to migrate overnight into the funnel stem, from which the clean larvae were recovered. The infective larvae (L3) were stored (for less than a month) until used in the infection studies (Exp. B). The procedure for culture and harvesting of infective L3 larvae is shown in the following figure (Figure 3.4).

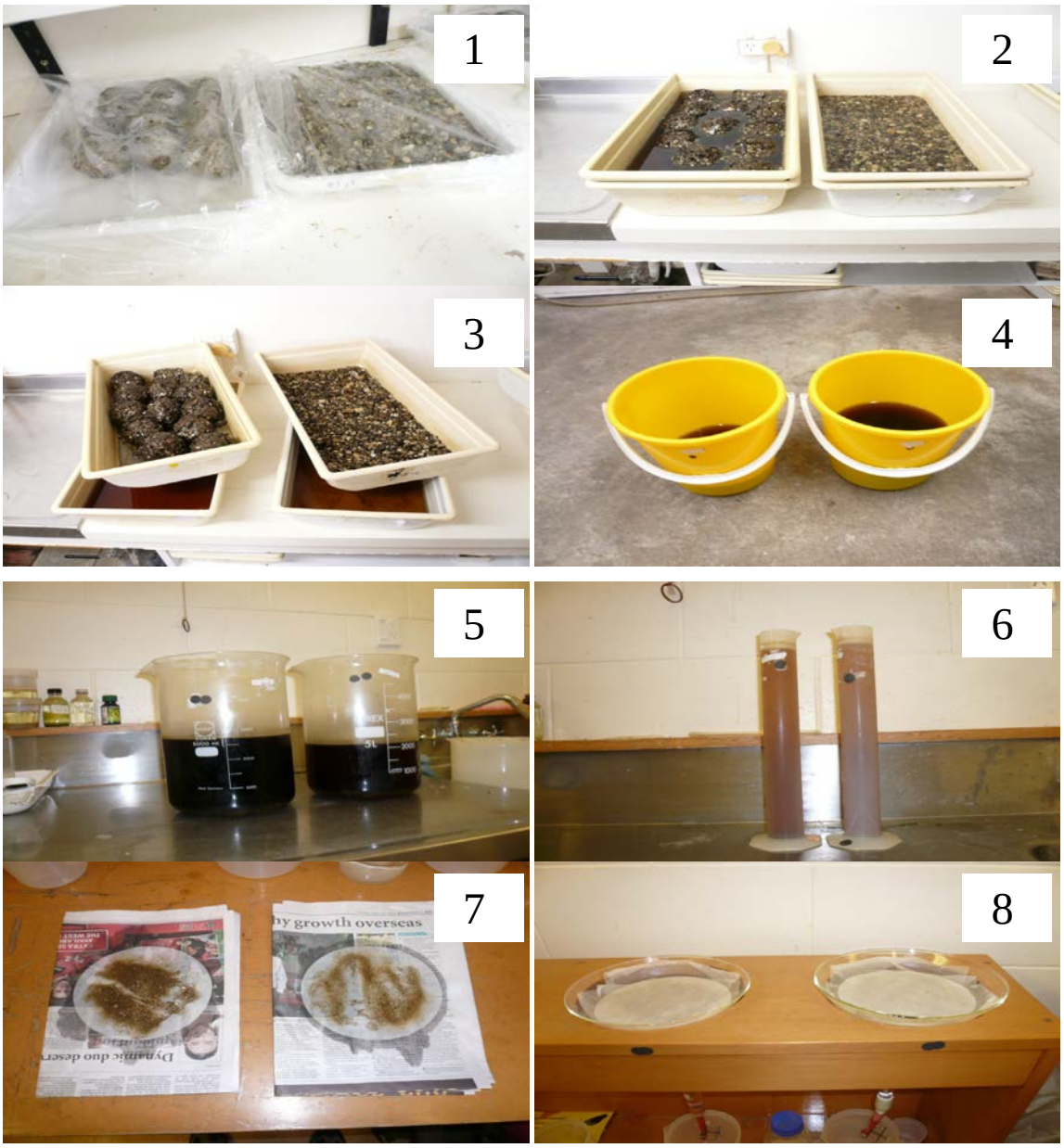

Figure 3.4 (1) Culture of faeces/vermiculite mixture on plastic trays covered with punctured plastic sheets. (2) Cultured faeces/vermiculite mixture flooded with water. (3) Lift off sieve tray to collect fluid in the collection tray. (4) Collection of fluid from tray into labelled plastic buckets. (5) Collection of sediments from buckets into large beakers. (6) Further sedimentation into measuring cylinders. (7) Pouring cylinder contents onto Whatman filter paper (150mm). (8) Inversion of dried filter paper onto a 2-ply tissue paper over a Baermann filter funnel.

3.5 Collection and processing of samples

3.5.1 Blood samples

Blood samples were collected from the coccygeal vein of each cow on day -28 (28 days prior to artificial worm infection) and at days 0 (d0), 10 (d10), 38 (d38) and 48 (d48) after the artificial worm infection. A dilution of 1: 512 of the pooled serum samples was used for detection of anti- *Ostertagia* antibodies based on the ELISA (see section 3.2.2.1) performed on the pooled serum samples (pooled from five different sampling times for each of three groups i.e., GI, GII & GIII). The samples were analysed in duplicate.

3.5.2 Faecal samples

Faecal samples were collected from all animals on the day (d-28) of treatment with eprinomectin and also at days 0 (d0), 10 (d10), 38 (d38) and 48 (d48) after the artificial worm infection and nematode eggs counted (as per section 3.2.3).

3.5.3 Worm counts at necropsy

At conclusion of the trial (and lactation), 11 cull cows (2-GI, 4-GII & 5-GIII) were slaughtered and examined for worm burdens (as per section 3.2.6.1 & 3.2.6.2).

3.6 Statistical analyses

Data were summarised and the raw figures were prepared in Microsoft Excel. Statistical analyses were performed with the software package of Genstat Release 15.1 (15th edition), VSN International Ltd and Minitab (Version 16). The FEC data from Experiment A were analysed using the non-parametric method- the Kruskal-Wallis test as the data were not transformable to a normal distribution and equal variances. The difference between the younger and older cows with regard to FEC was evaluated using the Chi-square test. The pasture larval data from Experiment A were analysed using the repeated measures analysis- the Greenhouse-Geisser epsilon ANOVA model. The milk yield data from Experiment A were analysed using the parametric method- the Wald statistic. The ELISA data from Experiment A and Experiment B were analysed using a balanced ANOVA. The post-mortem worm counts data from Experiment A and Experiment B were analysed using one way ANOVA. The relationship between different diagnostic indicators was evaluated with a Pearson correlation test. The significance level was set at $p<0.05$.

Chapter 4

Results

Experiment A

4.1 Faecal Egg Counts (FEC)

Mean monthly faecal egg counts (FEC) from cows of the high stock efficient (HSE) and low stock efficient (LSE) herds on the Lincoln University Research Dairy Farm (LURDF) from approximately one month after calving till the end of lactation are shown in Figure 4.1 & Appendix A.

4.1.1 HSE/LSE group means

At each collection point the majority of cows in both groups did not excrete detectable levels of nematode eggs in the faeces. The overall mean FEC for the HSE and LSE groups over the trial period were 9.7 and 13.1 eggs per gram (EPG) of faeces, respectively. The mean faecal egg counts from the HSE cows at the start of the trial 4.8 EPG in October, 2011, rose to 14.5 EPG in November, 2011 (late spring), dropped to about 11 EPG in December, 2011 (early summer) and then dropped below 10 EPG during May, 2012 (late autumn). The mean faecal egg counts from the LSE cows were above 10 EPG during the whole trial with the exception of 8.6 EPG in December, 2011. The individual faecal egg counts ranged between 0–150 EPG for both the HSE and LSE groups. There was neither a significant difference (p=0.26) in mean FEC between cows maintained in the HSE and LSE groups at any sampling period during the trial, nor a significant change over time (p=0.90).

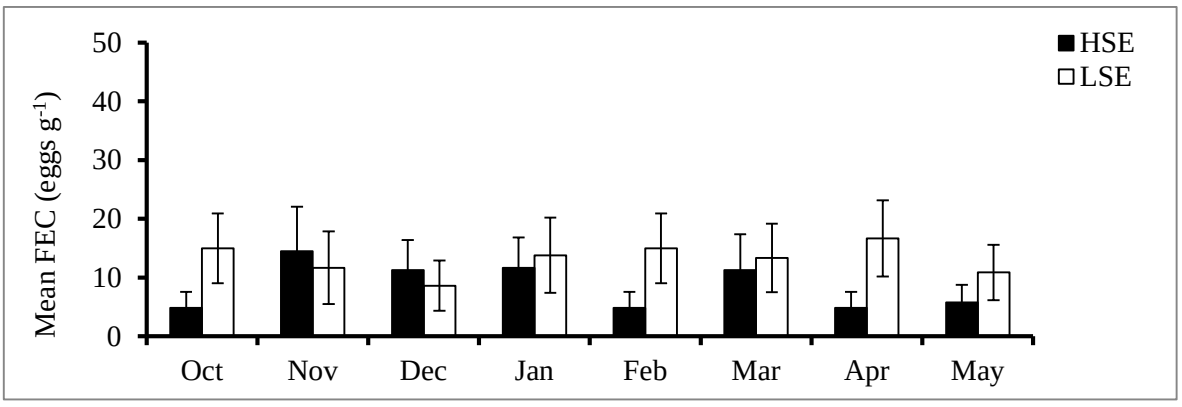

Figure 4.1 Seasonal pattern of faecal egg counts (FEC) of cows maintained in the HSE (n=31) and LSE (n=30) groups (FEC ± SEM).

4.1.2 Number of cows with positive faecal egg counts (FEC)

During the course of this trial, no nematode eggs were observed in the faeces of 29 (HSE: 14 + LSE: 15) out of 61 cows. The faecal egg output from the FEC+ve animals was intermittent except for one cow from the LSE group which shed worm eggs on all occasions during the trial.

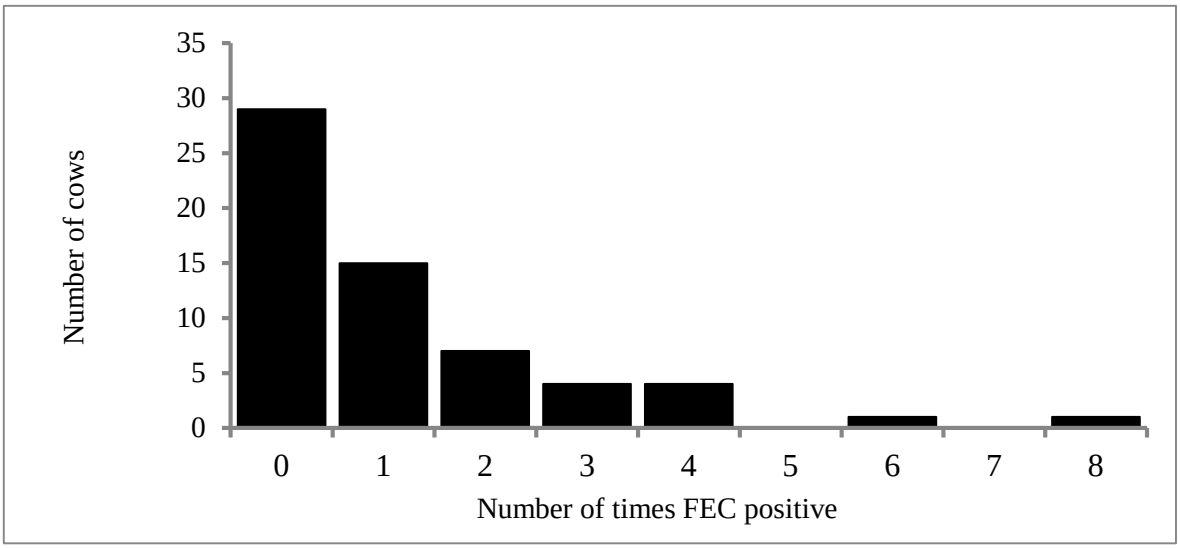

Figure 4.2 Number of cows from both the HSE and LSE groups with or without positive FEC when measured monthly over eight occasions.

