EFFECT OF SUCKLING ON RESPONSE TO NEMATODE PARASITES IN YOUNG LAMBS

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Abstract

The series of experiments described in this thesis were designed to investigate the role of suckling or late weaning in the response of young lambs to nematode infection. All experiments were conducted outdoors with grazing animals and no supplementation but for suckled groups of lambs whose counterparts were weaned to ryegrass – white clover swards. The parasite of interest was mainly *Teladorsagia circumcincta* solely but with mixed infection of *Trichostrongylus colubriformis* in one instance.

In Chapter 3 (first experiment), the hypothesis that milk *per se* may have a direct effect on nematode development, rather than an indirect effect through enhancement of host immunity by superior nutrient supply was tested. Sixty, twinborn lambs were used, allocated to one of eight groups formed by either dosing lambs from 42 days of age or not with the equivalent of 1000 or 250 L_3 *T. circumcincta* larvae d^{-1} until five days before necropsy, while a twin was either weaned at 39 days of age, suckled as single or twin until necropsy on day 84. The possibility that weaning one of a twin set onto pasture in close proximity to the ewe would cause abnormal ewe and lamb behaviour was tested by replicating the work in twins maintained as twins but in which one twin received equivalent of 250 and the other 1000 L_3 *T. circumcincta* larvae d^{-1}. This showed no abnormal ewe nursing or lamb suckling behaviour as a result of

weaning a twin in a set. Relatively low faecal egg counts (FEC) and a two to three fold lower worm burdens suggest suckling could reduce larval establishment. Inability to detect peripheral titres of immunoglobulins supports this conclusion. An intra worm-population regulation of *T. circumcincta*, indicated by a pattern of greater egg-laying by a numerically smaller but physiologically better developed nematode population in suckled lambs measured in eggs *'in utero'* and worm length made interpretation of FEC difficult. Suckling significantly improved weight gain and carcass weights, but early weaning did not reduce resilience to infection.

In Chapter 4 (second experiment), 40 pairs of twin lambs, average age of 39 days, were either infected with the equivalent of 1000 L_3 *T. circumcincta* larvae d^{-1} or not, while one twin was weaned and the other allowed to continue suckling. Necropsy was carried out on groups of five and six lambs from each of the uninfected and infected treatments, respectively, at mean age of 84, 112, and on six lambs from each group at 140 days of age. This serial slaughter allowed further confirmation of the hypothesis in Chapter 3 but also investigated the long-term effect of suckling on resistance or resilience of lambs at the trial when immune responses were anticipated to be developing. An *in vitro* direct larval challenge (IVDC) study, to monitor larval establishment, was carried out on tissue explants from necropsied lambs. Suckled lambs consistently showed lower FEC ($P < 0.05$) and worm burdens ($P < 0.05$) at every phase of the trial. Within the infected groups, *% in vitro* larval rejection suggested earlier immune responses in the weaned lambs by day 84, which was not consistent with lower worm burdens in suckled lambs but appeared similar in the subsequent necropsies. Lambs continued to show better growth due to suckling while weaning did not reduce the resilience of lambs confirming observations in Chapter 3.

The immunoglobulin profile suggested the commencement of immune responses in lambs from the period after the 84th day necropsy, with significantly greater ($P < 0.01$) IgA titre in the infected groups, and the suckled lambs towards the end of the trial on day 140. A vaccinating effect of early exposure to parasites was coincidentally revealed as a result of unintentional pasture larval contamination, seen in suckled non-infected lambs shedding fewer eggs and harbouring fewer worms during the later necropsies compared with their weaned non-infected counterparts.

In Chapter 5 (third trial), 93 pairs of twin lambs, 47 pairs of which received a vaccinating mixed infection of *T. circumcincta* and *T. colubriformis* larvae (60 L_3 / kg W / d) at ratio 40:60, respectively during the period 36 – 103 days of age, were either weaned early on day 51 or later on day 108. All lambs were drenched on day 108 and groups received challenge infections from day 116, at same rate with the vaccinating infection, or not, which ceased five days before respective necropsies. Necropsies were carried out on selected lambs on days 108, 184 and 218. The direct effect of milk on larval establishment appeared to feature only in the *T. circumcincta* populations on slaughter day 108. The long-term benefit of late weaning for development of resistance was conditional on lambs receiving the vaccinating infection, and appeared to be more pronounced in the small intestine, reflected by a greater reduction of *T. colubriformis* populations in that organ than of *T. circumcincta* populations in the abomasum. A negative consequence of enhanced immune response was the suggestion of an increased metabolic cost in reduced performance of lambs.

In conclusion, the work provides support to the hypotheses that: (a.) suckling may reduce the establishment of nematode larvae through the direct effect of milk, (b.) may

enhance rapid development of host immunity to infection, and (c.) it further suggests that lack of larval experience during suckling may have long term negative implications for host resistance. Finally, it suggests that milk may play little role in the enhancement of host resilience to infection and, on the contrary, that additional metabolic cost may be associated with a more rapid development of immunity resulting from larval challenge while suckling.

Keywords: Lambs, weaning, suckling, milk, resistance, resilience, nematode *Teladorsagia circumcincta*, *Trichostrongylus colubriformis*, IgA, IgM, antibody, abomasum, small intestine *in vitro* larval rejection

Dedications

In the Name of Allah, the Most Gracious, the Most Merciful

"And verily! In the cattle, there is a lesson for you. We give you to drink of that which is in their bellies, from between excretions and blood, pure MILK; palatable to the drinkers" - Al-Quran - Surat-ul-Nahl :66 (Chapter 16 verse 66)

This work is dedicated to my Creator, ***ALLAH***, Glory to Him in the Most High,

and

to the memories of my late Dad, Shefiu Olatunde Iposu and his late friend, Olatunde Sosanya, may Allah bless their souls

and

to those who fondly refer to me as 'Prof', may your wishes come to pass☺

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Chapter 1

General Introduction

A range of nematode species of the gastrointestinal (GI) tract of grazing ruminants are known to cause colossal losses to economies that have ruminant stock as their mainstay. In Kenya, haemonchosis causes an annual loss of US$26 million in sheep and goats, and returns could be increased by as much as 470% by controlling the disease (Maichomo *et al.* 2004). Approximately $1.7 billion are spent annually throughout the world, in an effort to reduce the deleterious effects of helminth parasites (Lanusse & Prichard 1993). With a sheep population of over 40 million and being the world biggest exporter of lamb, the problem of production losses posed by various GI nematode infections should be a major issue for New Zealand economy, and has been estimated to cost more than NZ$350 Million annually in lost production and anthelmintic costs (Rattray 2003).

Several options are open for coping with the problem of nematode parasitism, and have been the subject of research over the years. These include genetic selection for sheep with greater ability to cope with worm challenge (Morris *et al.* 2004; Davies *et al.* 2005; McEwan 2006); vaccination (Stankiewicz *et al.* 2000; Hein & Harrison 2005); biological control of free-living larval stages (Niezen *et al.* 1996) or parasitic stages (Waller *et al.* 2004); adopting various grazing-management strategies that are aimed at reducing contact between the host animal and the infective stages of the parasite on pasture (Barger 1999; Coop & Sykes 2002; Healey *et al.* 2004); feeding plants that contain condensed tannins (Niezen *et al.* 2002a; Tzamaloukas *et al.* 2006); anthelmintic control or drenching (Leathwick *et al.* 2001; Sutherland *et al.* 2003;

Sargison *et al.* 2005); and nutritional modulations, especially with respect to protein (Coop *et al.* 1995; Sykes & Coop 2001; Walkden-Brown & Kahn 2002; Steel 2003; Houdijk *et al.* 2005). While these approaches have recorded some success, these have been mostly under experimental conditions; their successful long term application to field situations is still not assured. Notably, the continued use of anthelmintics is becoming unsustainable, as nematode resistance to the three main classes of broad-spectrum anthelmintics is now common in small ruminants throughout many areas of the world (Pomroy & Whelan 1993; Waller 1997; Hughes *et al.* 2004; Sargison *et al.* 2005). The increasing popularity of organic farming and the consumer concern for chemical residues in foodstuff are making chemical approaches to parasite control less popular.

The need to develop sustainable approaches to parasite control has generated interest into research on the possibility of using nutritional interventions, among other strategies, to complement and therefore reduce the heavy reliance on chemical control. Nutritional interventions are usually aimed at either boosting the natural immunity or resistance of the host to infection, or enhancing their resilience in the face of exposure to infection. These two phenomena, "*Resistance*" and "*Resilience*", represent the desirable animal (host) response to nematode challenge (Albers *et al.* 1987), which are often explored in developing strategies to control or manage GI nematode infection. Resistance to GI nematode parasitism has been defined as the ability of a host to prevent or limit the establishment or development of infection, largely a function of the host's immune system. It is usually measured in live animals by counting the number of nematode eggs in faecal sample, often refer to as 'faecal egg counts' (FEC); and on post mortem, by counting the number of worms and differentiating the

developmental stages, often called 'worm counts or burdens', measuring the length of worms, counting the number of eggs in the uterus of adult female worms (called 'eggs *'in utero'*'), or measuring various systemic and local indicators of host immune response. Resilience have been defined as the ability of the host to maintain a reasonable level of production under parasitic challenge (van Houtert & Sykes 1996; Morris *et al.* 2004), usually measured by live weight gain, milk production, wool yield, and various blood indices such as serum protein, serum albumin, packed cell volume (PCV), haemoglobin concentration, etc.

For the young lamb, a natural and readily available means of nutritional intervention that can be explored is the act of suckling. The protection that milk feeding could afford suckling animals may be viewed from the following perspective:

1. Milk may contain components with useful antiparasitic properties against GI nematodes, which may have a practical application in the field.
2. Milk feeding can create an environment in the underdeveloped GI tract that may be unfavourable for parasite development.
3. Milk feeding affords the young animal a nutritional advantage, particularly with respect to metabolisable protein, which may help in developing resistance or resilience to GI nematode parasitism.
4. A good milk supply may delay pasture consumption and therefore exposure to significant larval number.

The series of experiments reported in this thesis were designed to test the hypothesis that milk can be beneficial for the development of resistance, or resilience of young lambs to the abomasal nematode, *Teladorsagia circumcincta*, and a concurrent

infection with the intestinal genus, *Trichostrongylus colubriformis* with the aim of developing a lamb production system utilizing minimal anthelmintic treatment.

Chapter 2

Literature Review

2.1: The New Zealand sheep industry and problem of nematode parasitism

Sheep have had a significant role in the development of the New Zealand economy over the past 100 years, and the impact of nematode parasitism on the economics of sheep production has been recognised since the end of the 19th century (Gilruth, 1895 cited by Vlassoff & McKenna (1994). Sheep were introduced into New Zealand in significant numbers after 1814 principally from Britain and Australia. As the indigenous mammalian fauna of New Zealand comprised only three species of bats, the parasite fauna present today is primarily composed of those parasites that survived the journey in introduced domestic stock (Vlassoff & McKenna 1994). New Zealand's warm temperate climate and ample rainfall facilitate the development of pastoral farming systems with year-round grazing, but also favoured the establishment of parasites.

To date, 29 species of nematodes have been recorded as parasitising sheep (Table 1), and with unhindered movement of livestock between districts and islands most of the 29 species can be found in all parts of the country (Vlassoff *et al.* 2001). Some of the listed species are considered as incidental infections in sheep (e.g. *Ostertagia ostertagi* and *Trichostrongylus capricola*); they are usually acquired when cross-grazing sheep with cattle and goats, respectively. *Oesophagostomum columbianum* has only been recorded in animals imported from Australia and is not considered to be endemic. Nematode species of economic importance, known to be associated with clinical diseases and production losses in sheep include *Haemonchus contortus*, *Ostertagia*

Table 2.1: Checklist of Nematode Parasites of Sheep in New Zealand

Lung	
Dictyocaulus filaria	*Muellerius capillaris*
Protostrongylus rufescens	
Abomasum	
Haemonchus contortus[a]	*Teladorsagia circumcincta*[a]
Ostertagia trifurcata[a]	*Ostertagia pinnata*
Ostertagia crimensis	*Ostertagia ostertagi*
Trichostrongylus axei[a]	
Small Intestine	
Bunostomum trigonocephalum	*Capillaria bovis*
Cooperia curticei[a]	*Cooperia mcmasteri*
Cooperia oncophora	*Cooperia punctata*
Nematodirus abnomalis	*Nematodirus filicollis*[a]
Nematodirus furcatus	*Nematodirus helvetianus*
Nematodirus spathiger[a]	*Strongyloides papillosus*
Trichostrongylus capricola	*Trichostrongylus colubriformis*[a]
Trichostrongylus vitrinus[a]	
Large Intestine	
Chabertia ovina	*Trichuris ovis*
Oesophagostomum columbianum[b]	*Oesophagostomum venulosum*

[a] Currently recognized as important in causing disease and production losses.
[b] Only recorded in animals imported from Australia.
Source : Vlassoff *et al.* (2001)

(*Teladorsagia*) *circumcincta* and other *Ostertagia* species, and *Trichostrongylus axei*, all residents of the abomasum. Others are those found in the small intestine, such as, species of *Trichostrongylus*, *Nematodirus* and to a lesser extent *Cooperia* (Pomroy 1997). Adverse effects due to lungworm infections are less frequently seen in New Zealand sheep (Vlassoff *et al.* 2001).

Current prevalence and distribution of parasitic nematodes are largely governed by a combination of their ecological requirements for development and survival outside the host, farm management practices and prevailing local climate. Climatic variations throughout New Zealand are related to north-south temperature and west-east moisture gradients, but the differences between these extremes of rainfall and temperature are small. Seasonal conditions are favourable for the development of most parasite species for at least part of each year in all districts; however, there is sufficient specificity in the requirements of the different species to affect their national spread (Vlassoff 1982). Among the most economically important genera, *Ostertagia* and *Trichostrongylus* tend to predominate in all areas, while *Haemonchus* and *Cooperia* occur with greater frequency in the northern part of the North Island. *Nematodirus*, on the other hand, is of relatively greater importance in the South Island regions of Canterbury, Otago and Southland than in the North Island (Brunsdon 1967).

2.2: Organic lamb production and sustainability of the sheep industry: A case for parasitic control without anthelmintics use

The use of anthelmintics for the control of gastrointestinal (GI) nematodes, in order to maintain high levels of production, is a common feature of the sheep industry worldwide, and New Zealand in particular. Effective control measures are based on strategic and tactical drenching combined with sound epidemiological principles, including rotational grazing (Dash 1986). According to Vlassoff & McKenna (1994) the high efficacy and relatively low cost of modern broad-spectrum anthelmintics has allowed many farmers to rely almost exclusively on heavy drench usage to control nematode parasites in their flocks.

However, the increasing popularity of organic production and the desire of some farmers to take advantage of the premiums, and economic benefits, of organic farming are putting some question marks on the widely used practice of chemical approaches to parasite control. Of course, consumers around the world are becoming wary of the levels of chemical residues in their foodstuff, and in some instance, may be ready to pay a premium for products that can guarantee the lowest level of chemical residues. To ensure that the sheep farming in New Zealand meets the standard expected of such products, the principles of organic farming as specified by the Bio-Gro New Zealand Organic Production Standard (BIOGRO 2005) are: "Internal parasite elimination, by breeding resistant and /or resilient animals, grazing management and other non-chemical procedures is an objective of organic farming. Natural purgatives and homeopathics are permitted. Stock with unacceptable worm burdens may be treated with prohibited remedies followed by quarantine. All stocks so treated lose their certification for the quarantine period and subsequent 12 months". Market access for premium organic sheep-meat will therefore be maintained only if anthelmintic usage can be reduced and eventually eliminated.

In addition, the problem of anthelmintic resistance in GI nematodes of sheep, exemplified by the emergence of strains of Ostertagia that are resistant to ivermectin and moxidectin (Pomroy & Whelan 1993; Leathwick 1995), is becoming a threat to the sheep industry. According to Sykes *et al.* (1992), the target parasites of the available range of anthelmintics have such genetic diversity and short generation intervals that coupled with sometimes indiscriminate anthelmintic use, drug resistance has become a serious concern, and future efficacy is in doubt. In the words of Douch *et al.* (1996a), New Zealand and Australia are among the few countries where the

increasing prevalence of ovine nematode resistant to currently available anthelmintics poses a considerable threat to a major export industry, the sheep production industry. New classes of anthelmintics, which may be developed in the future, are likely to be expensive for farmers while the risk of the future emergence of resistant nematode strains remains. As the global concept of "Sustainability" becomes widespread, the reliance on chemical control of parasites needs to be reduced, particularly when anthelmintic resistance is becoming problematic. With this problem at hand, the sheep industry may be found wanting in the global concept of "Sustainability".

Economic consideration is also a strong point of argument against the use of anthelmintic control measures. Drenches (use of anthelmintics) are becoming a readily identifiable cost burden on the sheep industry (Beck *et al.* 1985), and devising methods for maximising efficiency through cost reduction is inevitable.

The question here then is: can parasite control programmes in the sheep industry exclude or reduce the use of anthelmintics in order to meet the standards set by organic farming, and also meet the expectations of "Sustainability"? The magnitude of parasite induced production losses that may result from anthelmintic failure or regulatory requirements to substantially reduce chemical usage can be enormous. Brunsdon (1982) estimated that in New Zealand, anthelmintics sustain about one-third of all sheep production; the worth then was put at about $1000 million per annum. Alternative methods of controlling anthelmintic-resistant nematodes are urgently required if these production losses are to be avoided.

2.3: Host-parasite relationship

An effective parasite control strategy will require a sound understanding of the dynamic processes of parasitic infections (epidemiology) which are the result of interactions within a three-component system comprising the host, the parasite(s) and the environment. According to Brunsdon (1982) each component has many factors that determine the type, timing, duration and severity of infection:

Host: species, age, sex, genetic resistance, and level of acquired resistance.

Parasite: life history, occurrence and duration of inhibited larval development, survival of larvae on pasture (that is, resistance to or dependence on environmental factors) and adaptation to host species (parasite strain).

Environment: climate, weather, season, type of vegetation and management factors (e.g. stocking rate, grazing rotations), which affect micrometeorological conditions in the larval microhabitat.

Two phenomena that are pertinent to the understanding of these interactions are Resistance, and Resilience of the host animal to parasitic infections. Jointly, they are used to explain the manners in which the host animal responds to the challenges of parasites, and therefore may be referred to as the host factor component of the interrelationship.

Resistance has been defined as the ability of a host to prevent or limit the establishment or development of infection, while resilience was defined to be the ability of the host to maintain a reasonable level of production under parasitic challenge (van Houtert & Sykes 1996).

Host resistance is often expressed singly or as a combination of the following responses *viz.* suppression of parasite egg production; retardation of parasite development; structural alterations, such as stunting and failure of parasites to develop particular anatomical features; metabolic alterations in the parasites; prevention or reduction in the rate of establishment in the host; and expulsion of adult worms (Brunsdon 1982). Resistant animals are therefore expected to harbour fewer and stunted parasites than susceptible animals. The faecal worm egg count (FEC) is conventionally used as an indication of an animal's worm burden, and the lower the measure the more resistance the animal is compared with other animals in the flock (Woolaston & Baker 1996). Malnourished or diseased animals are usually less able to develop and maintain a level of resistance sufficient to protect them from serious parasite infection.

Expression of host resilience is simpler, as an animal, whose production level is considered satisfactory under a condition of parasitism is said to be resilient compared with other animals, under the same condition, with unsatisfactory production levels.

A temporary decline in host resistance that leads to a rise in faecal egg count (FEC) occurs in ewes around the time of lambing: a phenomenon popularly refer to as the periparturient relaxation of immunity (PPRI). Among a number of causes of PPRI, the balance between the requirement and supply of metabolisable protein has been the most popular subject of investigation (Donaldson *et al.* 1998; Kahn *et al.* 1999; Houdijk *et al.* 2001a; Houdijk *et al.* 2001b; Kahn *et al.* 2003). Even though the PPRI has been considered a major source of nematode egg contamination of pastures

(O'Sullivan & Donald 1970), studies have shown that more than half of the eggs are not viable due to the harsh conditions of the winter season (Jorgensen *et al.* 1998).

The life cycle of nematode parasites (parasite factor) is an important feature of the epidemiology of infection (Figure 2.1). Most nematodes infecting ruminants in New Zealand are in the super families Trichostrongyloidea and Strongyloidea, and share the same basic life cycle (Pomroy 1997). They have a direct life cycle consisting of free-living stages on pasture (egg to infective third stage larvae, L_3) and, after ingestion by the animal, parasitic stages (fourth stage larvae, L_4 to adult) in the host GI tract (McClure *et al.* 2000).

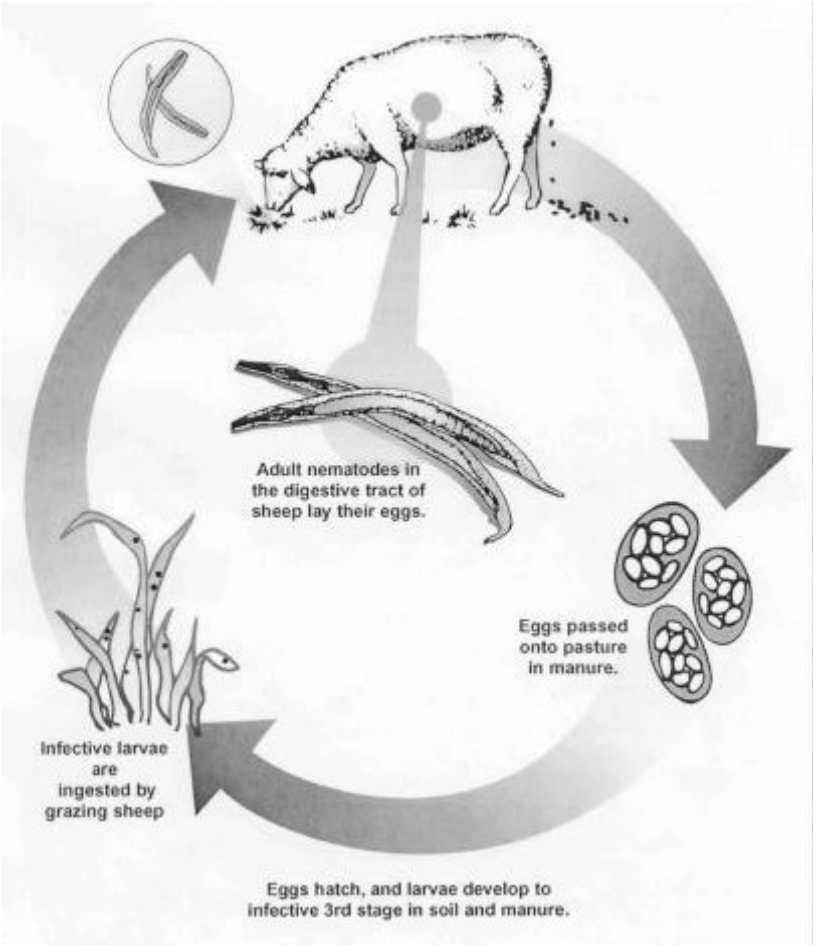


Figure 2.1: Direct life cycle of gastrointestinal nematodes in the sheep (Courtesy of Joseph Tritschler, Virginia State University; jtritsch@vsu.edu)

Typical strongylid eggs (about 80 by 40 microns) appear in faeces with the developing embryo in the multicelled morula stage (Pomroy 1997). They do not have a tissue migratory phase. There are, however, some species differences in their relationship with the host gut tissue. *Haemonchus* attaches to the mucosa of the abomasum and sucks blood; *Teladorsagia* and *Oesophagostomum* larvae penetrate into the abomasal glands or colonic mucosa before emerging and residing on the mucosal surface; and *Trichostrongylus* live in mucus-covered tunnels eroded on the surface of intestinal villi or abomasal folds (McClure *et al.* 2000).

The importance of the environment in the epidemiology of GI parasite infection is shown in the preferred developmental conditions of different species, which reflects their distribution and relative abundance from season to season and year to year. Parasites need to withstand a wide range of climatic conditions for a successful development of eggs and pre-infective stages into L_3 in faeces. For example, *Teladorsagia circumcincta* appears earlier in the season than *Trichostrongylus colubriformis*. While some development may occur within the temperature range of 4 – 35 ^{o}C, provided there is adequate moisture, optimum development occurs between 15 – 30 ^{o}C (Vlassoff *et al.* 2001). Once development to the L_3 stage is complete, larvae are considerably more resistant to adverse conditions. The seasonal pattern of variation in numbers of L_3 larvae on pasture generally consists of a small peak in spring/early summer, comprised of larvae that have survived over winter as well as larvae derived from the postpartum rise in FEC of ewes. A larger peak follows this in late summer/ autumn derived from eggs deposited by lambs in early summer (Vlassoff 1976).

2.4: Development and mechanisms of resistance to GI nematode parasite

The ability of sheep to acquire and express resistance to GI nematode parasites appears to be an exploitable feature for various alternative measures of parasite control. These measures include genetic selection, breeding, vaccination, and nutritional modulation of sheep for nematode resistance and/or resilience.

Immunological responsiveness against GI nematode infection can be expressed in different ways, and according to Balic *et al.* (2000) are generated only after a threshold level of stimulus to the host has occurred. The results of various studies have suggested the involvement of humoral and cell-mediated components of the immune system while changes in the mucosal tissue and associated lymphoid tissue have been consistently observed in relation to the development of immunity to GI nematodes in ruminants; however, much is still needed to be known about the mechanisms involved. For example, the immune mechanisms responsible for the delayed type expulsion of nematode larvae are unknown (Balic *et al.* 2002). The changes in the mucosal and associated lymphoid tissues have been summarised by Balic *et al.* (2000) to include: mucosal mast cell hyperplasia; appearance of globule leukocytes; eosinophilia; increased mucus production and the presence of inhibitory substances in the mucus; and the production of specific antibodies.

McFarlane (1997) grouped the responses of the immune system into innate (or non-specific), and acquired (or specific), which is further divided into humoral (antibody or immunoglobulin-Ig based) or cellular mediated mechanisms. Innate immunity comprises four types of defensive barriers: anatomic (such as the gut endothelial wall and mucus), physiologic (such as intestinal motility), phagocytic (ingestion and

killing) and inflammatory (allowing leakage of serum proteins). Acquired immunity reflects the ability to specifically recognise and selectively eliminate parasites in a process that entails antigenic specificity, diversity, memory, and self/non-self recognition (McFarlane 1997). In the context of GI parasite infection, these means the development of immunity against a wide range of antigens from specific worm species, independently of each other, although some non-specific immune factors produced by one species of helminth may interfere with the establishment or maintenance of another.

The role of serum antibodies (humoral immunity) in the expulsion of GI parasites is still uncertain. There is evidence for the involvement of elements of an immediate hypersensitivity response, whereby antigenic stimulation of IgE-sensitized mucosal mast cells leads to an accumulation of substances in mucus that may affect nematode survival (Smith 1988; Miller 1996). Emery *et al.* (1999) reported that antibody responses in trickle-immunised (protected) and challenge control (infected) neonates were almost exclusively of the IgG_1 isotype. According to Harrison *et al.* (2003), in a study to investigate the role of intestinal mucus antibody, against an L_3-specific surface antigen, in the immune rejection of *Trichostrongylus colubriformis*, immunoblotting showed that mucus and supernatants of centrifuged mucus from immune sheep contained IgG_1 and IgA antibodies that recognized a larval antigen with an estimated molecular weight of 35kDa.

The importance of mononuclear cells (lymphocytes and monocytes) in mechanisms of immunity has been demonstrated in sheep where lymphocytes derived from sites infected with GI parasites conferred resistance when transferred to parasite-naïve

recipients (Smith *et al.* 1984). The resultant immune responses may interfere with worm development by: rejection of incoming larvae, retardation of larval development, expulsion of adult worms, and interference with worm fecundity. As protection develops, animals sequentially acquire the ability to reject incoming larvae after 5 –7 weeks of continuous exposure, depress fecundity after 10 – 12 weeks, and finally to expel adult worms 16 – 20 weeks later (McClure *et al.* 2000). According to Sykes (1987) , sheep may not develop full resistance to GI parasites until 18 – 24 months of age.