4.1.3 Percentage of cows with positive FEC

The data were also analysed to determine the percentage of infected cows (positive FEC) in the HSE and LSE groups to see if there was any difference in FEC due to different stocking rates or time of the season. The percentage of cows with FEC+ve is shown in Figure 4.3.

The proportion of cows with FEC+ve was low in both the HSE and LSE farmlets throughout the trial. In October, 2011, 9.7% of the HSE cows sampled had positive FEC which increased to around 16% in December, 2011 (early summer) and dropped to around 11.5% in May, 2012 (late autumn), whereas, initially, 20% of the LSE cows sampled had positive FEC which dropped to around 13% in early summer and then increased to around 17% in late autumn. There was a trend for the LSE herd to have a higher percentage of cows with positive FEC than the HSE ($p=0.093$).

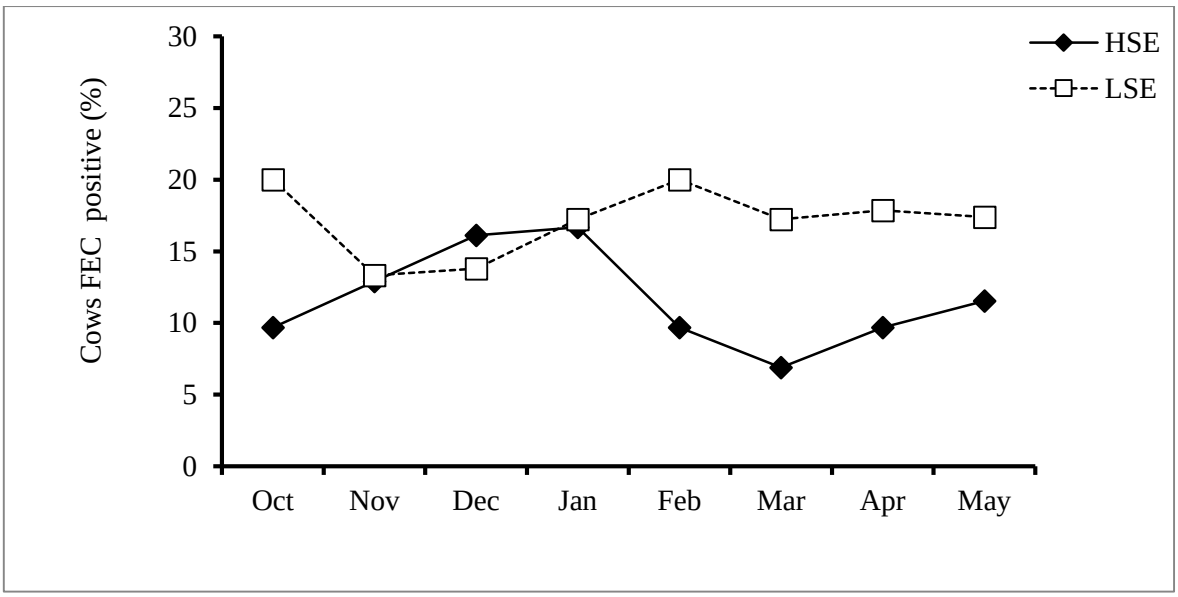

Figure 4.3 Percentage of cows with positive FEC in the HSE and LSE groups at each sampling time during the trial.

4.1.4 Age effect on FEC

Pooled FEC data from the HSE and LSE groups revealed a trend (Chi square=3.6; p=0.059) for a greater percentage of younger cows shedding worm eggs (cluster of 2-4 years) when compared with older (cluster of 5+ years) animals, during the trial.

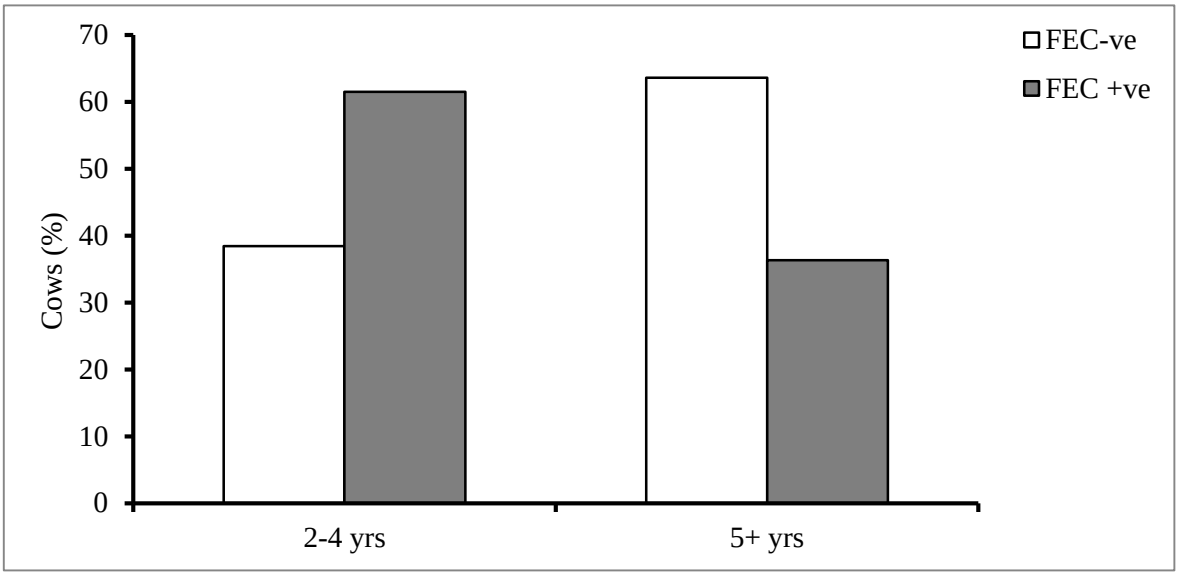

Figure 4.4 Percentage of cows with FEC–ve and FEC+ve pooled from the HSE and LSE groups, in relation to age.

4.2 Milk yield status of the HSE and LSE cows with regard to faecal egg counts (FEC)

Faecal egg counts (FEC) from both the HSE and LSE cows were categorised into three clusters (0, 50 and 100 EPG) and daily milk yield of the individual HSE and LSE cows corresponding to the above clusters were compared over the eight months of the trial. Faecal egg counts of >100 EPG (150 EPG) were recorded on only few occasions during the trial (refer to Appendix A, Table A.1) and therefore, included in the cluster of 100 EPG.

In the clusters of cows excreting 0, 50 & 100 EPG, the highest mean daily milk volume (L/day) of individual cows recorded at the start of the trial in October, 2011, was 25.67, 27.16 and 25.63, respectively. The lowest daily milk volume (L/day) for the same clusters of cows recorded at the end of the trial in May, 2012, was 12.65, 12.58 and 13.18, respectively. There was no significant association between FEC and milk production. The significant variation in milk yield over the milking season fitted a typical lactation curve (time effect with $p<0.05$).

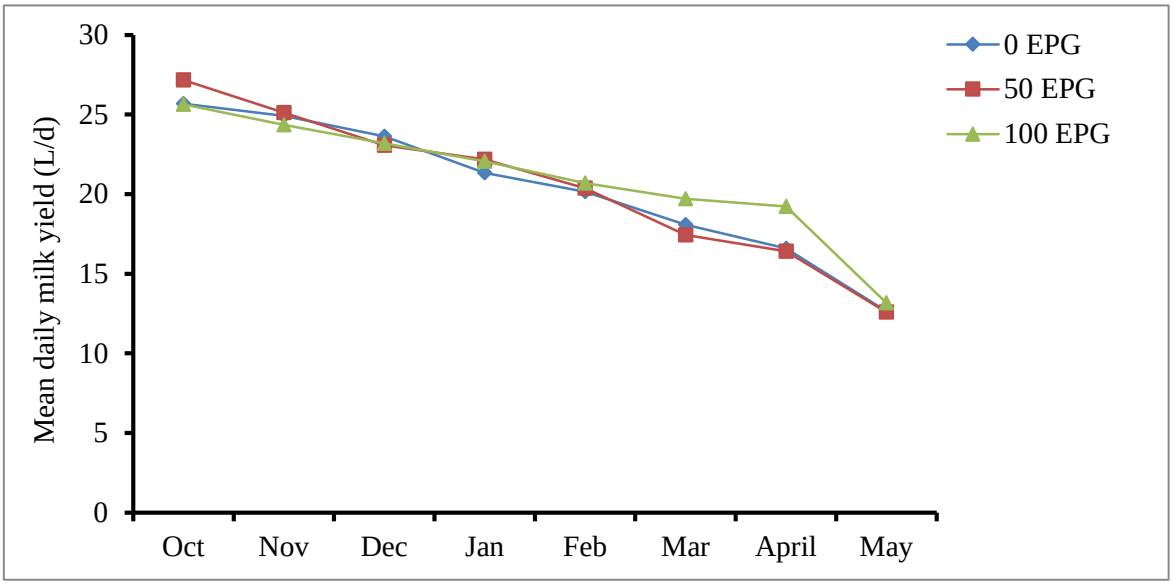

Figure 4.5 Variation in mean daily milk yield of the HSE and LSE cows during the trial, in relation to faecal egg counts.

4.3 Pasture L3 larvae levels

The mean infective L3 larvae levels of parasitic nematodes for the four monitor paddocks on the Lincoln University Research Dairy Farm (LURDF) are shown in Figure 4.6. The average grazing rotation was 18 days in summer and 31 days in autumn for the HSE cows, whereas for the LSE cows it was 21 days in summer and 33 days during autumn. Grazing rotation was based on a threshold of residual pasture cover of 1450 kg DM/ha for the HSE and 1600 kg DM/ha for the LSE herd. The highest infective pasture larvae (L3) level measured during

February, 2012 in the HSE and LSE farmlets was 244.8 (range 0–244.8) and 182.5 (range 0–182.5) L3 per kg fresh herbage, respectively. These larvae levels were recorded on old pasture (sown 2.5 years previously) from both the HSE and LSE farmlets, whereas infective L3 larvae were found only once from the new pasture (sown 6 months previously) on the HSE farmlet, during late February, 2012. There was significantly greater level of L3 larvae ($p<0.05$) on the old (versus new) pasture and a trend ($p=0.09$) for more L3 larvae in the HSE (versus LSE) older pasture.

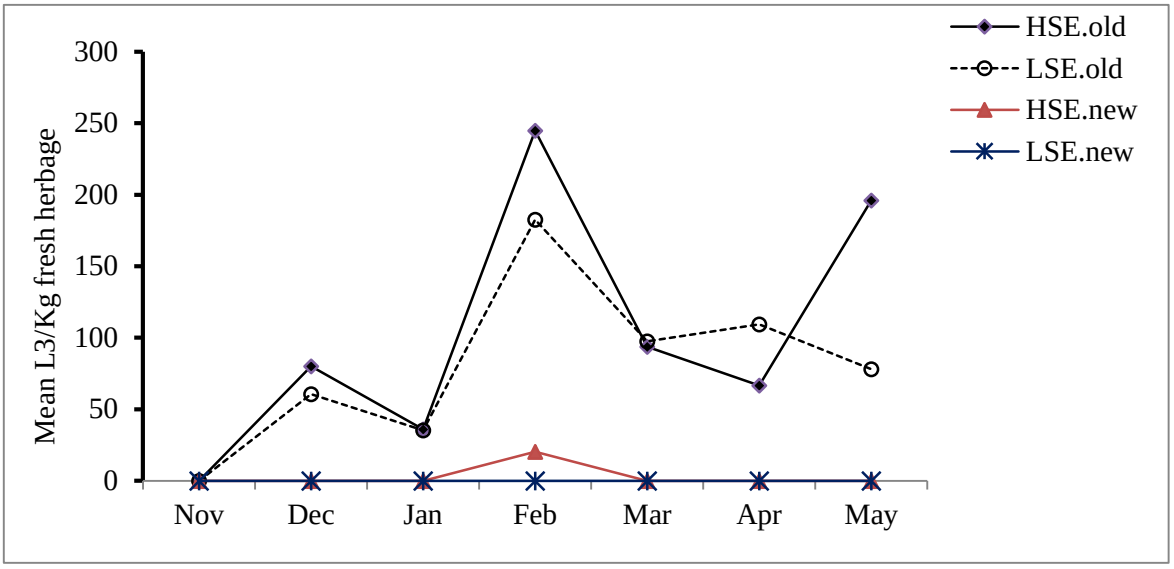

Figure 4.6 Pasture L3 levels on the monitor paddocks of the LURDF throughout the lactation season.