Parasite species differ in certain aspects of immunity. A peculiar spontaneous elimination of adult parasites occurs with *Nematodirus battus* in lambs leading to a high degree of protection (Lloyd & Soulsby 1987). Blood sucking *Haemonchus contortus* is more affected by systemic immunity than the mucosal burrowers such as *Trichostrongylus colubriformis* and *Teladorsagia circumcincta*, which are especially affected by local immunity (McFarlane 1997).

A phenomenon of host reaction to GI nematode infestation in ruminants is the classical “self cure” reaction (Lloyd & Soulsby 1987). It consists essentially of a sudden loss of parasitic infestation in grazing sheep, manifested by a sharp decline in faecal egg counts (Stewart 1950). Opposing views on this concept have emerged from various studies but what appears consistent is that “self cure” may not be related to a previous infestation (Gordon 1948; Stewart 1950), and the host is not necessarily protected from subsequent infestation as the larvae which stimulated the loss of the existing infestation develop to maturity themselves (Gordon 1948; Stewart 1953; Adams 1983). In essence, the phenomenon of “self cure” and “resistance” are

different. It has also been described as the rapid loss of an established burden of adult parasites in suitably infected and sensitised hosts following the ingestion of a challenge dose of infective larvae of the homologous species (Stoll, 1929, cited by Lloyd & Soulsby (1987). The initiation is antigen-specific, requiring local abomasal release of antigens during the molt of larvae from the third to the fourth stage. The effector mechanisms appear to be associated with an immediate-type hypersensitivity reaction, which once initiated by specific antigenic stimulation, act non-specifically. For example, a challenge with *Haemonchus contortus* larvae given to *Haemonchus contortus*-sensitized and infected sheep will induce self-cure, not only of the population of adult *Haemonchus contortus* but also concurrent infections with other nematodes in the abomasum and small intestine, that is, *Trichostrongylus axei, Teladorsagia circumcincta* and *Trichostrongylus colubriformis* (Lloyd & Soulsby 1987). This lack of specificity is dependent on the habitat of the latter parasites being the same or posterior in the GI tract to that of the parasite inducing the response, since self-cure initiated by *Trichostrongylus colubriformis* larvae and affecting *Trichostrongylus colubriformis* adults in the small intestine did not induce self-cure of *Haemonchus contortus* adults in the abomasum (Lloyd & Soulsby 1987). The concept has also been related to a rise in gut and blood histamine levels (Douch *et al.* 1984) though there was no evidence that the increase in histamine level is the direct cause (Stewart 1953).

In summary, it is apparent that significantly different expressions and manifestations of resistance to GI nematode parasites do exist, and the mechanism by which sheep acquire immunity to GI nematodes is complex and yet to be fully understood. Typically, substantial immunity to incoming larvae develops after about two months in

continously infected lambs (Barger *et al.* 1985), manifested by worm exclusion or expulsion probably brought about by a combination of immunologically specific (e.g., parasite specific T cells and antibodies) and non-specific components (e.g., mucus and inflammatory medicators) interacting in concert (Smith 1988). The manifestation may involve rejection of larvae or adult populations depending on nematode species, age and infection history of host, or immunological mechanisms involved. For example, in naïve sheep infected with 50,000 *T. circumcincta* larvae, lymphoblast response began some eight days post infection and was maintained for at least three weeks (Smith *et al.* 1983). No significant changes in the content of IgA or IgA anti-worm antibody were detected in the lymph, but pepsinogen concentrations were significantly raised from day 12 onwards. Whereas, sheep previously infected with 2000 larvae per day for two months, and which were immune to challenge infection, showed a vigorous secondary immune response in the lymph, which consist of an immediate-type hypersensitivity reaction followed by a cellular response , and large increases in total and specific anti-worm IgA, in that order (Smith 1988). The immediate-type hypersensitivity reaction was detected, 24 – 72 hours after homologous larval challenge (50,000 larvae) in sheep immune to *T. circumcincta, as* increases in concentration of mast cell protease and pepsinogen in the gastric lymph (Huntley *et al.* 1987). It has been observed that sheep mast cell protease (SMCP) levels in GI mucus, a measure of mast cell degranulation, will gradually increase following mast cells / globule leucocytes induction and granulation by incoming larvae and adult nematodes (Huntley *et al.* 1987) but will drop quickly after termination of infection to pre-infection levels (Balic *et al.* 2000). Such cellular response has been observed to reach a

peak three days after challenge and is composed of a large transient increase in the output of lymphoblastic and IgA containing cells in the lymph (Smith 1988).

Apparently nematode-resistance is such a complex phenomenon, which according to Douch *et al.* (1996a), is associated, irrespective of host's age, with increased numbers of mast cells and eosinophils in the GI mucosa, elevated levels of nematode-specific antibodies in serum and the presence in GI mucus of molecules having antiparasitic activity.

2.5: Nutritional modulation for host resistance and resilience

In farm animals, there has been a recognition of, and tradition of investigation into, the links between nutrition and susceptibility to infections, with particular emphasis on interactions between nutrition and gastro-intestinal nematode infection in ruminants (Brunsdon 1964; Downey *et al.* 1972; Dobson & Bawden 1974; Sykes 1987; Coop & Kyriazakis 1999; Sykes & Coop 2001; Niezen *et al.* 2002b; Kahn *et al.* 2003; Steel 2003; Houdijk *et al.* 2005; Kyriazakis & Houdijk 2006). This has lead to a growing interest into research on the enhancement of immune competence and disease resistance status with nutritional interventions in "normal" animals, as well as nutritional intervention to support animal function in the face of infection. This is stimulated in parts by the need to develop sustainable approaches to parasite control that are less reliant on frequent anthelmintic use.

An understanding of the pathophysiological consequences of GI nematode infections for the ruminant host is important in considering nutritional modulation as a strategy for parasite control. According to Walkden-Brown & Kahn (2002) the key distinguishing features of the pathogenesis of GI nematode are marked reductions in

voluntary feed intake of the host, and profound disturbances in the metabolism of protein, energy and some major minerals due in large part to the mounting of a gut-based immune response. In addition to causing production loss, these and other pathological changes, can result in clinical diseases distinguished by hypoproteinaemia and edema, anaemia, diarrhoea, and osteoporosis, depending on the species of nematode involved. According to Sykes (2000), a reduction in feed intake can reach 50 %, even in sub clinical cases; and the extent of its contribution to reduced energetic efficiency appears to be greater in infections of the abomasum than in infections in the small intestine (Sykes 1994; Coop & Kyriazakis 1999). Increase in endogenous protein loss (up to approximately 30 g protein/day) from the small intestine accounts chiefly for the commonly observed depression in protein retention (Rowe *et al.* 1988; Sykes 2000). Hypophosphataemia with resultant impairment of calcium and phosphorus deposition in the skeleton has been revealed by numerous studies of intestinal parasitism (Sykes *et al.* 1975, 1977; Wilson & Field 1983).

It is a well-established fact that the nutritional status of the host can influence the rate of acquisition of immunity to parasite, and that protein is of particular importance. Wagland *et al.* (1984) reported that, after vaccination, lambs on high plane of nutrition had higher titres of antibodies to parasite and after challenge had lower worm egg outputs and lower worm burdens than lambs on a low plane of nutrition. van Houtert *et al.* (1995) reported that, in six month old Merino weaners infected with *Trichostrongylus colubriformis* and fed a roughage diet supplemented with either 0, 50, or 100 g/day fish meal (to increase metabolizable protein supply), resistance developed most rapidly in animals fed the largest amounts of fish meal such that worm burdens were significantly reduced by 15 weeks post infection.

The phenomenon of the periparturient breakdown of immunity to parasites in ewes has attracted the attention of many researchers in recent times. It has been suggested that this phenomenon has a nutritional basis, relating to the allocation of scarce nutrients in the animal. The common hypothesis is that periparturient breakdown of immunity to parasites would result from prioritised scarce nutrient allocation to reproductive functions, like late pregnancy and lactation, rather than to immune functions (Coop & Kyriazakis 1999). Coop and Kyriazakis (1999) reviewed various evidences from the literature on the interactions between host nutrition and parasitism in ruminants and developed a framework on the relationships between changing nutrient demands, particularly energy and protein, of the animal during its growth and reproductive phases and the influences these could have on the resilience and resistance of the host to parasites. They stated that immunity to parasitic infection is a function, which competes for nutrient resources against the requirement of the host to maintain other body functions, and that prioritisation towards immune function will therefore depend on the phase of development of the host. It was argued that during periods of high nutrient demands, such as rapid growth and late pregnancy/lactation, the requirements of immune function might take a lower priority over the ability of the host to maintain the functions of growth and reproduction and to stabilise other physiological processes with a higher priority for survival. These were the periods when nutrient supplementation was shown to have beneficial effects on the host-parasite interaction.

The view above has been supported by recent studies on protein supplementation in parasitised periparturient sheep (Donaldson *et al.* 1998; Kahn *et al.* 1999; Houdijk *et al.* 2000; Donaldson *et al.* 2001; Houdijk *et al.* 2001a), in which protein

supplementation resulted in decreased faecal egg count and worm burden, and an increased concentration of globule leucocytes in the abomasal mucosa of periparturient ewes. Donaldson *et al.* (2001) fed three groups of pregnant ewes with diets containing fishmeal, to supply about 0.95 of estimated metabolisable energy requirement and an estimated 0.86, 1.12 and 1.43 of the predicted metabolisable protein requirement, respectively. It was revealed that resistance increased in a linear fashion with increasing level of supplementation, and that the metabolisable protein requirement of twin-rearing sheep in late pregnancy and early lactation, to maintain maximum immunity in the face of a nematode larval challenge may be close to 350 g/day. An earlier study by Houdijk *et al.* (2000) revealed that an increase of metabolisable protein supply from 190 to 330 g/day to lactating ewes, infected with *Teladorsagia circumcincta*, reduced nematode egg excretion but did not affect total worm burden. In a similar study, Houdijk *et al.* (2003) maintained that a metabolisable protein supply of 330 g/day might have been insufficient to reduce worm burden. They concluded that the contrast between effects of metabolisable protein supply on faecal egg count and worm burden points towards the possibility that if different effector responses regulates fecundity and worm expulsion, then they would differ in their sensitivity towards changes in the degree of nutrient scarcity.

In a review, Houdijk *et al.* (2001a) discussed the concept of metabolisable protein requirements for reproduction vis-à-vis that for expression of immunity to *Teladorsagia circumcincta*. The estimation of metabolisable protein requirement for expression of immunity was derived by comparing whole-animal quantities of effector cells and/or molecules in non-infected naïve sheep and infected immune sheep. These include quantitative data relating to circulating lymphocytes and Ig, mucosal mast cells

and sheep mast cell proteases, and plasma loss. At a systemic level, the most pronounced differences between infected immune sheep and parasite-free sheep were an increased concentration of lymphocyte and IgA in the gastric lymph. It was calculated that the amount of metabolisable protein required for maintaining this difference would be about 50mg/kg body weight$^{0.75}$. The leakage of considerable quantities of plasma into the gastrointestinal tract was also considered as one of the key features of gastrointestinal parasitism. Plasma nitrogen leakage into the gut was considered as an irreversible loss that needs to be replenished, resulting in an estimated metabolisable protein requirement of approximately 650 mg/kg body weight$^{0.75}$. Without taking into account the metabolisable protein requirement for mucus production, the requirement for the expression of immunity to *Teladorsagia circumcincta* in sheep was estimated to be approximately 0.7 g/kg metabolic weight per day.

The roles of mineral nutrition in parasitism have not received as much research attention as with protein. Coop and Field (1983) reported a reduction in worm burden of young sheep, which were trickle-infected with *Trichostrongylus vitrinus*, when dietary phosphorus was increased from 1.9 to 2.8 g/kg DM. Copper has been known to have anthelmintic properties for many decades. Dosing young sheep with copper oxide wire particles considerably reduced establishment of a subsequent challenge dose of two abomasal parasites, while it had no effect on establishment of an intestinal parasite (Bang *et al.* 1990b). Ferguson *et al.* (1989) reported a higher FEC and plasma pepsinogen concentration in cobalt-deficient young sheep.

From the foregoing, it is clear that protein nutrition is an important modulator of disease resistance, operating via non-immune mechanisms, and both the innate and adaptive branches of the immune system. It is also a potentially important modulator of disease resilience. The question then is in what form can protein be supplied to the animal in order to achieve a high efficiency of utilisation? This question is more critical for young lambs and periparturient ewes whose nutritional demands become elevated, particularly for metabolisable protein.

The present study will be focussing on the importance of the milk diet for enhancement of resistance and resilience to nematode parasite in young lambs.

2.6: The milk diet as a protein source for enhancement of resistance or resilience in suckling lambs

Nutrient requirements of young lambs and periparturient ewes are higher than those of other age and physiological categories of sheep. According to Freer *et al.* (1997), the metabolisable energy (ME) and metabolisable protein (MP) requirements of a 50 kg single-bearing ewe maintaining maternal live weight increase 3.0 and 5.4 fold by 3 weeks postpartum and peak lactation, respectively. The protein demand relative to energy demand (expressed as gram MP per Mega joule of ME) is greatest in the young growing lamb and declines with increased lamb body weight – *viz.* about 11, 8.8, 7.5 and 6.3 g MP MJ^{-1} ME at 10, 20, 30 and 40 kg live weight, respectively (Orskov 1992). Coincidentally, the young growing lamb is most susceptible to nematode infection, and PPRI in ewes has been associated with high FEC and larval contamination of pasture.

The importance of increasing protein supply to enhance the resistance of sheep to parasite attack has been well documented. According to Donaldson *et al.* (1998), the resistance to establishment of nematode larvae is directly proportional to estimated MP supply in ewes in the prepartum period and the MP requirement for maintenance of immunity may be as great as 350 g MP per day, 20 % higher than current estimates of requirement (AFRC, 1993). The challenge here is how to meet these high demands of MP bearing in mind that such high MP requirements are substantially greater than what can be supplied to the animals from pasture (Orskov 1992). Orskov (1992) had earlier demonstrated the inefficiency of microbial protein production (from feeding on pasture) relative to the needs of various categories of livestock, particularly during periods of heightened MP demand. The use of supplementary protein sources that will ensure ample supply of rumen undegradable protein to the abomasum for a more efficient protein utilisation was suggested.

Studies that are more recent have demonstrated the use of various supplements and forage plants containing condensed tannins in ensuring adequate amount of rumen undegradable (by-pass) protein. These include fishmeal (Donaldson *et al.* 2001), lotus [*Lotus pedunculatus*; (Niezen *et al.* 1998)], *Dorycnium pentophyllum* and *D. rectum* (Niezen *et al.* 2002b), Sulla [*Hedysarum coronarium*; (Niezen *et al.* 2002a)], and Chicory [*Cichorium intybus*; (Marley *et al.* 2003)]. Apart from the protein binding effect of condensed tannins, ovicidal and larval development inhibitory activity were also implicated for the effect of some of the forage plants (*Dorycnium pentophyllum* and *D. rectum*; (Niezen *et al.* 2002b). However, these findings are obviously relevant to parasitism in adult sheep. Studies on the improved protein nutrition of the young lamb are scarce in this respect. The option of weaning the lamb early to take advantage

of supplementation may not be acceptable as the danger of early exposure to larvae may surpass such benefit. Moreover, while lambs will commence eating of herbage by the third or fourth week of life and the rumen capable of microbial digestion of roughage at six weeks of age, milk still represent their major source of digestible protein and energy (Joyce & Rattray 1970)

The milk diet provides an opportunity for enhancing the nutrition of suckling lambs. According to Geenty & Sykes (1983), milk contains a ratio of amino acid to ME of 10.7 g to 1 MJ that, in addition to its high nutrient density and ready accessibility to the lamb, can meet the high protein requirement. However, the production of milk by the ewe dam must be adequate to meet the nutrient requirement of the suckling lamb whose growth, development, and health depend largely on milk for the first several weeks of life. Various studies have investigated the relationship between ewe's milk production and pre-weaning live-weight gain of lambs (Peart *et al.* 1972, 1975; Torres-Hernandez & Hohenboken 1980; Godfrey *et al.* 1997; McKusick *et al.* 2001), and the relative contribution of milk and herbage to the nutrient need of growing lambs (Joyce & Rattray 1970; Doney *et al.* 1983; Ramsey *et al.* 1994; Degen & Benjamin 2005). In practice, milk production is often expressed as 'Lactation curve', which is a mathematical analysis of milk production records over time in order to understand the complex physiological mechanisms that underlie the milk secretion process (Cappio-Borlino *et al.* 2004). According to Cappio-Borlino *et al.* (2004) lactation peak is expected within 3-4 weeks of lambing. The milk yield of ewes is known to be influenced by genotype of ewe, nutrition during pregnancy, and litter size. In a 105-day lactation-period study of the relationships between ewe milk production (MP), percentage milk protein (MMP) and percentage milk fat (PMF) to pre-weaning weight

gain (G) of single and twin lambs Torres-Hernandez & Hohenboken (1980) reported large and significant correlations between lamb weight gain and ewe milk production around peak lactation, which generally decreased as lactation progresses. Single lambs were observed to gain more weight than did individual twin lamb; however, the total gain of a twin set was much greater than the gain of a single lamb. A range of 3.4 – 7.1 with a mean of 5.5 g milk g^{-1} lamb gain in weight has been estimated to be the efficiency of transformation of milk into lamb gain in previous studies on ewe lactation and lamb growth (Butterworth *et al.* 1968; Peart *et al.* 1972, 1975). In a review, Treacher & Caja (2002) reported studies indicating that lambs consuming only milk gain 160-170 g d^{-1} per kg of liquid milk, which is equivalent to about 6.0 kg of milk kg^{-1} of gain or about 1 kg gain kg^{-1} milk dry matter consumed.

Besides the nutritional advantage that milk could confer on growing lambs, it has also been demonstrated that milk feeding could have some health benefit, even beyond the first few days of colostrum feeding. In a study to compare the susceptibility to parasitism by *Teladorsagia circumcincta* of lambs fed entirely with bovine milk or weaned on to solid feed at 3 weeks of age, Zeng *et al.* (2001) found that milk-fed lambs are less susceptible to parasitism. According to them, a variety of explanations has been offered for the resistance of milk-fed animals to parasitism. These are: the high pH of milk protects against nematodes in milk-fed calves (Rohrbacher *et al.* 1958); increased gut motility causes expulsion of nematodes from skim-milk-fed pigs (Spindler *et al.* 1944); or a less developed rumen in milk-fed calves (Satrija *et al.* 1991). To these, Zeng *et al.* (2001) added another reason that there may be a direct effect of the milk on the parasites or an unfavourable environment in the pre-ruminant stomachs. To date, no study has been carried out to investigate the resistance, or

otherwise, of lambs raised exclusively on milk from their dam. It may also be worthwhile to investigate the extent to which the milk diet confers a nutritional advantage on the lamb.

Chapter 3

Effects of suckling, and infection with the abomasal nematode parasite, *Teladorsagia circumcincta*, on the parasite status of young lambs during the six to twelve weeks post lambing period

3.1. Introduction

The development of anthelmintic resistance in gastrointestinal (GI) nematodes of sheep is a serious issue to the sheep industries in several countries (Mitchell *et al.* 1991; Chandrawathani *et al.* 1999; Van Wyk *et al.* 1999; Leathwick *et al.* 2001; Von Witzendorff *et al.* 2003; von Samson-Himmelstjerna & Blackhall 2005), and has stimulated the search for sustainable approaches to GI nematode control.

Immune unresponsiveness of very young lambs (Dineen *et al.* 1978; Smith *et al.* 1985; Kambara *et al.* 1993) and the consequent susceptibility to parasitic infection is a considerable problem for farmers. The development of successful vaccines against nematode parasites has proved elusive (Hein & Harrison 2005) and, while genetic selection in sheep has led to increased capability to limit nematode egg output (McLean *et al.* 2006), the achievement of adequate nematode parasite control without recourse to anthelmintics remains a goal for the future. Moreover, the incorporation of desirable traits, such as, minimal contamination of pasture with nematode eggs, maximal animal productivity, and minimal anthelmintic intervention - all in one sheep breed is still uncertain (Morris *et al.* 2004). Therefore, it would be worthwhile to explore other possibilities for limiting the effect of GI nematode infection in very young lambs.

One such strategy is the possibility of advantage conferred on young mammals by milk consumption. Studies in sheep (Watson & Gill 1991b; Zeng *et al.* 2001), cattle

(Rohrbacher *et al.* 1958; Satrija *et al.* 1991; Boom *et al.* 2004), rabbit (Rohrbacher *et al.* 1958), horse (Leese 1943), and pigs (Shorb & Spindler 1947) have all demonstrated lower worm burdens in young mammals fed milk than in those weaned to solid feed or grass. While the exact mechanisms are unclear, such benefits could accrue through a direct effect of milk on the nematode or indirectly through enhancement of the host immune response or of host resilience to the pathological effects of infection. Direct effects could operate through adverse effects of milk components, for example, of oligosaccharides on the adhesion of pathogens to host mucosa (Hakkarainen *et al.* 2005), or of milk proteins and components associated with milk proteins on motility of nematode larvae (Zeng *et al.* 2003). Indirect effects could operate via the superior amount and quality of protein supplied by milk, which is protected from degradation in the rumen by the oesophageal groove reflex, promoting greater or more rapid development of host immunity or greater host resilience to the pathogenic effects of infection (Bown *et al.* 1991; Sykes & Coop 2001).

This chapter describes a study designed primarily to test the hypothesis that milk has a direct effect on the establishment of infection with *T. circumcincta* in lambs during the six to 12-week period after lambing, but also provided some information on the effects of suckling on resilience to infection, and data on peripheral immunoglobulins to monitor possible immune response.

3.2. Materials and methods

3.2.1. Management of experimental animals

Thirty twin-bearing Coopworth ewes were selected, after scanning for number of lambs carried, and dosed with a controlled-release anthelmintic capsule [Extender^{TM} 100 (3.85 g Albendazole with a minimum release of 0.5 mg kg^{-1} LW^{-1} day^{-1}) Captec New Zealand Ltd, Auckland, New Zealand], 21 days prior to lambing to minimise pasture larval contamination. The ewes and offspring were then grazed on a freshly sown annual ryegrass sward (*Lolium perenne*) that was confirmed to have low levels of pasture larval contamination when tracer lambs, which had been drenched and then grazed on the same pasture, returned zero FEC after 21 days. The sward had an estimated yield of 2000 kg DM ha^{-1} at the beginning of the trial, and 1200 kg DM ha^{-1} at the end (C. M. Logan; personal communication, April 2004). At parturition, lambs were weighed and tagged for identification with the lambing dates recorded.

3.2.2. Experimental design

At the lambs' median 39 days of age, ewes and their twin lambs were allocated to a control ('not infected') group, two infected groups, and one group of infected and twin-suckled lambs as shown in Table 3.1. The infected groups received equivalent of either 250 or 1000 L_3 larvae of *T. circumcincta* per day, approximately 14 and 54 L_3 kg LW^{-1} d^{-1}, respectively, from day 42 of age onwards. A member of each set of twins of the control group and the two infected groups were then weaned, leaving the other member with the dam to continue suckling (single-suckled) until necropsy at day 84 of age (42^{nd} day of infection). A member of each set in the twin-suckled group received equivalent of either 250 or 1000 L_3 larvae per day. These replicated the suckled

(single-suckled) and infected groups. Lambs were allocated to treatment groups hierarchically by live weight (LW; mean: 18.4 ± 0.5 kg on day 42 post-lambing) and groups balanced for lambing date. A portion of the 'parasite-safe' paddock was fenced to rear the weaned lambs. The two flocks i.e. suckled lambs along with their dams and weaned lambs, were "set stocked" at 15 animals (ewes or lambs) per hectare in their respective portion of the paddock till necropsy.

Table 3.1: Experimental design showing lamb treatment groupings formed with lambs weaned (W-) at 39 days of age, or single-suckled (SS-) throughout the trial period, and either not infected (-0), infected with equivalents of 250 (-250) or 1000 (-1000); or twin-suckled and infected with 250 (TS250) or 1000 (TS1000) L_3 *T. circumcincta* larvae d^{-1} from day 42 of age onwards, respectively.

No. of ewes	No. of lambs	Lamb groups	Larval dose/ d	Group acronyms
7	7	weaned	0	W0
	7	suckled	0	S0
8	8	weaned	250	W250
	8	suckled	250	S250 (SS250)
8	8	weaned	1000	W1000
	8	suckled	1000	S1000 (SS1000)
7	7	twin-suckled	250	TS250
	7	twin-suckled	1000	TS1000

From the start of the experiment (42nd day after lambing) lambs were weighed weekly with an electronic scale, and the infected groups were given the average daily larval dose (details described later). Faecal and blood samples were collected at weekly intervals. All lambs were slaughtered for necropsy on day 84 (five days after

last larval dose), and carcass weight measured. The detailed experimental procedures are described in the following sections.

3.2.3. Parasitology and necropsy procedure

Larval dosing: The infective larvae used for dosing the infected lambs were sourced by passing mono-specific culture of *T. circumcincta* through parasite-naïve lambs that were reared indoor; faeces of the lambs were then collected and cultured for larvae (R. W. McAnulty, personal communication, Nov. 2003). The larvae were transferred onto filter papers (one filter paper per dose) from a suspension of known larval content using a pipette. The filter papers were then rolled up and administered to lambs using a balling gun (Donaldson *et al.* 2001). Larval doses were administered, for convenience, three times each week – on Mondays, Wednesdays, and Fridays – to give the average daily dose. However, the last dose was given five days to the necropsy date (day 37 of the infection period).

Faecal egg counts (FEC): Faecal samples were collected *per* rectum by grabbing with gloved fingers, and placed in labelled jars that were identified for each lamb. Sample of faeces (1.7 g) was soaked with 5 mL of water in separate jars, and kept at $4^{o}C$ overnight. The number of nematode eggs in the faecal samples (FEC) was estimated using a modification of the McMaster method (M.A.F.F. 1987) and expressed as eggs per gram of fresh faeces (epg). Briefly, the soaked faecal sample was homogenised with an electrical stirrer for 25 seconds after adding 46 mL of saturated NaCl. A Pasteur pipette was used to fill both chambers of a moistened McMaster slide with the faecal suspension. Eggs present floated to the surface of the salt solution in the chambers and stuck to the cover glass. The number of eggs present in both chambers

of the slide were counted under a microscope, totalled, and multiplied by 100 to give the e.p.g for that sample. The method described had a sensitivity of one egg counted representing one hundred eggs per gram in the sample.

Post-mortem worm counts: Post-mortem abomasum and small intestinal contents were collected for the enumeration of abomasal and intestinal worms, respectively, in accordance with the methods described by Donaldson *et al*. (2001). Lambs to be slaughtered were removed from the paddock and fasted from the previous day. Necropsy was done by stunning the lamb with a captive bolt gun, followed immediately by severance of the neck to achieve bleeding through the carotid arteries and jugular veins, and removal of the visceral mass. The abomasum and first five metres of small intestine, from the duodenal end, were removed from the visceral mass and open ends tied up to retain the contents. The abomasum was cut open along the lesser curvature over a container in which the contents were collected, avoiding spillage as much as possible. A stream of water was used, along with gently rubbing of the fingers, to wash and remove adhering worms from the abomasal tissue. The contents and washings were then transferred to a beaker and made up to a volume of two litres. The contents of the beaker were then thoroughly mixed and four 50 mL sub samples withdrawn and placed in a labelled container, to which 20 mL of formalin (10 %) was added (to make a volume of 220 mL). From the 220 mL sample, four 5 mL abomasal aliquots (20 mL) were taken into a petri dish, after mixing, and worms counted under a microscope. The 20 mL aliquot represented a one hundred and tenth of the original sample, and each worm counted therefore represents 110 worms in final abomasal worm count. This was taken as the abomasal wash count. After washing, the abomasal tissue was cut up into pieces and subjected to peptic digestion (HCL/pepsin

solution) for 16 - 20 h. After digestion, all material was passed through a 45-micron sieve and washed with a jet of water. The material collected on the sieve was fixed in formalin (10 %) and stored pending examination. A one-tenth aliquot of the digested sample was examined under the microscope. The number of worms counted was then multiplied by ten, to give a total number of worms in the abomasal digest. The final abomasal worm count was taken as the sum of the abomasal wash and abomasal digest counts (R. W. McAnulty, personal communication, Jan. 2003).