4.4 Enzyme-linked immunosorbent assay (ELISA) to detect anti-*Ostertagia* antibody levels (OD) in milk and serum

The trim milk samples were pooled from all cows within a treatment group and within each sampling time and anti-*Ostertagia* antibody levels measured at 16-fold dilutions (refer to Appendix D; Table D.1). The serum samples were also pooled from all cows within a treatment group and within each sampling time and anti-*Ostertagia* antibody levels measured at 512-fold dilutions (refer to Appendix D, Table D.1). The OD values for pooled milk including corrected milk OD (see section 3.2.2.1) and OD values for pooled serum are shown in the following figures (Figures 4.7 & 4.8, respectively).

4.4.1 Optical density (OD) of pooled milk samples

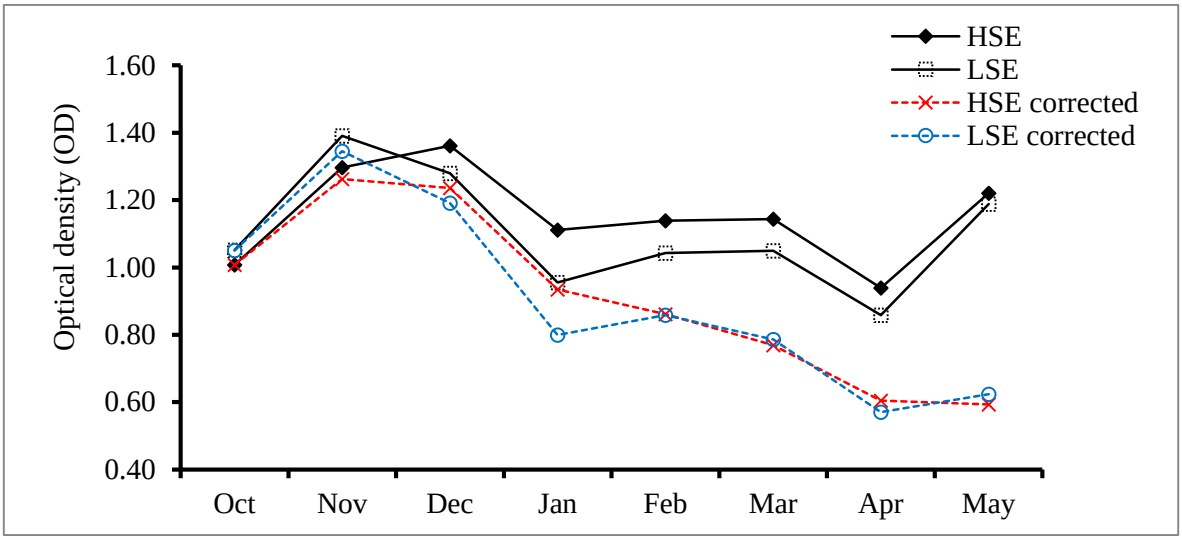

Figure 4.7 Optical density of pooled milk samples from the HSE and LSE cows measured over the trial period. Corrected milk OD was derived (based on milk OD, adjusted for daily milk yield).

4.4.2 Optical density (OD) of pooled serum samples

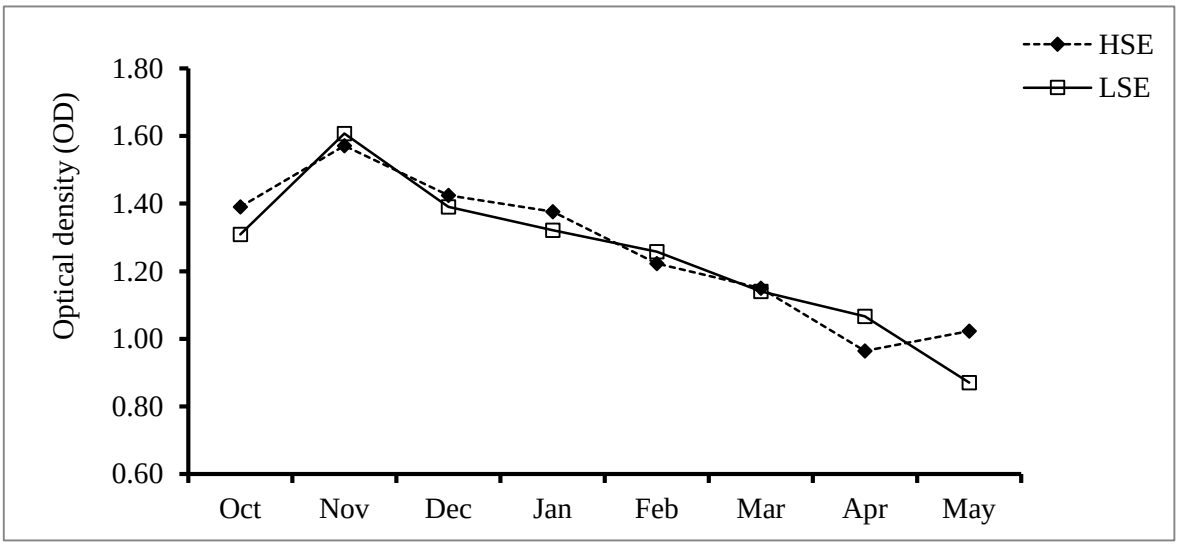

Figure 4.8 Optical density of pooled serum samples from the HSE and LSE cows measured over the trial period.

For the HSE group, milk OD values ranged between 0.94–1.36 and serum OD values ranged between 0.96–1.57. For the LSE group, milk OD values ranged between 0.86–1.39 and serum OD values ranged between 0.87–1.61. The results from milk and serum showed significant differences with time ($p<0.001$) but not between the treatment groups (HSE & LSE). The correlation between milk and serum OD in both the HSE and the LSE groups was positive but

statistically non-significant (HSE: r=0.503, p=0.204; LSE: r=0.486, p=0.222). The correlation between the corrected milk OD and serum OD was significantly positive in both the groups (HSE: r=0.958, p<0.01; LSE: r=0.900, p<0.01).

4.5 Worm counts at necropsy

Mean worm burdens in the HSE and LSE groups were 4,940 (range 600–13,420) and 13,396.67 (range 2,080–34,080), respectively (Figure 4.9, Appendix E). The L3/arrested L4 numbers in the HSE and LSE groups were 2,390 (range 500–6,070) and 8,160 (range 1,060–19,910), respectively. There was no significant difference between the HSE and LSE groups with regard to mean worm burden (p=0.492) or number of mean L3/arrested L4 (p=0.405).

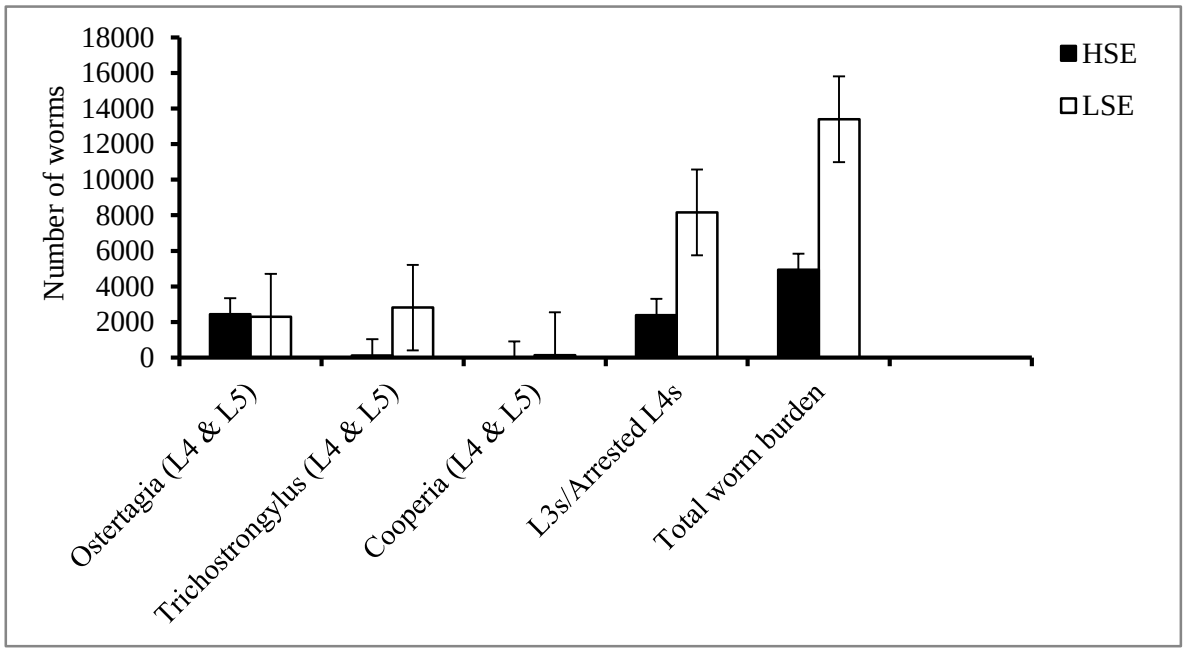

Figure 4.9 Mean worm burden in animals from the HSE (n=3) and LSE (n=3) groups showing different worm genera (worm burden ±SEM).

Relationship between faecal egg counts, pasture L3 counts, anti-*Ostertagia* antibody levels (OD) in milk/serum, milk yield and worm burden in the HSE and LSE groups

As infective L3 larvae were recovered only once from the new pasture during the trial these data were excluded from the analysis. Correlation was sought between the monthly data of mean pasture L3 levels from the old pasture and the FEC data measured one month after the pasture L3 samples were taken, which included the 3-4 week ‘prepatent period’ where ingested larvae mature into adults and produce eggs detectable in the animal faeces. The

results showed evidence of a positive correlation (r=0.868, p=0.056) in the HSE group but did not show any correlation (r=0.054, p=0.932) in the LSE group (Figure 5.0, Table 4.1).

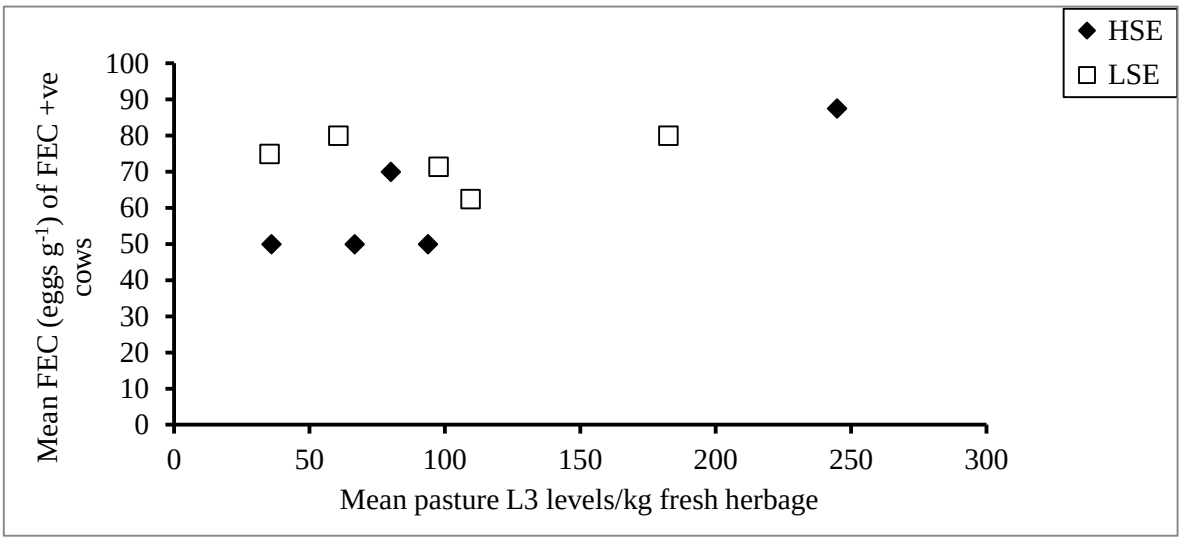

Figure 5.0 Correlation between mean FEC of FEC +ve cows and mean pasture L3 larvae levels in the HSE (n=31) and LSE (n=30) groups over the trial period.

Table 4.1 Pearson correlation between indicators of parasitism and milk yield

HSE					
Mean values	FEC	Pasture	Milk OD	Milk OD (corrected)	Serum OD
Pasture L3	r=0.868 p=0.056				
Milk OD	r=0.171 p=0.686	r= -0.064 p=0.892			
Milk OD (corrected)	r=0.271 p=0.516	r= -0.257 p=0.623	r=0.597 p=0.118		
Serum OD	r=0.210 p=0.618	r= -0.510 p=0.242	r=0.503 p=0.204	r=0.958 p=0.000	
Daily milk yield	r=0.162 p=0.701	r= -0.588 p=0.165	r=0.176 p=0.677	r=0.891 p=0.003	r=0.909 p=0.002
LSE					
Mean values	FEC	Pasture	Milk OD	Milk OD (corrected)	Serum OD
Pasture L3	r=0.054 p=0.932				
Milk OD	r= -0.817 p=0.013	r= -0.524 p=0.227			
Milk OD (corrected)	r= -0.480 p=0.228	r= -0.152 p=0.774	r=0.759 p=0.029		
Serum OD	r= -0.196 p=0.641	r= -0.482 p=0.274	r=0.486 p=0.222	r=0.900 p=0.002	
Daily milk yield	r= -0.089 p=0.833	r= -0.363 p=0.423	r=0.339 p=0.412	r=0.865 p=0.006	r=0.908 p=0.002

Faecal egg counts (FEC) were negatively correlated with milk OD (r= -0.817, p=0.013) in the LSE group (Table 4.1). There was a significant positive relationship between daily milk yield and serum OD [(HSE: r=0.909; p=0.002), (LSE: r=0.908; p=0.002)] in both the treatment groups (Figure 5.1, Table 4.1).

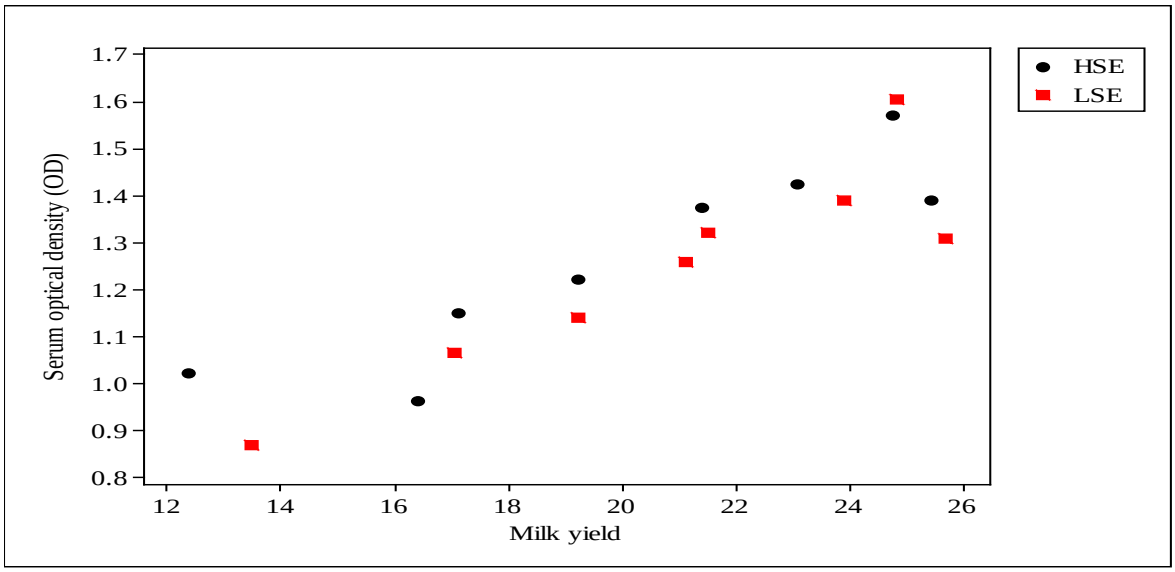

Figure 5.1 Correlation between milk yield and anti-*Ostertagia* antibody levels (OD) in serum during the trial.

Relationship between anti-*Ostertagia* antibody levels (OD) in serum and worm burdens from individual slaughtered animals

The correlation between anti-*Ostertagia* antibody levels (OD) in serum (pooled from last two samplings) and worm burdens in these animals was negative (r= -0.805, p=0.054).

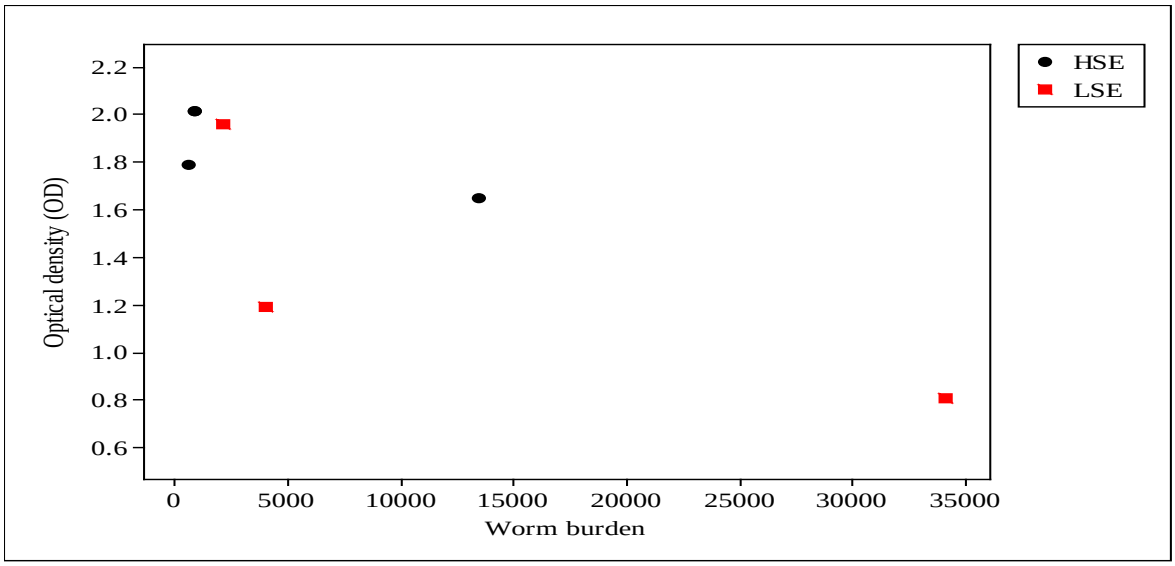

Figure 5.2 Correlation between anti-*Ostertagia* antibody levels (OD) in serum and worm burden.

Experiment B

4.6 Faecal egg counts (FEC)

The faecal egg counts from the majority of the experimental cows were negative (<50 EPG). The percentage of cows with FEC+ve was calculated for each treatment group (n=20) from each time point; there was a significant difference on the percentage of cows showing positive FEC over time ($p<0.05$) but not between the treatment groups ($p=0.700$). The percentage of cows from GI, GII and GIII that were FEC+ve is shown in the following table (Table 4.2).

Table 4.2 Percentage of cows showing positive FEC

Groups	d-28	d0	d10	d38	d48
G I (Control)	15	0	0	0	0
G II	5.3	10	10	0	0
G III	10.5	10.5	0	10	5

4.7 ELISA assays to detect anti-*Ostertagia* antibody levels (OD) in serum

The serum samples were pooled from all cows within each treatment group of GI, GII & GIII at each time point and anti-*Ostertagia* antibody levels measured at 512-fold dilutions (refer to Appendix D, Table D.2). The serum OD values are shown in the Figure 5.3.

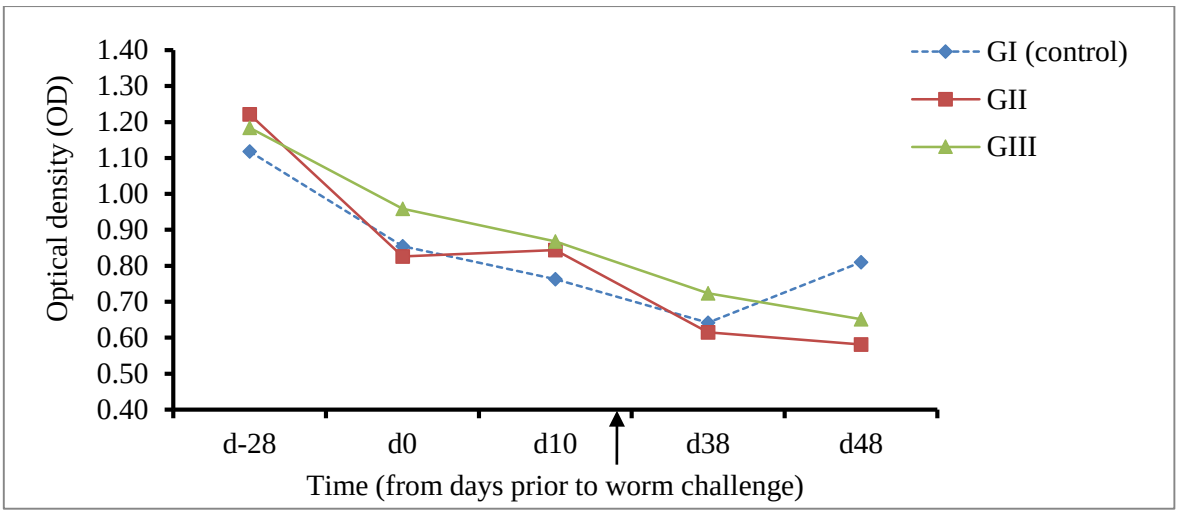

Figure 5.3 Optical density (OD) of pooled serum from cows in GI, GII and GIII prior to, and during, artificial worm challenge. The arrow indicates when the cows were dried off (day 16).

The lactating cows were dried off at day 16 and moved to the Ashley Dene Pastoral Systems Research Farm, where they were winter-grazed on kale. Serum OD values decreased in all treatment groups following an anthelmintic treatment at day -28; GI: 1.12–0.81; GII: 1.22–0.58 and GIII: 1.18–0.65. There was a significant difference ($p<0.001$) in mean OD of pooled serum over time during the trial but not between the treatment groups ($p=0.479$).

4.8 Worm counts at necropsy

Mean worm burdens in GI, GII and GIII were 9,970 (range 2,910–17,030); 3,418 (range 1,600–7,300) and 4,904 (range 100–12,560) respectively (Fig. 5.4). The numbers of mean L3/arrested L4 in GI, GII and GIII were 7,850 (range 1,940–13,760); 2,002 (range 850–5,100) and 4,068 (range 0–10,720) respectively. There was no significant difference between the treatments (GI, GII and GIII) with regard to total worm burden ($p=0.370$) or number of mean L3/arrested L4 ($p=0.337$).

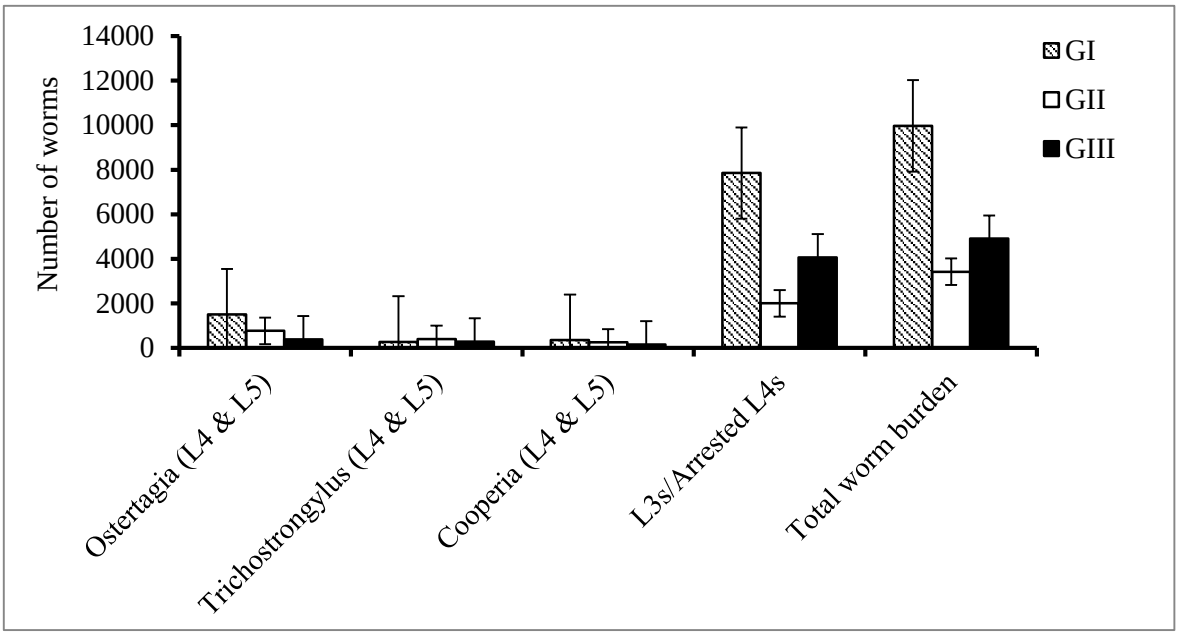

Figure 5.4 Mean worm burdens in animals from GI (n=2), GII (n=4) and GIII (n=5) groups showing different worm genera (worm burden ±SEM).