Intestinal worm counts were determined as described above for the abomasum and recorded as small intestinal worm counts. This was further differentiated into various nematode species counts (R. W. McAnulty, personal communication, Dec. 2003).

Abomasal digesta pH was measured, immediately after opening the abomasum (approximately 10 min after slaughter), by dipping the electrode of a pH meter (Orion TM model 720A) into the digesta content.

Carcass weights: This was measured on a hanging scale immediately after the removal of the visceral mass and the cutting of the legs from the hocks.

Worm eggs *'in utero'* (EPU): The first twenty-five adult female worms encountered while viewing the abomasal aliquot under a microscope were selected on a slide. Two drops of lactophenol (10 g Phenol dissolved in 10 mL distilled water + 10 mL Glycerine + 10 mL Lactic acid) were placed on the selected worms on the slide and held for 24 h; this made the cuticle of the worms to be transparent and eggs within visible for counting under the microscope. The slide was then mounted on a microscope and eggs within each worm were counted and recorded individually;

resulting in 25 counts for each lamb, the average of which was recorded as the eggs *'in utero'* (epu) for each lamb (R. W. McAnulty, personal communication).

Worm length: The slides containing the 25 adult female worms were mounted on a microscope-camera-projector network and the images of the worm projected onto a screen. The length of individual worms were measured using a map wheel that was calibrated with a one-millimetre micrometer (Olympus^{TM}), similar to the technique described by Donaldson *et al.* (2001).

3.2.4. Blood sampling and analyses

Blood samples were taken weekly from each lamb for serum and plasma to measure total plasma protein, plasma albumin, plasma pepsinogen, total serum immunoglobulin (Ig), immunoglobulin A (IgA), and immunoglobulin M (IgM). Samples were drawn via jugular venipuncture into two vacutainers (5 mL each; Becton Dickinson, VACUTAINER Systems, Rutherford, New Jersey, USA; ref. # 367886), one plain for serum harvest, and the other Lithium heparinized for plasma harvest. Heparinized samples were immediately centrifuged at 2000 *g* for 20 min using a MSE Mistral 3000 Tabletop Centrifuge (Global Medical Instrumentation, Inc. Minnesota, USA), and plasma withdrawn. Blood samples for serum harvest were stored at 4^{o}C overnight, and then spun at 2000 *g* for 20 min. Plasma and serum in separate labelled tubes for each lamb were stored at –20^{o} C until analyses.

Plasma protein and albumin: These were measured in plasma on a Cobas Mira Plus™ auto-analyser (Roche Diagnostics GmbH, Mannheim, Germany) by colorimetric assay via a Biuret reaction for total plasma protein (Roche Unimate 7™

Total protein kit # 11929917, Roche Ltd, Basel) and a Bromocresol reaction for plasma albumin (Roche Unimate 7™ albumen kit # 11970569, Roche Ltd, Basel).

Plasma pepsinogen: This was measured by a modification of the micro method as described by Dorny and Vercruysse (1998) and expressed as units of tyrosine (U tyr - micromoles of tyrosine released l^{-1} of plasma min^{-1}). Briefly, 50 µL of serum sample and 250 µL of 2 % bovine serum albumin [2 g bovine serum albumin to 100mL distilled H_2O with 0.1M glycine-NaCl-HCl buffer (0.89 mL HCl to 80 mL distilled H_2O, with 0.58 g glycine + 0.75 g NaCl added and adjusted to pH 2.5 with 0.01M HCl and made up to 100 mL)] were added into a 1.5 mL conical tube (Eppendorf™). The mixture was whirled on a vortex and incubated for 24 h at 37^{o}C followed by the addition of 500 µL of 4 % TCA (4 g trichloroacetic acid in 100 mL distilled H_2O) and centrifugation for 5 min at 10,000 g. A 20 µL aliquot of supernatant, following centrifugation, was put into flat-bottomed microtitre plate (in triplicate wells per sample) followed by 200 µL of 0.25 M NaCl (1.46 g NaCl to 100 mL distilled H_2O) and mixed for 2 min. This was followed by the addition of 30 µL of 25 % Folin and Ciocalteu's reagent (made up daily as one part Folin and Ciocalteu's reagent to three parts of distilled H_2O), mixing for 2 min and incubating for 30 min at room temperature. Plates were then read for optical density (OD) at 680 nm using a microplate reader (Floustar, BMG Lab Technologies, Germany). Also, an unincubated serum duplicate was measured simultaneously with the plates.

A substrate blank, basically water, and a set of tyrosine standards (0.1, 0.2, and 0.3 µmol mL^{-1} solutions), prepared from a 0.01 M tyrosine stock standard (0.181 g tyrosine in 100 mL 0.1 M HCl), were subjected to the same treatment as the plasma

supernatant above in order to generate a standard curve. The standard curve was used to derive pepsinogen concentrations in the samples as units of tyrosine (U tyr) – i.e. Micromoles of tyrosine released per litre of serum per minute. The equation according to Dorny and Vercruysse (1998) is as follows:

U tyr = (OD sample-Blank) × F × 11.11

Where,

U tyr - Micromoles of tyrosine released per litre of serum per minute

OD sample – Mean optical density from 3 measurements

Blank – mean optical density for serum blank

F – calculation factor from tyrosine standards =

[(0.1/OD1) + (0.1/OD2) + (0.1/OD3)]/3

Where,

OD1 – optical density of the 0.1 µmol mL^{-1} tyrosine standard

OD2 – optical density of the 0.2 µmol mL^{-1} tyrosine standard

OD3 – optical density of the 0.3 µmol mL^{-1} tyrosine standard

11.11 – conversion factor for serum dilution and incubation time.

Immunological parameters: *Teladorsagia circumcincta*-specific L_3 total Ig, IgA, and IgM were measured by colorimetry using an enzyme-linked immunosorbent assay (ELISA) as described by Xie *et al.* (2004). Briefly, each well of Costar microplates 9017 (Corning Incorporated, U.S.A) was coated with 100 µL secretory/excretory antigen (AgResearch, Wallaceville Animal Research Centre, New Zealand) of *T. circumcincta* L_3 larva (2 µg mL^{-1}) at 37°C for 2 h. Plates were then washed 3 × with 0.05 % Tween 20 in 10 mM phosphate buffered saline, pH 7.2

(PBST) and unoccupied surface of the plate was blocked by immersion of the plate in 5 % bovine skim milk powder/PBST for at least 5 min. Plates were then washed 6 × in PBST and then stored at - 20^{o}C until required (R. S. Green, personal communication). To measure total Ig, a 1:200 dilution of serum was pipetted into the wells of the antigen-coated Costar plates in triplicate at 100 µL per well. Plates with contents were then incubated at room temperature for 2 h followed by flushing of the wells with PBST to wash off the content (serum dilution). At this stage, the specific antibodies in the serum sample were expected to have bonded to the coated antigen. This was followed immediately by pipetting 100 µL of a 1:4000 dilution of an enzyme-conjugated antibody [Horseradish peroxide (HRP) conjugated rabbit anti-sheep immunoglobulins; Code # P 0163, DakoCytomation^{TM}, Denmark] into the wells and incubating at room temperature for another 1h, which was then followed by another washing of the wells. At this stage, the enzyme-conjugated antibody is expected to bond with the specific antibody that earlier bonded the coated antigen. This was followed by pipetting 100 µL of a prepared substrate [400 µg mL^{-1} o-phenylenediamine (OPD, Sigma) in citrate buffer pH 5.0 and 0.03 % H_2O_2] into the wells. The enzyme of the conjugate catalysed the substrate (o-phenylenediamine), in the presence of the hydrogen peroxide (H_2O_2), into the colour form in the dark for 15 min before the reaction was stopped by adding 1.5 M H_2SO_4. The colour was then read for optical density (OD) at 492 nm using a microplate reader (Floustar, BMG Lab Technologies, Germany). Data for total Ig were expressed as the mean OD value of the triplicate wells, and adjusted with a standard serum sample that was subjected to the same assay above on each plate. The principle of the ELISA is that the OD of the colour developed is directly related to the concentration of the specific antibody in the

samples, since the non-specific substances and the excess reagents were removed during the washing, and all reagents, except the antibody in the serum were prepared at a certain concentration.

The above protocol was followed for measuring IgA and IgM; except that serum dilutions were 1:10 and 1:20 (to allow for appropriate colour development relative to serum antibody concentrations) and enzyme-conjugated antibodies used were HRP-conjugated rabbit anti-sheep IgA (Cat. # A130-108P, Bethyl Laboratories Inc, USA) and HRP-conjugated rabbit anti-sheep IgM (Cat. # A130-109P, Bethyl Laboratories Inc, USA), while colours were developed in 40 min and 12 min, respectively.

3.2.5. Statistical Analyses

Data were analysed using the Genstat suite of statistical packages (GenStat Release 7.2 Copyright 2004, Lawes Agricultural Trust, Rothamsted Experimental Station). All data were tested for normality, and only FEC and abomasal worm counts required log-transformation [LOG_{10} (count+1)] before analysis, and are presented as back-transformed means. All parameters measured weekly underwent sequential comparison of ante-dependence structures for repeated measures before being analysed by the Restricted Maximum Likelihood (REML) routines. Data from lamb pairs with weaned members (46 lambs) were analysed separately for the effects (main and interactions) of time (for parameters measured weekly), suckling (weaned/suckled), and infection (0, 250, or 1000 larvae d^{-1}). Those from the 14 twin-suckled and infected lambs and their corresponding single-suckled and infected groups (among the 46 lambs) were analysed separately in a 2 × 2 factorial design for the effects (main and interactions) of time, suckling category (single, or twin-suckled), and

infection (250, or 1000 larvae d^{-1}). The FEC up to the 14^{th} day (day 56 after lambing) was excluded from the statistical analysis because of the zero counts of most lambs. Correlation coefficients were used to describe the relationships between various parasitological parameters, weekly average daily weight gain, and IgA (Minitab Release 14).

This experiment was carried out following approval of the Lincoln University (New Zealand) Animal Ethics Committee: Application no. 4.

3.3. Results

3.3.1. Clinical observations

None of the lambs showed any overt sign of infection, such as breech soiling. One lamb was culled (ID # 42 in the TS250 group of lambs) on day 16 of the trial (day 58 after lambing) on humane grounds due to trauma caused to the rectum during faecal sampling. Data from this animal were included in the statistical analysis using estimates of missing values.

3.3.2. Faecal egg count

The changes in mean FEC of the lambs are given in Figure 3.1. There was a time × rate of infection interaction for the W-/S- ($P < 0.001$) and SS-/TS- ($P = 0.021$) groups of lambs as a consequence of direct relationship between larval dosage and FEC peaks that occurred earlier on days 63 and 70 in the 1000 larvae d^{-1}-infected lambs, but later on day 77 in the 250 larvae d^{-1}-infected lambs. Overall, nematode eggs first appeared in the faeces of five lambs (two of the W-/S- and three of the TS- groups of lambs, respectively) on day 56, and were present in faeces of most lambs by day 63 (i.e. days 14 and 21 of infection, respectively). This was followed by FEC peaks from day 63 to 77, which declined to 200 epg or less by day 84 for all infected groups except in the TS250 lambs (Figure 3.1a) where FEC declined to 688 epg. The two uninfected groups (W0 and S0) had a similar trend of FEC throughout the 42-day trial (infection) period, which remained below 30 epg (Figure 3.1b) indicating minimal intake of larvae from pasture. There were no significant effects of suckling, or suckling category on FEC ($P > 0.05$), but a trend for greater FEC peaks in twin-suckled lambs than their single-suckled counterparts (Figure 3.1a).

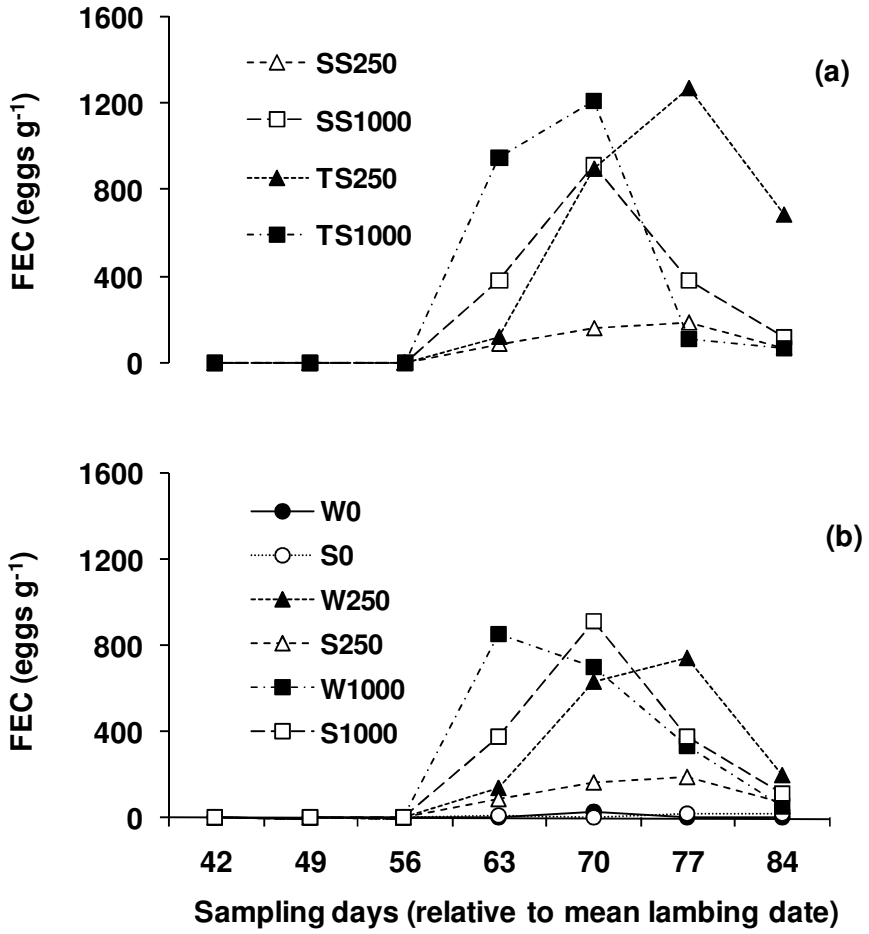


Figure 3.1: Mean FEC of (a) lambs single-suckled (SS-) or twin-suckled (TS-) and (b) those weaned (W-) at 39 days of age or suckled (S-) throughout the trial period, and infected with equivalent of 0 (-0), 250 (-250), or 1000 (-1000) L_3 *T. circumcincta* larvae d^{-1} from day 42 of age onwards, respectively.