Relationship between FEC (% cows with FEC+ve) and anti-*Ostertagia* antibody levels (OD) in serum

From each of the three experimental groups the percentage of cows with positive FEC was compared with the anti-*Ostertagia* antibody levels (OD) in pooled serum. The result was a significant positive correlation (r=0.559, p=0.030).

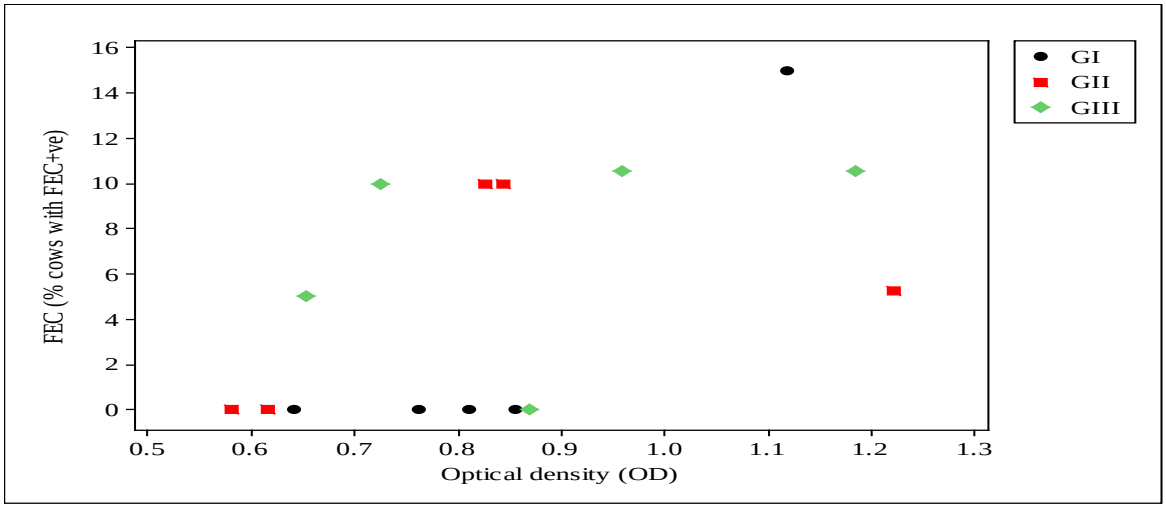

Figure 5.5 Correlation between FEC and anti-*Ostertagia* antibody levels (OD) in serum during the trial.

Relationship between anti-*Ostertagia* antibody levels (OD) in serum and worm burdens from individual slaughtered animals

When the worm burdens of the eleven cull cows (GI: two, GII: four & GIII: five) were compared with the anti-*Ostertagia* antibody levels (OD) in serum (pooled from last two samplings) there was no significant correlation (r= -0.331, p=0.320).

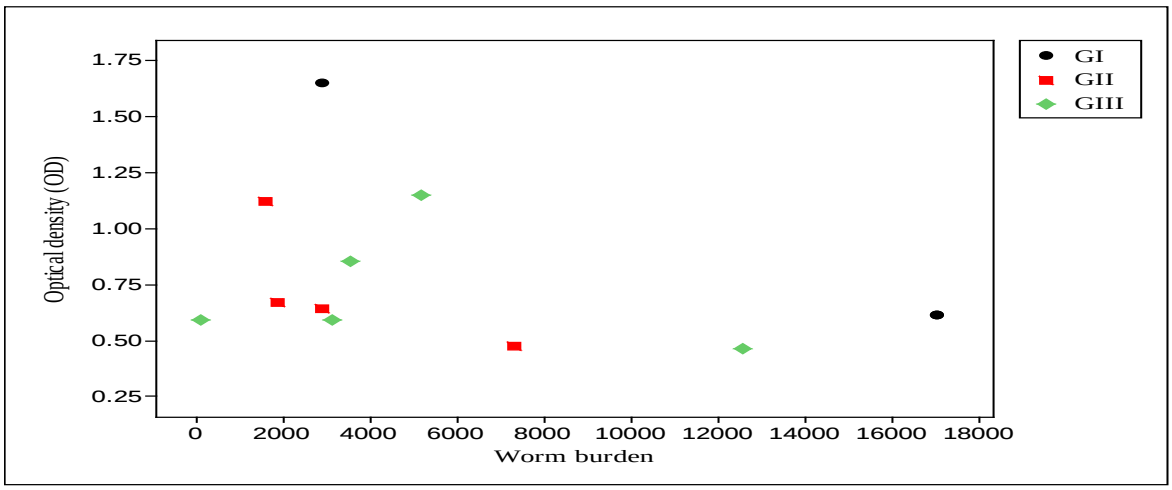

Figure 5.6 Correlation between anti-*Ostertagia* antibody levels (OD) in serum and worm burden.

Chapter 5
Discussion

The present study was conducted with the objectives of seeking associations between serum or milk antibody levels to gastrointestinal nematode parasites and infective L3 levels on pasture, worm burdens and faecal egg counts (FEC) in a New Zealand grazing situation. The initial studies of two herds grazing at high or low stock density were monitored regularly for parasite burdens (Exp. A). During the grazing period, monthly data were collected on faecal egg counts (FEC), infective L3 levels on pasture and, serum and milk antibody levels against gastrointestinal nematodes. Because the initial parasitology assays revealed low pasture larval (L3) contamination (<100 L3/kg fresh herbage) and low FEC (<100 eggs per gram) in the herds (HSE & LSE), a second experiment (Exp. B) was started where cows were artificially infected with additional L3 larvae. The purpose of truncating L3 infection in one group (GII) and allowing the infective L3 to mature in another group (GIII) was to verify whether the uptake of L3 larvae or developing worms within the host triggered the antibody response.

In this study, anti-*Ostertagia* antibody levels were measured in milk and serum using dilutions higher than the manufacturer's (Svanova Biotech AB, Uppsala, Sweden) recommendations (milk: 16-fold; serum: 512-fold) in order to have a linear relationship between optical density (OD) and antibody concentration. The continual exposure of the experimental animals to nematode parasites in this life-long pasture grazing system was seemingly reflected in the presence of high antibody levels. For example, an OD>1 or ODR>0.80 where optical density ratio (ODR) = (OD sample – OD negative control)/ (OD positive control – OD negative control) in milk and serum, was present in the majority (milk: 81; serum: 87%) of the pooled samples. In a previous trial conducted on round-the-year pasture grazed lactating cows in Argentina, Mejia et al. (2011) demonstrated that the majority (89%) of the milk samples (undiluted) had high anti-*Ostertagia* antibody levels (ODR=0.88). The higher level of acquired immunity in older cattle grazing on pasture is due to repeated host-parasite interactions (Sanchez et al., 2004b). Eysker et al. (2002) indicated a significant positive relationship between IgG levels determined by a crude *Ostertagia ostertagi* ELISA in adult dairy cows and the number of infective larvae on pasture. This relationship could not be established in the present study. In this study, the serum OD peaked during late spring (late November) and then declined gradually with time. This could possibly be triggered by re-exposure of the host (lactating cows) to infective L3 larvae on pasture after a non-contact

period (approximately 8 weeks) of winter grazing on kale/fodder beet. Although the cause of the gradual decline of anti-*Ostertagia* antibody levels in serum over the trial period remains unclear (Figure 4.8), it was likely that L3 levels on pasture throughout the trial were insufficient to elicit a detectable humoral immune response in immunocompetent adult cattle. Milk OD did not decline gradually unlike serum OD but varied significantly ($p<0.01$) with time. Sanchez et al. (2004b) described that the correlation between antibodies measured in serum and milk of individual housed cows was around 0.5 as in our experiment and, hence, correction factors should be taken into account when comparing them. Charlier et al. (2009) pointed out that several factors, such as lactation stage, age, the presence of mastitis and serum antibody levels may play crucial roles in determining the milk antibody titre of which serum antibody titre is the most influential factor. Caffin et al. (1983) suggested that milk yield as a dilution factor causes variations in the milk IgG concentrations and therefore, higher concentrations of IgG might be expected towards the end of the lactation. The results of the present study showed that corrected milk OD (based on milk OD, adjusted for milk volume at each sampling time) decreased with progress of the lactation season (Figure 4.7) and was positively correlated with serum OD (HSE: $r=0.958$, $p<0.01$; LSE: $r=0.900$, $p<0.01$). The present study showed that there was a significant positive correlation between anti-*Ostertagia* antibody levels (OD) in serum and milk yield for both the HSE and LSE groups (Table 4.1). As discussed earlier, in both the treatment groups (HSE & LSE) the serum OD declined with time and this is likely to be due to a low level of infective larval ingestion leading to a decline in immunity over the lactation period. The milk yield declined with time following a typical lactation curve. A positive correlation between corrected milk OD and serum OD along with the typical lactation curve clearly indicated that the positive relationship between serum OD and milk yield was a reflection of the seasonal change in milk production rather than a direct link between them.

When animals in Experiment B were treated with eprinomectin pour-on (Eprinex; Merial, NZ) at the beginning of the trial (d-28), serum OD values decreased, presumably reflecting a decline in the resident worm population. This is also likely to reflect insufficient pasture larval challenge to maintain OD values, an effect which was compounded by the removal of the animals to a winter crop (kale) at day 16 of the trial. In Experiment A, the average L3 level on pasture was approximately 100 L3 per kg fresh herbage. Based on this, it was assumed that the animals in Experiment B were ingesting a maximum of 8000 L3 per day (100 L3/kg fresh herbage × 80 kg fresh herbage intake/day). The artificial infection dose of 50×10^3 L3/cow, therefore appeared to be a substantial addition for an artificial worm infection. However, the

artificial infection with additional infective larvae in two treatment groups (GII & GIII) had no effect on serum antibody titre. The single infection dose of 50×10^3 L3/cow may not have been adequate to cause a change in serum antibody titre. Foregoing this the assay seemed to detect antibodies to adult worms reflecting the fact that the crude antigens in the assay are derived from the adult *Ostertagia ostertagi* and it may have minor cross reactivity with infective larval forms. Those animals in GI that were not artificially infected with L3 larvae and kept as controls had an increase in anti-*Ostertagia* antibody levels after 48 days which likely reflects maturation of ingested infective L3 into adult worms on top of any remaining resident worm population. When artificial and natural infections in GII animals were artificially truncated with eprinomectin, any late rise in antibody levels was prevented. Similarly, the animals in GIII that had been artificially infected with L3 larvae once had insufficient time for ingested parasites to have matured into adults and be recognized by the immune system.

Charlier et al. (2009) stated that ELISA based on a crude worm extract might cross-react with other helminths. Overall in the study of animals with truncated infections (Experiment B), there was no significant relationship between anti-*Ostertagia* antibody levels (OD) in serum and worm burdens from the slaughtered adult cows. However, the results showed a negative correlation between anti-*Ostertagia* antibody levels (OD) in serum and total worm burden from the animals in Experiment A (Figure 5.2). As discussed previously crude antigens used in the *Ostertagia ostertagi*-Ab ELISA Kit (Svanova Biotech AB, Uppsala, Sweden) were extracted from the adult *Ostertagia ostertagi* parasites and possibly the ELISA could not detect the serum antibodies generated by the developing larval forms which comprised the large proportion of the total worm burden. As the majority of the worms were undifferentiated L3/arrested L4 forms it was impossible to assess cross reactivity with other genera. However, the interpretation of the results from this study must be made with caution as these data were collected from a very few animals and statistical significance was difficult to achieve in this regard.

In an earlier review from New Zealand, Brunsdon (1983) stated that high burdens of arrested *Ostertagia spp.* larvae (50–95%) had been recorded from adult cattle. Although the arrested L4 larvae (EL4) were not speciated in the present study, the proportion of L3/arrested L4 numbers of the total worm burden from Experiment A appeared to be high (up to 60.9%). The overseas data suggested that high percentage of hypobiosis (up to 90% EL4 during the winter season) could be observed in adult cattle throughout the year (Agneessens et al., 2000) and

may be attributed to a higher level of acquired immunity in adult cattle compared with young calves, which inhibits the development of ingested L3 into adult parasites. The animals in the HSE group ingested more infective L3 (119 L3 per kg fresh herbage) compared with the LSE group (93 L3 per kg fresh herbage) but, interestingly, the mean worm burden in the HSE group (4940) appeared to be less than the LSE group (13396). The infective L3 larvae may have failed to establish possibly due to the host's resistance being boosted by the feed supplement (1.5 kg crushed barley per day). The worm counts at necropsy showed that most of the animals from both the HSE and LSE groups (66.7%) had low worm burdens (range 600–4030) with the exception of 13,420 worms from one cow in the HSE and 34,080 worms from one cow in the LSE group (Appendix E). The low worm burdens in 66.7% dairy cows was similar to a previous study by Borgsteede et al. (2000), where it was reported that 76% dairy cows had low to moderate (100–10,000) worm burdens. The grazing season of the cows on the trial above, conducted in the Netherlands (Borgsteede et al., 2000) was only six months (May to October) and shorter than in New Zealand.

The level of larvae infectivity on the current pastures was low. Overall mean larval levels on pasture grazed only by adult lactating cows were 119 and 93 L3/kg fresh herbage for the HSE and LSE farmlets, respectively with a peak of 244 and 182 L3 per kg fresh herbage on old pasture (Figure 4.6) and these did not differ significantly during the trial period. Under New Zealand climatic conditions, pasture larval levels in the areas grazed by young calves could reach up to 12000 L3 per kg fresh herbage, particularly in late autumn or winter (Vlassoff, 1975). In contrast, the number of infective parasite larvae (L3) on pasture grazed only by adult cows has been reported to be very low (<180 L3/kg fresh herbage) in the North Island of New Zealand (Bisset, 1995). Low pasture larval contamination in the present study could be due to the fact that there was no history of young calves being grazed on the new and old pastures. However, it is noteworthy referring to the statement by Bisset (1994) that over-wintering a milking herd on a separate 'run off' has been a common practice in the majority of New Zealand dairy farms and adult cows may encounter relatively high levels of larval challenge during winter as these pastures have frequently been previously grazed by young stock . These low figures were similar to an observation by McAnulty (2012, pers. comm.) who noted a low larval contamination of pasture (<200 L3/kg fresh herbage, range 0–300) grazed by adult lactating cows in the South Island of New Zealand. Although low in numbers on the monitored paddocks, parasite larvae were recovered consistently from the old pasture (sown in April, 2009) compared with the new pasture (sown in April, 2011) for both the HSE and LSE groups (Figure 4.6). There was a significant difference ($p<0.05$) in larval

contamination between the old and new pastures reflecting the establishment times (September, 2009 and September, 2011, respectively). In this trial, these pronounced variations of pasture L3 levels could also be attributable to factors other than the age of the fodder or class of the stock, which may include seasonal changes in the environment (temperature, rainfall etc.) and pasture composition as such variables can alter larval survivability and availability to grazing ruminants for infection or re-infection. On the LURDF, the old pasture comprised tetraploid ryegrass with AR37 endophyte (Tet AR37) and white clover (WC) and the new pasture comprised arrow perennial ryegrass with AR1 endophyte (Arrow AR1) and diploid white clover (Dip WC). These pasture varieties have been developed in New Zealand. It is possible that the differences observed in pasture larval levels between the old and new pasture could be influenced by the two different pasture types, although this was not able to be substantiated from the current investigations.

In this study, mean FEC of both the HSE and LSE cows were consistently low (<100 eggs per gram) throughout the trial. The low FEC are likely to be due to the development of immune competence in adult dairy cows. This is in agreement with Michel (1968) who reported that host immunity can considerably affect worm ovulation and FEC. The necropsy results from Experiment A showed that more than 50% of total worm burden was composed of immature/inhibited worms, a major cause for the low FEC (Appendix E). The minimum detection limit of worm eggs in faeces from the modified McMaster method used in this study, was 50 eggs per gram (EPG) and, in adult cattle, a more sensitive technique such as the 'Wisconsin sugar floatation technique' (Cox & Todd, 1962) could be more appropriate. However, such techniques are not very convenient due to their labour intensive nature. The overall mean faecal egg counts over the trial period were 9.7 EPG in the HSE and 13.1 EPG in the LSE group (Figure 4.1) and these results were comparable with mean faecal egg counts of 15 EPG in adult cows previously reported in Canterbury, New Zealand by Guerra (1999). Host age is considered an important intrinsic factor for the development of acquired immunity to gastrointestinal parasites and host immunity affects parasite fecundity (Nansen, 1993; Vercruysse & Claerebout, 1997). Previous studies in the Netherlands (Borgsteede, 1978) and New Zealand (Guerra, 1999) documented that young cows (first calvers), had higher faecal egg counts compared to older cows. In the present study also, there was a trend (Figure 4.4) for a greater percentage of cows showing positive FEC in younger (cluster of 2- 4 years) than older cows (cluster of 5+ years) and, presumably this reflects either the time required for the development of immunity or the competition for nutrients between growth and development of immunity. In this experiment, neither the HSE nor the LSE group showed more than 20%

of cows with positive FEC (>80% FEC were negative) at any time point during the trial (Figure 4.3), which was similar to a previous study in Belgium conducted on dairy cows by Agneessens et al. (2000) where 86% were FEC negative. This was also comparable with another study in New Zealand by McPherson et al. (2001) where only 25.5% of adult dairy cows showed positive FEC.

Intermittent positive faecal egg counts were a feature of the present study. In the present study, only one cow (LSE) showed positive FEC on all eight sampling times during the trial (Table A.1, Appendix A). Intermittent worm egg shedding could possibly be due to the variable faecal consistency and faecal output over time. The faecal consistency was watery during early spring and dry in summer. The faecal consistency as well as the volume of faecal output varies with the seasonal variation in the forage quality and this could have an effect on the concentration of worm eggs in faeces. From the results of the FEC and worm counts at necropsy it could be stated that FEC appeared to be a poor indicator for the presence or level of parasite infection in adult cattle. The present study supports the findings of previous studies in New Zealand (Brunsdon, 1971), Belgium (Agneessens et al., 2000) and the Netherlands (Borgsteede et al., 2000) that mean FEC of adult dairy cows are generally low despite nearly all pastured cows in a herd being, presumably, infected with gastrointestinal nematode parasites up to some level.

Faecal egg counts (FEC) are a reflective rather than a predictive measurement of gastrointestinal nematode infection in ruminants (Litherland & Deighton, 2008) but may have little diagnostic value in bovine ostertagiasis. The principle behind this technique depends on the assumption that there is a definable relationship between worm egg shedding per gram of freshly voided faeces and total worm burdens. However, the validity of this assumption has been questioned over recent years with the growing awareness that FEC may be influenced by a number of variables such as the volume of faecal output, the fecundity of different worm species and host immunity (McKenna, 1997). Bovine ostertagiasis commonly occurs as a mixed infection mostly with *Trichostrongylus spp.* and *Cooperia spp.* under New Zealand climatic conditions and the host immunity may not have the same degree of effects on worm egg production from these nematode parasites as from *Ostertagia spp.* Therefore, in mixed infections, repeated FEC may provide more useful information on herd parasite status (Baker, 1988). The measure of FEC still remains a common laboratory technique in the diagnosis of gastrointestinal parasitism as it is cheap and easy to perform repeatedly without the need to sacrifice any livestock and appears likely to remain so in the absence of any suitable

alternatives. The results from the present study showed that percentage of cows with positive FEC was positively correlated with anti-*Ostertagia* antibody levels (OD) in serum (Figure 5.5) and may be a more convincing indicator for herd parasitism when compared with the level of worm eggs in the faeces of individual animals.

In Experiment A, it was evident (Table A.1, Appendix A) that most of the experimental animals showed negative FEC (<50 EPG) and, therefore, it was difficult to compare FEC data with pasture larval levels for the detection of any association between these two parameters. Hence, mean faecal egg counts of only FEC+ve cows were compared with mean pasture larvae levels on both the HSE and LSE farmlets. The results did not show any correlation ($r=0.054$, $p=0.932$) in the LSE group but showed a positive correlation ($r=0.868$, $p=0.056$) in the HSE group. The larval counts conducted only over one season is a shortcoming of the study, and it would have been a more meaningful quantitative estimation if continued to the following lactation. The animals in the HSE group, due to the high stocking rate, grazed pasture closer to the soil surface leaving a lower grazing residual (1450kg DM/ha) compared with the LSE group (1600kg DM/ha). This may support Vlassoff's (1982) observation that ruminants that grazed pasture closer to the soil surface ingested more infective L3 larvae as they were concentrated on the first 2 cm (from soil) of plants. In an overseas report by Eysker and Meurs (1982), it was stated that an adult cow with an estimated faecal excretion of 30 kg and a low mean value of 5 worm eggs per gram of faeces (5 EPG), produced a considerable level of contamination (150,000 worm eggs/cow/day). Considering only 10% of these worm eggs develop into infective L3 larvae, the resultant pasture larval contamination for a stocking rate of 3.5 cows/ha would be 52,500 L3/ha/day. Therefore, despite their low faecal egg output adult cows may produce a high level of pasture larval contamination. However, an in-depth study of nematode epidemiology in New Zealand cattle is needed before making any remarks between the relationship of FEC and infective L3 larvae levels on the pastures grazed by adult cattle.

Individual daily milk yield (L/day) was recorded from the HSE and LSE cows during the trial (Table B., Appendix B) and an attempt was made to investigate any negative effect of FEC on milk production. In this trial, it was evident that the measured FEC had no significant association with milk yield. As discussed earlier, FEC appeared to be a poor indicator of subclinical worm infection in adult cattle and therefore, may not be able to provide useful information on parasite-induced production losses. Perri et al. (2011) demonstrated that high FEC, especially around peripartum period caused loss of milk production. In contrast, the

results of the current experiment are in agreement with Barger and Gibbs (1981), who suggested that there was no apparent relationship between FEC and milk production from housed cows.

In summary, the infective larval infestation of pasture grazed only by adult cattle seems to be low under New Zealand conditions. The results of the present study indicate that milk OD needs to be corrected for milk yield to be positively correlated with serum OD. There was no consistent relationship between anti-*Ostertagia* antibody levels in serum and mature worm burdens from a small subset of slaughtered animals. Hence, at the present time it is premature to recommend the use of the ELISA kit, with diluted milk samples using a correction factor, as a routine diagnostic procedure for GI parasitism in intensively grazed adult cattle.

Chapter 6
Conclusion and scope for future research

This thesis reports on a study that was undertaken to test the hypothesis that worm burden or the level of challenge with infective third-stage larvae (L3) is linked to serum or milk antibody levels, faecal egg counts (FEC) or milk yield. A secondary objective of this study was to identify any influence of high or low stocking density grazing on gastrointestinal worm infections in dairy cows. While a link between serum antibodies (ELISA) to GI parasite (*O. ostertagi*) antigen and worm burden, rather than intake of infective larvae, seemed likely, this could not be confirmed from the present study due to a few numbers of animals (17 cull cows) being sacrificed for post-mortem worm assessment (small sample size within each treatment). There was no significant relationship between pasture L3 levels, milk yield or mean FEC. The mean FEC was negatively correlated with antibodies to gastrointestinal parasites in milk in pasture-fed/unsupplemented animals. The use of individual FEC as an indicator of gastrointestinal parasitism in adult cows has to be questioned. The study did not reveal any significant difference between the HSE and LSE groups with regard to FEC, infective L3 levels on pasture, anti-*Ostertagia* antibody levels in milk or serum and mean worm burdens. Forbes et al. (2008) reported that even a modest access to the outside exercise yard or paddock resulted in cattle being exposed to *Ostertagia ostertagi*, manifested by high antibody levels in milk. In New Zealand, dairy cows are grazed on pasture year-round, unlike Europe or North America where dairy herds are kept in 'total confinement' or 'semi confinement'. This continual exposure to nematode parasites on pasture has led to our research animals (and presumably other New Zealand dairy herds) having high anti-*Ostertagia* antibody levels in milk or serum which necessitates the use of higher dilutions in the ELISA assay than that recommended by the manufacturer. Intensive dairy farming across New Zealand is growing at a rapid rate and a clear understanding of gastrointestinal parasitism in pasture grazed cattle is paramount in order to use anthelmintics sustainably and continue dairy trade profitably.