3.3.3. Worm counts, eggs 'in utero' (EPU), and female worm length

The data for worm counts, EPU, and female worm length are shown in Table 3.2. Total worm counts and number of immature worms (L_3 and L_4 larvae) were significantly influenced by rate of infection for both the W-/S- ($P < 0.001$; $P < 0.001$) and SS-/TS- ($P = 0.012$; $P < 0.001$) groups of lambs, respectively, reflected in number of worms recovered being directly proportional to level of larval dose. The number of mature worms showed a similar trend, although only the difference between the 250 and 1000 larvae-infected lambs and zero-infected

Table 3.2: Worm counts (Log-transformed values, with back-transformed means in parenthesis), eggs *'in utero'*, and worm length of lambs as influenced by suckling (weaned/suckled), infection (0, 250, or 1000 larvae d^{-1}), and suckling category (single- or twin-suckled).

Lamb treatment groups (n)	Worm count			Eggs *'in utero'*	Worm length (mm)
	L_3 & L_4 larvae	Adult Worms	Total		
W0 (7)	**0.15^{c} (0)**	**1.68^{c} (47)**	**1.69^{d} (48)**		
S0 (7)	**0.64^{c} (3)**	**2.10^{bc} (126)**	**2.13^{cd} (135)**		
W250 (8)	**1.91^{b} (80)**	**3.38^{a} (2398)**	**3.42^{ab} (2650)**	**41^{ab}**	**10.7^{ab}**
S250 (8)	1.53^{by} (33)	2.83^{abx} (679)	2.88^{bcy} (766)	56^{ay}	11.2^{axy}
W1000 (8)	**3.59^{a} (3850)**	**3.80^{a} (6275)**	**4.05^{a} (11333)**	**15^{c}**	**9.7^{c}**
S1000 (8)	3.36^{ax} (2289)	3.24^{ax} (1754)	3.81^{ax} (6399)	29^{bcz}	10.2^{bcz}
SEM[1]	**0.216**	**0.173**	**0.168**	**3.9**	**0.19**
TS250 (6)	2.09^{y} (121)	3.50^{x}(3187)	3.52^{xy} (3334)	75^{x}	11.8^{x}
TS1000 (7)	3.41^{x}(2582)	3.14^{xy} (1367)	3.80^{x} (6375)	23^{z}	10.5^{yz}
SEM[2]	0.200	0.210	0.137	4.8	0.17

^{abcd} - Column means for the W-/S- (**values in bold**) lambs bearing similar superscript are not significantly different ($P > 0.05$).

^{xyz} **-** Column means for the SS-/TS- lambs bearing similar superscript are not significantly different ($P > 0.05$).

SEM[1] - Standard error of means for W-/S- lambs.

SEM[2] - Standard error of means for SS-/TS- lambs.

lambs was statistically significant. There were no significant effects of suckling, or suckling category ($P > 0.05$), however, there was a trend for 2 – 3.5 fold reduction in number of worms in suckled (766 and 6399) compared with weaned (2650 and 11333 worms) lambs at the 250 and 1000-larval doses, respectively.

For the W-/S- groups of lambs, suckling significantly influenced EPU ($P = 0.020$), reflected by a greater EPU in worms recovered from the S- than from W- lambs. Also, the rate of infection significantly affected EPU ($P < 0.001$) in both W-/S- and SS-/TS- groups of lambs, reflected by greater EPU in worms recovered from 250 than from 1000 larvae d^{-1}-infected lambs. There was a suckling category × rate of infection interaction in the comparison SS-/TS- group of lambs, reflected by a greater EPU in worms recovered from TS- than from SS- group of lambs at 250-larval dose but similar EPU for both groups at the 1000-larval dose.

Female worm length was significantly influenced by rate of infection in both W-/S- ($P = 0.005$) and SS-/TS- ($P < 0.001$) groups of lambs, reflected by longer worms in 250- than in 1000 L_3-infected lambs from both treatments. A trend for longer female worms in suckled lambs was not statistically significant ($P = 0.16$). There was no significant effect of suckling category on female worm length ($P > 0.05$).

3.3.4. Immunology

Mean total antibody (optical density) against *T. circumcincta* is shown in Figure 3.2. with high optical density indicating high antibody levels. There was a time × rate of infection interaction ($P = 0.034$) in W-/S- group of lambs, reflected by antibody levels tending to fall for the first 21 days of infection (up to day 63) and then to rise, with lower absorbance in 1000 L_3 infected lambs. For the SS-/TS- group of lambs, a significant time effect ($P < 0.001$) was similarly reflected in antibody levels falling till day 70 and then rising till day 84. There were no significant effects of suckling, or suckling category on total antibody ($P > 0.05$).

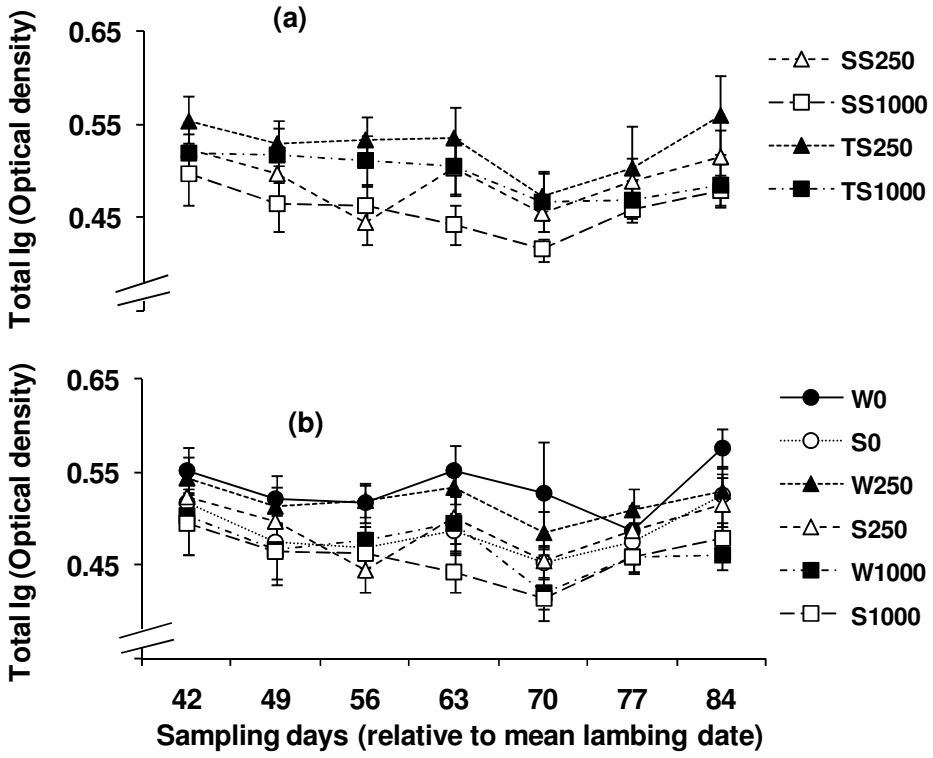


Figure 3.2: Mean L_3 *T. circumcincta*-specific total antibody (Ig) absorbance of (a) lambs single-suckled (SS-) or twin-suckled (TS-) and (b) those weaned (W-) at 39 days of age or suckled (S-) throughout the trial period, and infected with 0 (-0), 250 (-250), or 1000 (-1000) L_3 *T. circumcincta* larvae d^{-1} from day 42 of age onwards, respectively.

Mean L_3 parasite-specific IgA absorbance is shown in Figure 3.3. For the W-/S- lambs, there was a time × suckling × rate of infection interaction ($P = 0.009$) reflected in the trend for increase in levels with time and especially in infected than uninfected lambs after day 63. A similar trend was observed in the SS-/TS- lambs in which a time × rate of infection interaction was reflected by higher levels in 250 than in 1000 L_3-infected lambs on day 49 and a reversal on day 70. There was no significant effect of suckling category on IgA level ($P > 0.05$).

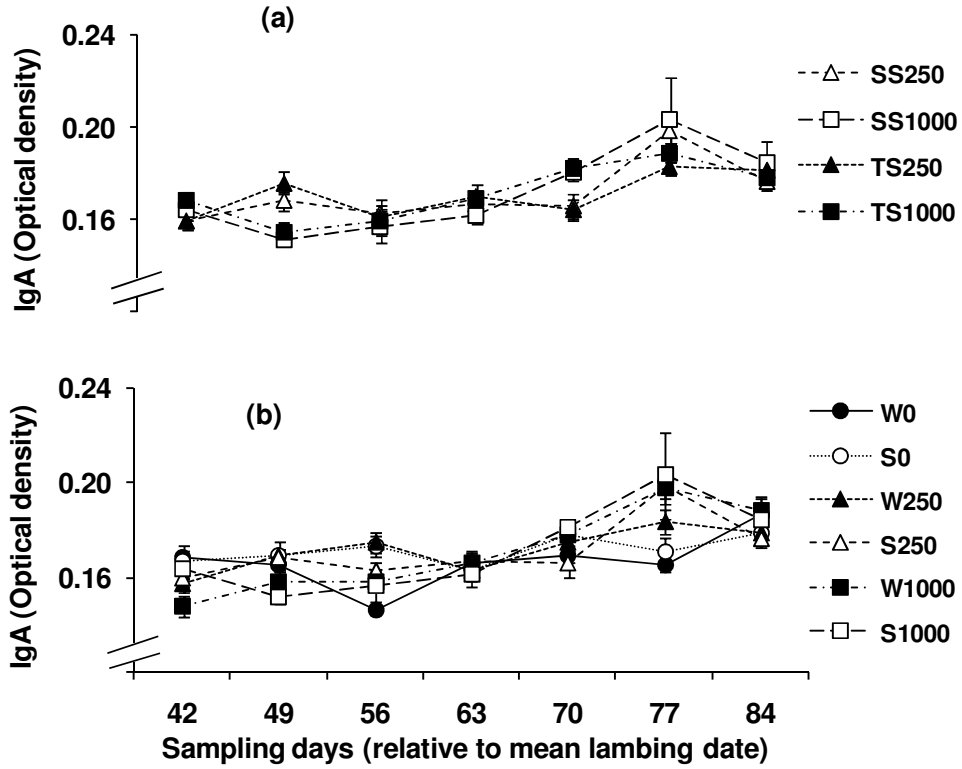


Figure 3.3: Mean L_3 *T. circumcincta*-specific IgA levels of (a) lambs single-suckled (SS-) or twin-suckled (TS-) and (b) those weaned (W-) at 39 days of age or suckled (S-) throughout the trial period, and infected with 0 (-0), 250 (-250), or 1000 (-1000) L_3 *T. circumcincta* larvae d^{-1} from day 42 of age onwards, respectively.

Mean L_3 parasite-specific IgM absorbance is shown in Figure 3.4. There was a significant effect of time ($P < 0.001$) reflected in a rise in IgM levels of all lamb groups up to day 77 followed by a drop on day 84, except for the W250 lambs. There were no significant effects of suckling, rate of infection, or suckling category on IgM level ($P > 0.05$).

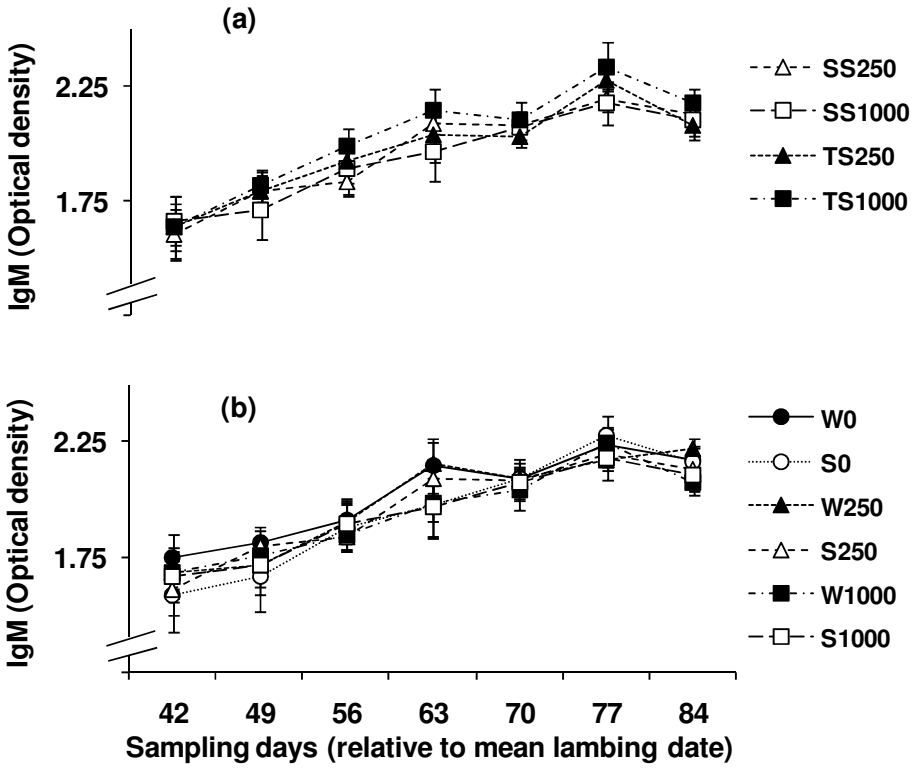


Figure 3.4: Mean L_3 *T. circumcincta*-specific IgM absorbance of (a) lambs single-suckled (SS-) or twin-suckled (TS-) and (b) those weaned (W-) at 39 days of age or suckled (S-) throughout the trial period, and infected with 0 (-0), 250 (-250), or 1000 (-1000) L_3 *T. circumcincta* larvae d^{-1} from day 42 of age onwards, respectively.