In conclusion, the use of bulk-tank milk (BTM) antibody levels to predict GI parasite burdens or the usefulness of anthelmintic treatment in New Zealand dairy herds needs to be modified and/or validated. From the present study, it appears that the difference in stocking rate has no obvious impact on gastrointestinal worm infection in adult dairy cows. However, our studies have limitations and further research on the use of diagnostic indicators in pasture-grazed New Zealand dairy herds is recommended.

Chapter 7
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Appendix A

Faecal egg counts (FEC)

Table A.1 FEC from Experiment A

Cow ID#	Groups	Age	Breed	EPG[Oct]	EPG[Nov]	EPG[Dec]	EPG[Jan]	EPG[Feb]	EPG[Mar]	EPG[Apr]	EPG[May]
38	HSE	3	J8 F6	50	100	0	0	0	0	0	0
53	HSE	5	J	0	0	0	0	0	0	50	0
64	HSE	5	F8 J5	0	0	0	0	0	0	0	0
74	HSE	2	F	0	50	0	0	0	0	0	0
77	HSE	7	F	0	0	0	0	0	0	0	0
78	HSE	5	F7 J1	0	0	0	0	0	0	0	0
87	HSE	8	F11J5	0	0	100	100	0	0	0	50
90	HSE	6	F10J6	0	0	0	0	0	0	0	*
96	HSE	2	J12F4	0	0	0	0	0	0	0	50
99	HSE	10	F6	0	0	0	0	0	0	0	0
102	HSE	2	F	0	0	0	0	0	0	0	0
104	HSE	2	F	0	0	0	0	0	50	0	50
106	HSE	2	F	0	0	50	50	50	100	0	0
107	HSE	2	F	0	0	50	*	0	0	0	*
110	HSE	2	F	0	0	0	0	0	0	0	0
114	HSE	2	F	50	150	0	50	0	150	0	0
116	HSE	3	F	0	0	50	0	0	0	0	*
117	HSE	3	F12J4	0	0	0	0	0	0	0	0
118	HSE	3	F10J6	0	0	0	0	0	0	0	0
119	HSE	3	F	0	0	100	0	0	0	0	0
120	HSE	3	F12J4	0	0	0	0	0	0	0	0
123	HSE	7	F	0	0	0	100	0	0	0	0
126	HSE	8	F	0	0	0	0	0	0	50	0
128	HSE	6	F	0	0	0	0	0	0	0	0
129	HSE	4	F	0	0	0	0	0	0	0	0
135	HSE	4	F	50	150	0	0	50	0	0	0
136	HSE	3	F	0	0	0	0	0	0	0	*
137	HSE	3	F	0	0	0	50	0	0	0	*

* Sample missed, HSE-high stock efficient, LSE-low stock efficient,
F- Friesian, J-Jersey, EPG- eggs per gram,

Cow ID#	Groups	Age	Breed	EPG[Oct]	EPG[Nov]	EPG[Dec]	EPG[Jan]	EPG[Feb]	EPG[Mar]	EPG[Apr]	EPG[May]
138	HSE	3	F	0	0	0	0	50	0	0	0
141	HSE	5	F13J3	0	0	0	0	0	0	0	0
143	HSE	4	F13J3	0	0	0	0	0	50	50	0
5	LSE	3	J10F6	100	50	50	150	50	50	50	50
8	LSE	5	F12J4	0	0	0	0	0	0	0	*
21	LSE	5	F8 J7	0	0	0	0	0	0	0	0
25	LSE	3	F	0	0	0	0	0	0	0	0
51	LSE	5	F12S4	0	0	0	0	0	0	0	0
61	LSE	5	F9 J7	0	0	0	0	0	0	0	0
72	LSE	8	F	0	0	0	0	0	0	0	0
76	LSE	5	F11J5	50	0	50	50	0	0	50	0
81	LSE	3	F13J3	0	0	0	50	50	50	0	0
88	LSE	2	J13F3	0	0	0	0	0	0	0	0
103	LSE	2	F	0	0	0	0	0	0	0	0
105	LSE	2	F	50	0	0	100	0	100	100	*
108	LSE	2	F	0	0	0	0	0	100	0	0
109	LSE	2	F	0	0	0	0	0	0	0	*
111	LSE	2	F	0	0	0	0	50	0	50	0
112	LSE	2	F	100	50	50	0	100	100	150	0
113	LSE	2	F	0	0	0	0	0	0	0	0
115	LSE	3	F	0	0	100	0	100	0	0	50
122	LSE	3	F12J4	0	0	0	0	100	0	50	*
124	LSE	3	F12J3	0	0	0	50	0	0	0	0
125	LSE	7	F	0	0	0	0	0	0	0	100
127	LSE	9	F	0	0	0	0	0	0	0	0
130	LSE	3	F	0	0	0	0	0	0	0	*
131	LSE	3	F	0	0	0	0	0	0	0	0
132	LSE	3	F	0	0	0	0	0	0	50	0
133	LSE	7	F	0	0	0	0	0	0	0	50
134	LSE	4	F	100	150	0	0	0	0	0	0
139	LSE	5	F13J3	50	100	*	*	0	0	0	*
140	LSE	5	F	0	0	0	0	0	0	0	0
142	LSE	4	F13J2	0	0	0	0	0	0	0	*

* Sample missed, HSE-high stock efficient, LSE-low stock efficient,
F- Friesian, J-Jersey, EPG- eggs per gram,

Table A.2 FEC from Experiment B

Cow ID#	Treat Groups	EPG1 (d-28)	EPG2 (d0)	EPG3 (d10)	EPG4 (d38)	EPG5 (d48)
4	Group I (C)	0	0	0	0	0
6	Group I (C)	0	0	0	0	0
12	Group I (C)	0	0	0	0	0
16	Group I (C)	0	0	0	0	0
19	Group I (C)	0	0	0	0	0
26	Group I (C)	0	0	0	0	0
27	Group I (C)	0	0	0	0	0
28	Group I (C)	100	0	0	0	0
31	Group I (C)	0	0	0	0	0
39	Group I (C)	0	0	0	0	0
40	Group I (C)	0	0	0	0	0
45	Group I (C)	0	0	0	0	0
57	Group I (C)	0	0	0	0	0
60	Group I (C)	50	0	0	0	0
62	Group I (C)	0	0	0	0	0
69	Group I (C)	0	0	0	0	0
80	Group I (C)	0	0	0	0	0
92	Group I (C)	0	0	0	0	0
93	Group I (C)	0	0	0	0	0
97	Group I (C)	50	0	0	0	0
1	Group II	0	0	0	0	0
7	Group II	0	0	0	0	0
10	Group II	*	0	0	0	0
17	Group II	0	0	0	0	0
23	Group II	0	0	0	0	0
29	Group II	0	0	0	0	0
30	Group II	0	50	50	0	0
32	Group II	0	0	0	0	0
34	Group II	0	0	0	0	0
35	Group II	0	50	100	0	0

d-28: 28 days prior to artificial worm challenge, d0: time of artificial worm challenge, d10: 10 days post challenge, d38: 38 days post challenge, d48: 48 days post challenge.
* Sample missed, C: control.

Cow ID#	Treat Groups	EPG1 (d-28)	EPG2 (d0)	EPG3 (d10)	EPG4 (d38)	EPG5 (d48)
41	Group II	0	0	0	0	0
44	Group II	0	0	0	0	0
47	Group II	0	0	0	0	0
49	Group II	0	0	0	0	0
54	Group II	0	0	0	0	0
58	Group II	100	0	0	0	0
73	Group II	0	0	0	0	0
75	Group II	0	0	0	0	0
86	Group II	0	0	0	0	0
100	Group II	0	0	0	0	0
15	Group III	0	0	0	0	0
18	Group III	0	0	0	0	50
22	Group III	0	0	0	0	0
24	Group III	0	0	0	50	0
33	Group III	0	0	0	0	0
37	Group III	0	0	0	0	0
43	Group III	100	50	0	100	0
46	Group III	0	0	0	0	0
48	Group III	0	0	0	0	0
50	Group III	0	0	0	0	0
52	Group III	0	50	0	0	0
55	Group III	0	0	0	0	0
63	Group III	0	0	0	0	0
66	Group III	0	0	0	0	0
67	Group III	0	0	0	0	0
89	Group III	0	0	0	0	0
91	Group III	50	0	0	0	0
98	Group III	0	0	0	0	0
101	Group III	0	0	0	0	0
121	Group III	*	*	0	0	0

d-28: 28 days prior to artificial worm challenge, d0: time of artificial worm challenge, d10: 10 days post challenge, d38: 38 days post challenge, d48: 48 days post challenge.
* Sample missed.

Appendix B

Milk yield

Table B. Daily milk yield (L/d) of HSE and LSE cows during the trial

Cow ID	Herd	Milk (L/d)	Milk (L/d)	Milk (L/d)	Milk (L/d)	Milk (L/d)	Milk (L/d)	Milk (L/d)	Milk (L/d)
		Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
5	LSE	26.64	26.82	24.99	22.27	20.19	16.34	13.84	9.45
8	LSE	27.37	26.04	24.94	21.72	20.47	17.49	14.71	10.97
21	LSE	26.35	24.73	25.59	23.36	22.95	20.93	18.32	14.58
25	LSE	31.01	29.44	26.44	24.38	24.03	20.51	17.71	12.85
36	HSE	29.29	23.26	20.33	20.05	19.54	17.02	14.91	10.12
38	HSE	23.61	22.04	19.41	18.26	15.43	13.26	11.59	7.91
51	LSE	31.59	29.56	27.24	22.36	20.67	18.54	16.01	12.25
53	HSE	26.64	29.20	26.91	23.65	21.03	18.12	17.70	13.58
61	LSE	31.13	30.62	29.65	27.31	27.46	26.01	23.11	18.49
64	HSE	22.67	20.69	20.52	19.70	18.40	16.01	15.18	10.85
72	LSE	33.96	30.53	27.24	23.86	23.72	20.99	18.73	14.47
74	HSE	16.39	16.40	14.86	14.09	12.51	11.35	9.91	7.06
76	LSE	31.11	30.42	27.91	26.75	25.58	22.52	20.18	16.31
77	HSE	28.20	28.64	26.32	23.05	19.77	17.44	16.13	11.44
78	HSE	28.99	28.42	27.27	24.63	21.89	20.33	19.76	15.67
81	LSE	22.13	21.47	20.25	19.00	18.29	16.53	14.84	11.30
82	HSE	28.53	30.16	30.30	28.35	25.91	20.87	19.48	16.83
87	HSE	32.93	32.05	28.68	26.46	24.14	22.60	21.09	14.18
88	LSE	15.69	15.32	14.59	12.87	11.75	10.70	9.33	7.40
90	HSE	33.48	31.76	27.76	25.96	23.34	20.68	-	-
96	HSE	17.85	16.81	16.74	16.90	14.50	13.31	12.35	9.58
99	HSE	23.87	23.75	22.53	20.47	18.40	15.85	15.09	10.91
102	HSE	24.19	21.91	20.31	17.72	16.88	14.93	15.11	11.87
103	LSE	22.77	22.00	20.43	18.23	19.32	18.50	16.49	12.35
104	HSE	20.94	20.34	19.72	17.46	15.67	12.92	12.41	10.71
105	LSE	26.72	23.54	22.46	21.31	20.57	20.55	19.20	
106	HSE	16.42	18.01	17.79	16.95	14.94	14.34	13.84	10.03
107	HSE	24.43	23.61	21.93	20.50	18.81	17.73	16.94	
108	LSE	21.56	20.75	20.03	18.05	17.73	16.92	15.45	12.21
109	LSE	21.24	19.38	16.82	10.18	13.37	12.35	12.55	-
110	HSE	21.78	21.29	19.12	17.06	16.05	14.79	-	-
111	LSE	22.02	21.76	21.37	19.80	19.98	18.00	16.39	12.67
112	LSE	21.37	22.27	22.49	19.07	19.28	17.76	16.54	13.34
113	LSE	18.87	17.62	17.14	15.53	16.07	14.90	13.30	9.63
114	HSE	21.95	18.90	18.39	16.92	14.76	14.37	-	-
115	LSE	23.05	23.03	22.62	20.43	20.24	19.29	16.67	13.37
116	HSE	21.72	22.22	18.29	19.92	18.22	16.45	-	-
117	HSE	29.68	28.22	26.02	23.02	21.22	18.68	18.07	15.91
118	HSE	26.14	25.02	24.18	21.91	19.24	16.46	15.88	11.56

L/d- Litre per day

Cow ID	Herd	Milk (L/d)	Milk (L/d)	Milk (L/d)	Milk (L/d)	Milk (L/d)	Milk (L/d)	Milk (L/d)	Milk (L/d)
119	HSE	23.22	23.58	22.59	21.70	20.04	18.85	17.90	14.16
120	HSE	27.08	25.17	22.70	20.65	19.18	17.31	17.41	12.94
121	HSE	16.95	16.96	16.74	16.12	14.92	13.43	12.30	9.94
122	LSE	25.23	24.56	24.12	22.77	22.58	22.04	18.81	-
123	HSE	32.31	32.76	30.66	26.97	24.02	20.67	20.68	15.48
124	LSE		29.03	31.40	28.38	27.07	24.87	22.04	17.24
125	LSE	25.29	23.10	22.19	20.39	19.80	17.14	15.50	12.15
126	HSE	30.45	30.45	28.33	26.51	23.39	21.49	19.81	14.99
127	LSE	22.84	22.77	22.50	20.94	20.17	18.11	16.08	12.73
128	HSE	27.12	26.10	23.16	20.77	17.56	14.57	14.12	11.07
129	HSE	25.82	24.45	22.98	21.87	20.46	18.89	17.56	10.45
130	LSE	24.12	23.80	23.12	21.41	21.70	19.39	17.17	16.44
131	LSE	26.22	28.72	27.33	23.38	23.28	21.45	18.40	14.49
132	LSE	24.31	22.94	22.59	20.96	21.31	20.34	17.90	13.87
133	LSE	25.01	23.63	22.12	20.35	19.44	17.12	15.04	11.82
134	LSE	29.53	27.10	27.06	25.47	25.09	23.38	21.56	17.70
135	HSE	27.56	26.11	23.83	22.36	20.27	17.28	17.16	12.13
136	HSE	28.69	27.33	22.49	21.94	19.64	17.25	-	-
137	HSE	22.95	22.96	21.55	20.71	18.61	16.70	16.39	-
138	HSE	-	28.32	27.45	24.66	21.34	18.22	17.47	12.80
140	LSE	31.63	30.57	29.46	25.76	23.73	20.95	17.43	12.96
141	HSE	32.13	31.89	28.67	25.02	21.54	20.17	19.94	15.60
142	LSE	30.43	29.24	29.66	27.23	27.12	24.10	21.53	19.57
143	HSE	-	23.67	26.94	25.00	22.35	19.55	19.10	16.38

L/d- Litre per day

Appendix C

Infective nematode larvae (L3) on pasture

Table C. Pasture L3 larvae levels

Month	Paddock ID#											
	B4c (LSE, np)			B6d (HSE, np)			E2d (LSE, op)			D2a (HSE, op)		
	sampling date (SD)	FH (g)	L3/ kg FH	SD	FH (g)	L3/ kg FH	SD	FH (g)	L3/ kg FH	SD	FH (g)	L3/ kg FH
Nov, 2011	22/11/11	222	0	22/11/11	229	0	-	-	-	-	-	-
Dec, 2011	7/12/11& 27/12/11	270 & 242	0	7/12/11 & 29/12/11	268 & 280	0	2/12/11 & 23/12/11	237 & 253	42.2 & 79.1	2/12/11 & 22/12/11	255 & 236	117.6 & 42.4
Jan, 2012	12/01/12 & 26/01/12	298 & 227	0	12/01/12	280	0	18/01/12	284	35.2	26/01/12	278	35.6
Feb, 2012	13/02/12	255	0	1/02/12 & 27/02/12	229 & 247	0 & 40.5	14/02/2012	274	182.5	15/02/2012	286	244.8
Mar, 2012	2/03/12 & 24/03/12	292 & 259	0	19/03/12	273	0	5/03/12 & 25/03/12	255 & 258	117.6 & 77.5	5/03/12 & 25/03/12	260 & 277	115.4 & 72.2
Apr, 2012	15/04/12	236	0	15/04/12	269	0	3/04/12	274	109.5	6/04/12	300	66.7
May, 2012	-	-	-	-	-	-	5/05/12	256	78.1	10/05/2012	255	196.1

LSE- low stock efficient, HSE-high stock efficient, np- new pasture, op- old pasture, SD- sampling date, FH- fresh herbage, L3- third-stage larvae, g- gram, Kg- kilogram

Appendix D

ELISA assay

Table D.1 ELISA data (OD value) of pooled serum and milk samples from Exp. A

Protocol:				
C +ve	HB Dec	HB Apr	HM Dec	HM Apr
C +ve	LB Dec	LB Apr	LM Dec	LM Apr
C –ve	HB Jan	HB May	HM Jan	HM May
C –ve	LB Jan	LB May	LM Jan	LM May
HB Oct	HB Feb	HM Oct	HM Feb	C +ve
LB Oct	LB Feb	LM Oct	LM Feb	C +ve
HB Nov	HB Mar	HM Nov	HM Mar	C –ve
LB Nov	LB Mar	LM Nov	LM Mar	C –ve
Original assay, serum dilution used 1:512, milk dilution used 1:16				
1.5437	1.4233	0.9548	1.3625	0.9294
1.5047	1.3854	1.0704	1.2747	0.8647
0.2352	1.3847	1.0231	1.1042	1.2210
0.2251	1.3157	0.8620	0.9598	1.2197
1.3782	1.2438	1.0034	1.1339	1.5633
1.2810	1.2547	1.0483	1.0470	1.5476
1.5589	1.1451	1.2897	1.1332	0.2193
1.6025	1.1359	1.3877	1.0364	0.2100
Duplicate assay, serum dilution used 1:512, milk dilution used 1:16				
1.5915	1.4255	0.9741	1.3597	0.9484
1.5452	1.3945	1.0625	1.2834	0.8526
0.2173	1.3672	1.0231	1.1179	1.2197
0.2197	1.3276	0.8792	0.9510	1.1568
1.4023	1.2027	1.0121	1.1433	1.5763
1.3362	1.2617	1.0538	1.0394	1.5426
1.5829	1.1541	1.3034	1.1531	0.2242
1.6113	1.1446	1.3927	1.0634	0.2210

C+ positive control, C- negative control, HB: HSE pooled blood, LB: LSE pooled blood, HM: HSE pooled milk, LM: LSE pooled milk, OD: optical density, Exp. A- Experiment A

Table D. 2 ELISA data (OD value) of pooled serum samples from Exp. B

Protocol:				
C+	d-28 GIII	d10 GI	d38 GII	d48 GIII
C+	d-28 GIII	d10 GI	d38 GII	d48 GIII
C-	d0 GI	d10 GII	d38 GIII	
C-	d0 GI	d10 GII	d38 GIII	
d-28 GI	d0 GII	d10 GIII	d48 GI	
d-28 GI	d0 GII	d10 GIII	d48 GI	
d-28 GII	d0 GIII	d38 GI	d48 GII	
d-28 GII	d0 GIII	d38 GI	d48 GII	
Serum dilution used 1:512, sample run in duplicate				
1.6845	1.2015	0.7224	0.6159	0.6397
1.7326	1.1666	0.8027	0.6142	0.6639
0.2685	0.8538	0.8389	0.7372	
0.2709	0.8551	0.8492	0.7105	
1.0506	0.8343	0.8840	0.8038	
1.1867	0.8175	0.8519	0.8169	
1.2390	0.9505	0.6249	0.5649	
1.2032	0.9679	0.6580	0.5978	

C+ positive control, C- negative control, GI: group I, GII: group II, GIII: group III, d-28: 28 days prior to artificial infection, d0: day zero of artificial infection, d10: 10 days post infection, d38: 38 days post infection, d48: 48 days post infection, OD: optical density, Exp. B – Experiment B

Table D. 3 ELISA data (OD value) of final (2) serum samples from individual slaughtered animals (from Exp. A & Exp. B)

Protocol:				
C+	116 (HSE)	62 (GI)	75 (GII)	52 (GIII)
C+	116 (HSE)	62 (GI)	75 (GII)	52 (GIII)
C-	72 (LSE)	93 (GI)	86 (GII)	91 (GIII)
C-	72 (LSE)	93 (GI)	86 (GII)	91 (GIII)
110 (HSE)	105 (LSE)	44 (GII)	33 (GIII)	98 (GIII)
110 (HSE)	105 (LSE)	44 (GII)	33 (GIII)	98 (GIII)
114 (HSE)	109 (LSE)	54 (GII)	48 (GIII)	C+
114 (HSE)	109 (LSE)	54 (GII)	48 (GIII)	C-
Serum dilution used 1:512, sample run in duplicate				
2.0200	1.6405	0.6212	1.1514	0.6135
1.8250	1.6623	0.6109	1.0950	0.5730
0.1781	1.9862	1.7044	0.4661	0.8397
0.1848	1.9391	1.5978	0.4902	0.8688
2.0065	0.7961	0.6462	1.1978	0.5234
2.0265	0.8225	0.6414	1.1029	0.4129
1.7531	1.2459	0.6713	0.6003	2.0046
1.8224	1.1392	0.6668	0.5849	0.1882

C+ positive control, C- negative control, HSE: high stock efficient, LSE: low stock efficient, GI: group I, GII: group II, GIII: group III, OD: optical density. Exp. A: HSE & LSE; Exp. B: GI, GII & GIII.

Appendix E

Worm counts at necropsy

Table E. Quantification of L4/L5 (* differentiated worm genera) and L3/EL4 (undifferentiated) worms**

Cow ID#	Group	*Ostertagia**				*Trichostrongylus**				*Cooperia**				L3/EL4 (**)	Total worm burden
		Ab. wash	Ab. saline incub.	SI wash	SI saline incub.	Ab. wash	Ab. saline incub.	SI wash	SI saline incub.	Ab. wash	Ab. saline incub.	SI wash	SI saline incub.		
110	HSE	200	0	0	-	100	0	0	-	0	0	0	-	500	800
114	HSE	0	0	0	-	0	0	0	-	0	0	0	-	600	600
116	HSE	1100	5990	0	0	0	260	0	0	0	0	0	0	6070	13420
72	LSE	600	130	0	-	200	90	0	-	0	0	0	-	1060	2080
105	LSE	1700	4090	0	100	5900	1580	100	300	0	0	200	200	19910	34080
109	LSE	0	260	0	0	0	260	0	0	0	0	0	0	3510	4030
62	GI	1400	1330	0	200	0	40	100	0	0	0	100	100	13760	17030
93	GI	0	70	0	0	0	0	0	400	0	0	0	500	1940	2910
44	GII	200	170	0	0	100	600	0	60	0	0	900	0	850	2880
54	GII	700	40	0	0	0	90	0	0	0	0	0	0	1060	1890
75	GII	200	100	0	0	0	100	0	100	0	0	0	100	1000	1600
86	GII	700	950	0	0	100	50	0	400	0	0	0	0	5100	7300
33	GIII	400	290	0	-	400	70	0	-	0	0	300	-	3720	5180
48	GIII	100	100	0	0	0	10	0	100	0	0	0	0	2800	3110
52	GIII	0	0	0	0	0	0	0	100	0	0	0		0	100
91	GIII	0	270	0	0	0	0	0	100	0	0	100		3100	3570
98	GIII	600	190	0	-	600	50	0	-	0	0	400	-	10720	12560

Ab. Abomasum, SI-Small intestine, incub.-incubation, HSE- High stock efficient, LSE- Low stock efficient, GI-Group I, GII- Group II, GIII- Group III, L3- Infective third-stage larvae, L4- Fourth–stage larvae, L5- young-adult worm, EL4- Arrested fourth-stage larvae