3.3.5. Live and carcass weights

Changes in mean LW of lambs are given in Figure 3.5. For the W-/S- groups of lambs, a time × suckling interaction ($P < 0.001$) was reflected in all lambs progressively gaining weight but at a faster rate in the suckled groups from day 56 onwards regardless of infection (Figure 3.5b).

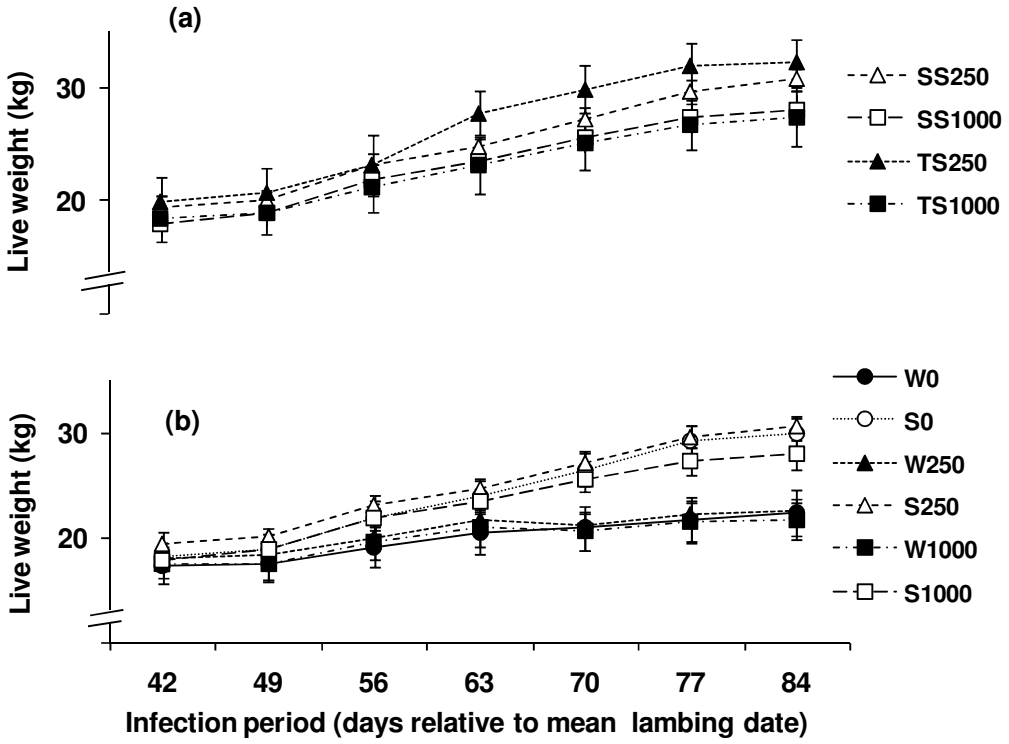


Figure 3.5: Weekly LW (kg) of (a) lambs single-suckled (SS-) or twin-suckled (TS-) and (b) those weaned (W-) at 39 days of age or suckled (S-) throughout the trial period, and infected with 0 (-0), 250 (-250), or 1000 (-1000) L_3 *T. circumcincta* larvae d^{-1} from day 42 of age onwards, respectively.

For the SS-/TS- groups of lambs, however, all groups progressively gained weight ($P < 0.001$) irrespective of suckling category (Figure 3.5a). Average daily weight gains were 122 ± 19, 282 ± 112, 110 ± 12, 268 ± 14, 98 ± 11, 240 ± 23, 291 ± 21, and 211 ± 18 g d^{-1} for the W0, S0, W250, S250, W1000, S1000, TS250, and TS1000, respectively.

The carcass weights of the lambs are given in Figure 3.6. Carcass weights (kg) were heavier ($P < 0.001$) for the suckled lambs across the infection rates with means being 13.7 ± 0.8 vs. 8.2 ± 0.8 kg for S0 and W0, 13.8 ± 0.6 vs. 8.5 ± 0.5 kg for the S250 and W250, and 12.2 ± 0.7 vs. 7.8 ± 0.8 kg for the S1000 and W1000 groups, respectively. There were no significant effects of infection rate, or suckling category on carcass weight ($P > 0.05$). The carcass weights for the TS250 and TS1000 lambs were 14.4 ± 0.9 and 12.3 ± 0.9 kg, respectively.

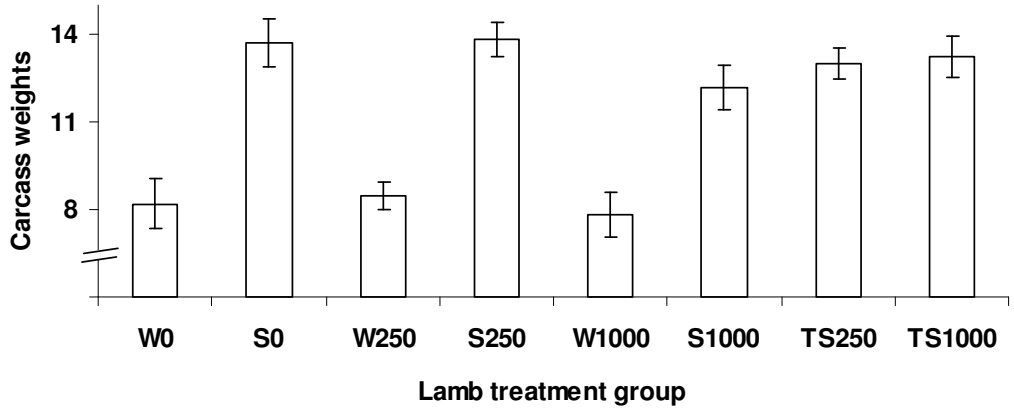


Figure 3.6: Mean carcass weights of lambs single-suckled (S-) or twin-suckled (TS-) and those weaned (W-) at 39 days of age or suckled (S-) throughout the trial period, and infected with 0 (-0), 250 (-250), or 1000 (-1000) L_3 *T. circumcincta* larvae d^{-1} from day 42 of age onwards, respectively.

3.3.6. Total plasma protein and plasma albumin

Mean plasma protein of lambs is shown in Figure 3.7. A significant effect of time ($P < 0.001$) on total plasma protein was reflected in a sharp rise between day 42 and 49, and relatively constant levels thereafter till the end of the trial on day 84 for both the W-/S- and SS-/TS- groups of lambs. There were no significant effects of suckling, rate of infection, or suckling category on total plasma protein ($P > 0.05$). Overall, mean plasma total protein in the study ranged from 50.6 to 77 g l^{-1}.

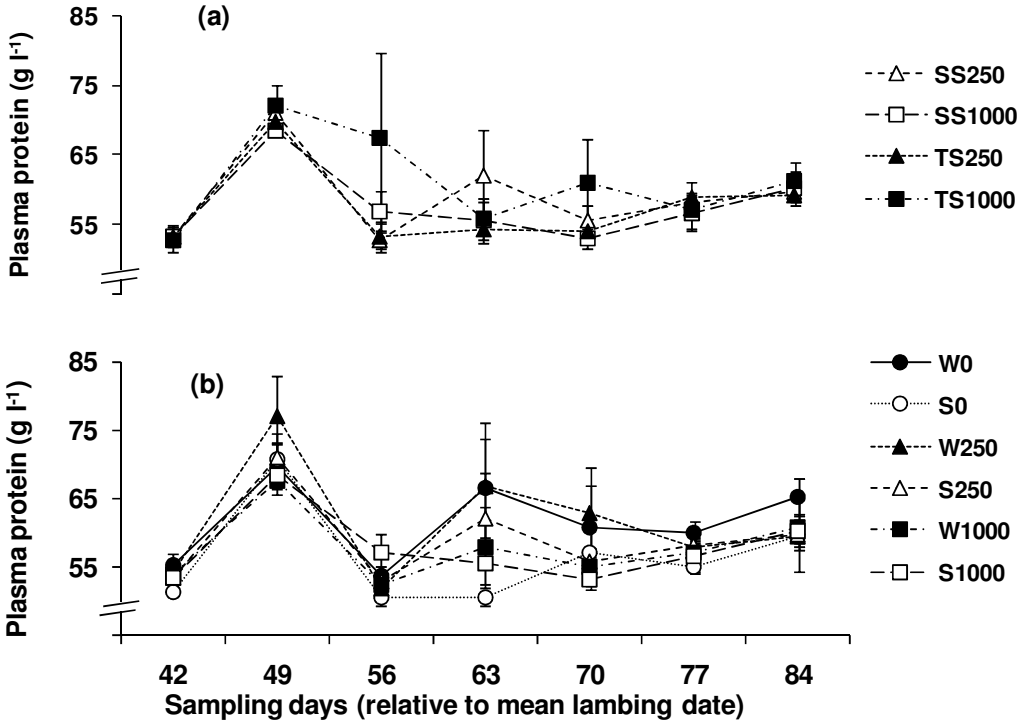


Figure 3.7: Mean plasma protein of (a) lambs single-suckled (SS-) or twin-suckled (TS-) and (b) those weaned (W-) at 39 days of age or suckled (S-) throughout the trial period, and infected with 0 (-0), 250 (-250), or 1000 (-1000) L_3 *T. circumcincta* larvae d^{-1} from day 42 of age onwards, respectively.

Mean plasma albumin of lambs is shown in Figure 3.8. A time × suckling interaction (P = 0.017) on plasma albumin was reflected by a drop in albumin with time and especially in weaned lambs after day 63. There were no significant effects of rate of infection, or suckling category on plasma albumin (P > 0.05). Overall, mean plasma albumin in the study ranged from 29.3 to 44.3 g l^{-1}.

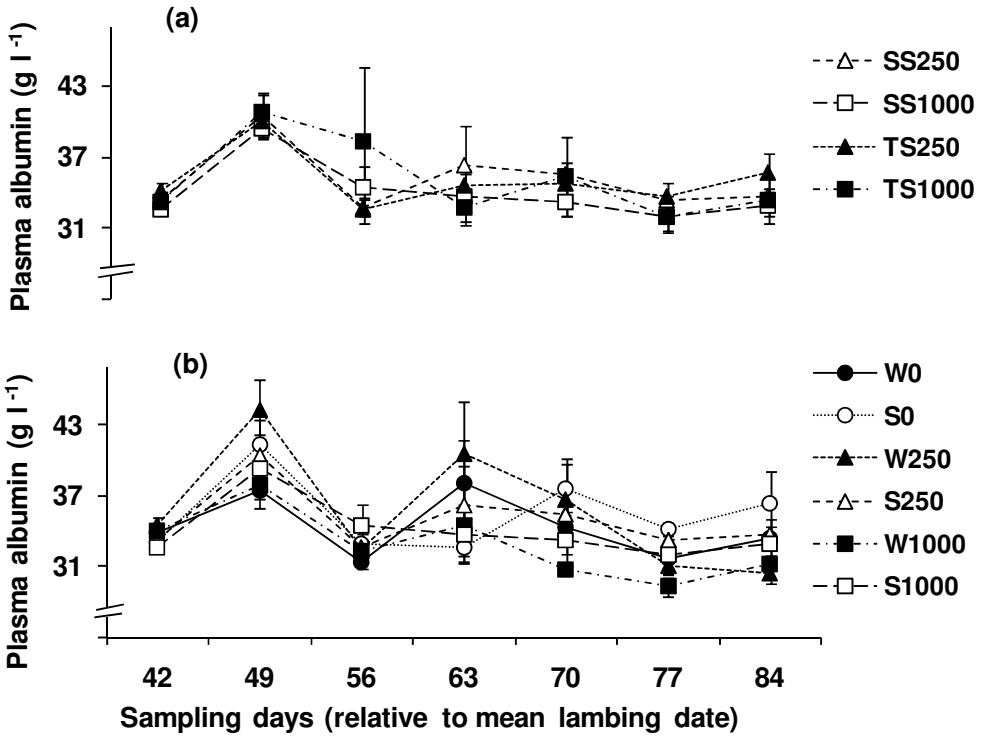


Figure 3.8: Mean plasma albumin of (a) lambs single-suckled (SS-) or twin-suckled (TS-) and (b) those weaned (W-) at 39 days of age or suckled (S-) throughout the trial period, and infected with 0 (-0), 250 (-250), or 1000 (-1000) L_3 *T. circumcincta* larvae d^{-1} from day 42 of age onwards, respectively.

3.3.7. Plasma pepsinogen and abomasal digesta pH

Mean plasma pepsinogen is given in Figure 3.9. For the W-/S- groups of lambs, a time × rate of infection interaction (P = 0.004) was reflected by a significant rise in plasma pepsinogen level in the 1000 L_3-infected lambs from day 56 onwards (Figure 3.8b).

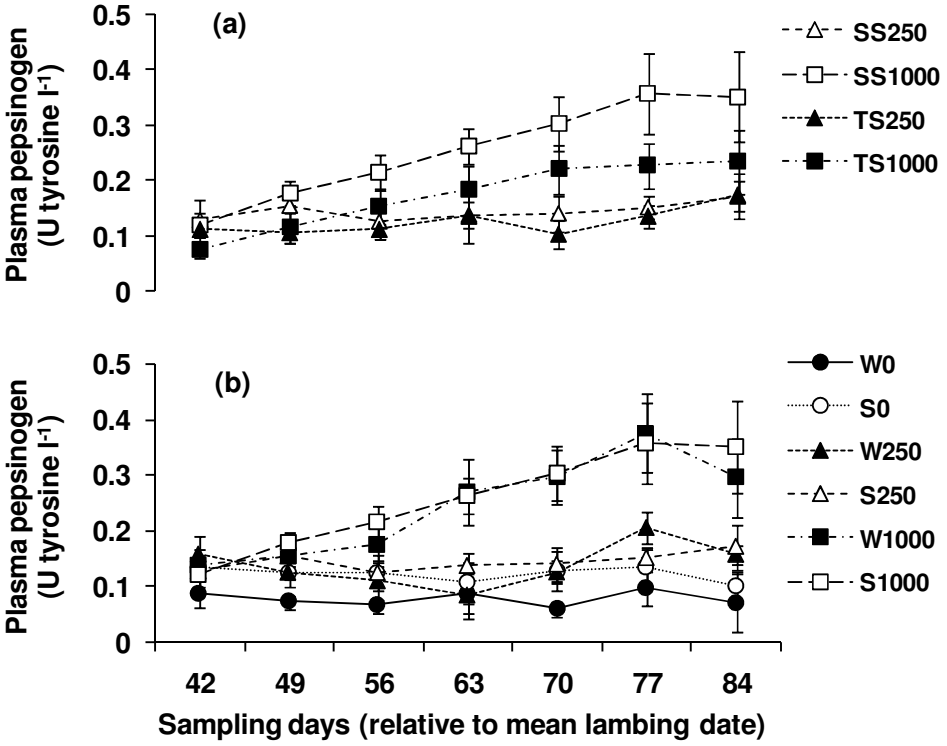


Figure 3.9: Mean plasma pepsinogen of (a) lambs single-suckled (SS-) or twin-suckled (TS-) and (b) those weaned (W-) at 39 days of age or suckled (S-) throughout the trial period, and infected with 0 (-0), 250 (-250), or 1000 (-1000) L_3 *T. circumcincta* larvae d^{-1} from day 42 of age onwards, respectively.

Values in uninfected lambs remained stable at an average of 0.1 U tyr l^{-1}, and there was a trend for slight increase (not significant) in those infected with 250 larvae d^{-1}. For the SS-/TS- groups of lambs, a similar time × rate of infection interaction (P = 0.014) was reflected by a significant rise in plasma pepsinogen level of the 1000 L_3-

infected lambs from day 77 onwards (Figure 3.8a). There were no significant effects of suckling, or suckling category on plasma pepsinogen level ($P > 0.05$).

Mean abomasal digesta pH of the lambs is given in Figure 3.10. A significant effect of rate of infection on abomasal digesta pH in the W-/S- ($P < 0.001$) and SS-/TS- ($P = 0.054$) groups of lambs was reflected by pH being directly proportional to the rate of infection (Table 3.2). A suckling × rate of infection ($P < 0.05$) interaction on pH was reflected by a significantly higher pH in the suckled lambs than their weaned counterparts at the 250 larvae dose level only. There was no significant effect of suckling category on abomasal digesta pH ($P > 0.05$).

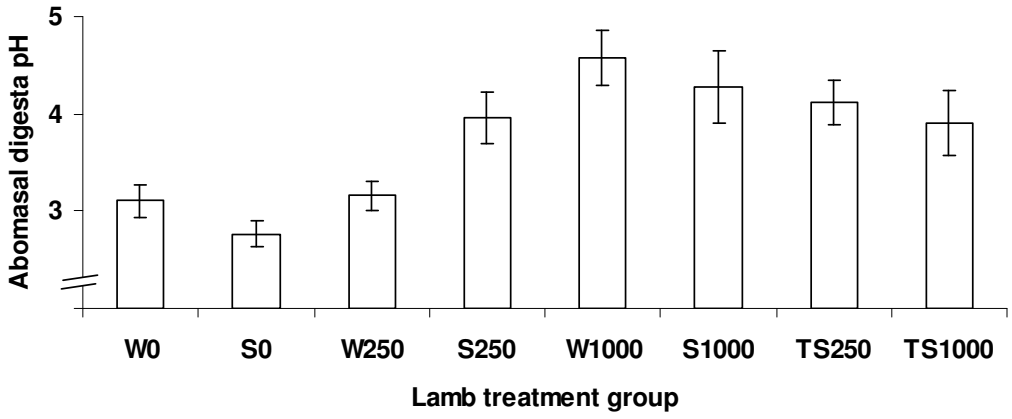


Figure 3.10: Mean abomasal digesta pH of lambs single-suckled (S-) or twin-suckled (TS-) and those weaned (W-) at 39 days of age or suckled (S-) throughout the trial period and infected with 0 (-0), 250 (-250), or 1000 (-1000) L_3 *T. circumcincta* larvae d^{-1} from day 42 of age onwards, respectively.

3.3.8. Correlations

Faecal egg count was positively correlated with total worm count (Table 3.3) from day 63 to necropsy on day 84 for both weaned and suckled lambs. Also, female worm length was positively correlated with FEC on days 77 and 84, respectively, for weaned

lambs but only on day 84 for suckled lambs. Egg *'in utero'* was positively correlated with FEC only on day 77 for weaned lambs and day 84 for suckled lambs. There were no relationships ($P > 0.05$) between weekly FECs and corresponding weekly serum IgA of lambs, except for a significant positive correlation on day 77 in weaned lambs.

Table 3.3: Correlation coefficients of the relationships between weekly FEC and post-mortem total worm count, female worm length, eggs *'in utero'*, and corresponding weekly serum IgA, respectively, of lambs weaned at 39, or suckled until necropsy at 84 days of age.

	FEC (Sampling days)						
Weaned lambs	**42**	**49**	**56**	**63**	**70**	**77**	**84**
Total worm count	#	#	-0.57^{**}	0.77^{***}	0.69^{***}	0.57^{**}	0.35^{ns}
Female worm length	#	#	#	-0.25^{ns}	0.46^{ns}	0.75^{***}	0.59^{*}
Eggs *'in utero'*	#	#	#	-0.32^{ns}	0.16^{ns}	0.52^{*}	0.45^{ns}
Serum IgA	#	#	-0.15^{ns}	-0.01^{ns}	0.03^{ns}	0.41^{*}	0.27^{ns}
Suckled lambs							
Total worm count	#	#	0.13^{ns}	0.65^{***}	0.77^{***}	0.65^{***}	0.56^{***}
Worm length	#	#	-0.01^{ns}	-0.45^{*}	-0.31^{ns}	0.16^{ns}	0.46^{*}
Eggs *'in utero'*	#	#	-0.06^{ns}	-0.47^{**}	-0.26^{ns}	0.32^{ns}	0.45^{*}
Serum IgA	#	#	0.19^{ns}	-0.04^{ns}	0.29^{ns}	0.16^{ns}	-0.06^{ns}

- no relationship due to zero FEC
ns – not significant $P > 0.05$
* - significant at $P < 0.05$
** - significant at $P < 0.01$
*** - significant at $P < 0.001$

The relationships between weekly average daily weight gain and the parasitological parameters of lambs (Table 3.4) were, in most cases, very weak and inconsistent, with insignificant low positive and negative correlations on different weeks of the 42-day trial. However, average daily weight gain correlated negatively ($P < 0.05$) with

corresponding weekly FEC on day 70 in weaned lambs, but only so with total worm count on day 77 ($P < 0.05$) in suckled lambs.

Table 3.4: Correlation coefficients of the relationships between weekly average daily weight gain and post-mortem total worm count, female worm length, eggs *'in utero'*, and corresponding weekly FEC, respectively, of lambs weaned at 39 days, or suckled until necropsy at 84 days of age.

	Average daily weight gain (Weekly)						
Weaned lambs	**42**	**49**	**56**	**63**	**70**	**77**	**84**
Total worm count	#	0.06^{ns}	0.24^{ns}	-0.06^{ns}	-0.19^{ns}	0.1^{ns}	-0.37^{ns}
Female worm length	#	-0.02^{ns}	-0.39^{ns}	-0.05^{ns}	-0.22^{ns}	0.19^{ns}	0.26^{ns}
Eggs *'in utero'*	#	0.14^{ns}	-0.34^{ns}	0.18^{ns}	0.02^{ns}	0.34^{ns}	0.22^{ns}
FEC	#	#	0.11^{ns}	0.03^{ns}	-0.44^{*}	0.05^{ns}	0.07^{ns}
Suckled lambs							
Total worm count	#	-0.07^{ns}	-0.16^{ns}	-0.04^{ns}	-0.13^{ns}	-0.35^{*}	-0.09^{ns}
Female worm length	#	0.11^{ns}	0.15^{ns}	-0.11^{ns}	0.30^{ns}	0.23^{ns}	0.04^{ns}
Eggs *'in utero'*	#	0.08^{ns}	0.27^{ns}	-0.07^{ns}	0.27^{ns}	0.38^{ns}	0.04^{ns}
FEC	#	#	0.19^{ns}	-0.21^{ns}	-0.17^{ns}	-0.11^{ns}	-0.05^{ns}

- no relationship
ns – not significant $P > 0.05$
* - significant at $P < 0.05$
** - significant at $P < 0.01$
*** - significant at $P < 0.001$

The relationship between serum IgA concentration and the parasitological parameters (Table 3.5) were, in most cases, weak and inconsistent, with insignificant low positive and negative correlations on different blood sampling days (for IgA measurement). Significant positive correlations were found between IgA and worm length, and eggs *'in utero'*, on sampling day 56 for weaned lambs, but were earlier for suckled lambs on day 49. Average daily weight gain of lambs had no relationship with

corresponding weekly serum IgA, except for a positive correlation ($P < 0.05$) on day 63 in suckled lambs.

Table 3.5: Correlation coefficients of the relationships between weekly serum IgA concentrations and post-mortem total worm count, female worm length, eggs *'in utero'*, and corresponding weekly average daily weight gain, respectively, of lambs weaned at 39, or suckled until necropsy at 84 days of age.

	Serum IgA (Sampling days)						
Weaned lambs	**42**	**49**	**56**	**63**	**70**	**77**	**84**
Total worm count	-0.64**	-0.07^{ns}	0.34^{ns}	0.09^{ns}	0.17^{ns}	0.48*	0.11^{ns}
Worm length	0.01^{ns}	0.46^{ns}	0.51*	-0.21^{ns}	0.36^{ns}	-0.21^{ns}	-0.01^{ns}
Eggs *'in utero'*	0.1^{ns}	0.54*	0.51*	-0.18^{ns}	0.28^{ns}	-0.4^{ns}	0.06^{ns}
Ave. daily weight gain	#	0.04^{ns}	-0.24^{ns}	0.15^{ns}	-0.21^{ns}	0.1^{ns}	0.05^{ns}
Suckled lambs							
Total worm count	0.18^{ns}	-0.06^{ns}	-0.34*	0.08^{ns}	0.28^{ns}	0.23^{ns}	-0.05^{ns}
Worm length	-0.11^{ns}	0.44*	-0.08^{ns}	0.10^{ns}	-0.46*	-0.08^{ns}	0.13^{ns}
Eggs *'in utero'*	-0.16^{ns}	0.54**	-0.08^{ns}	0.23^{ns}	-0.38^{ns}	-0.1^{ns}	0.12^{ns}
Ave. daily weight gain	#	-0.07^{ns}	0.1^{ns}	0.34*	0.08^{ns}	-0.25^{ns}	-0.03^{ns}

ns – not significant $P > 0.05$
* - significant at $P < 0.05$
** - significant at $P < 0.01$

Significant negative correlations were found between total worm count, and female worm length and eggs *'in utero'* (Table 3.6) in suckled lambs but not in weaned lambs. Female worm length and eggs *'in utero'* were positively and significantly correlated in both suckled and weaned lambs.

Table 3.6: Correlation coefficients of the relationships (pair wise) between total worm counts, female worm length, and eggs *'in utero'*, respectively, of lambs weaned at 39 days, or suckled until necropsy at 84 days of age.

Weaned lambs	Total worm counts	Female worm length
Female worm length	-0.08 ($P = 0.75$)	-
Egg *'in utero'*	-0.42 ($P = 0.11$)	0.71 ($P < 0.01$)
Suckled lambs		
Female worm length	-0.35 ($P = 0.08$)	-
Egg *'in utero'*	-0.4 ($P < 0.05$)	0.91 ($P < 0.001$)

3.4. Discussion

The data in the present study support the hypothesis that continued suckling can reduce establishment of *T. circumcincta* in young lambs. The twin-suckled groups were formed as a safe-guard against any problems that may arise from ewe behaviour and lamb suckling behaviour due to the weaning of one lamb in a twin set, and to investigate the possible effects of multiple suckling (single or twin-suckled) and therefore individual milk supply. These groups were observed to show similar results as their single-suckled counterparts among the W-/S- group of lambs, which suggests that the weaning of a twin has not confounded the results in the W-/S- design. This discussion will therefore focus more on observation from the W-/S- design with corroborations from the SS-/TS- design where necessary.

The 42-day length of the trial and low rates of infection (250 and 1000 L_3 larvae d^{-1}; approximately 14 and 54 L_3 larvae kg LW^{-1} d^{-1}, respectively) were chosen specifically to maximise the ability to test the hypothesis that milk *per se* may have a direct effect on nematode development, rather than an indirect effect through enhancement of host immunity by superior nutrient supply. Earlier studies, though with older lambs and higher rates of infection (30 - 180 L_3 larvae kg LW^{-1} d^{-1}), had suggested, on the basis of the timing of the asymptote of FEC after first exposure of naïve lambs to larvae, that host immunity could be anticipated to begin to limit the worm population after about six weeks of infection (Gibson & Parfitt 1976; Coop *et al.* 1977, 1982). In addition, the well established effect of dietary protein in enhancing the development of host immunity in previously naïve animals has only been observed after about 6 weeks of infection (Bown *et al.* 1991; van Houtert *et al.* 1995). Therefore, it seems plausible to suggest that a direct effect of milk on larval establishment had occurred in the

present study rather than an effect of enhanced host immunity through greater nutrient supply from milk. The very low FEC and worm burdens in the uninfected lambs (W0 and S0) confirmed that the pasture in this study was reasonably 'parasite safe'; conditions which would allow maximum expression of the effects of the contrived infection. The absence of clinical symptoms of infection such as breech soiling, or mortality suggests that all the lambs, even those weaned, were able to tolerate the level of infection adopted in this trial.

A feature of this study, however, was the evidence of strong intra worm-population regulation dynamics. This would have made interpretation of effects of continued suckling problematic if based simply on FEC. Faecal egg counts of weaned and suckled lambs infected with 1000 L_3 larvae d^{-1} were identical except for a trend for an earlier rise in the former (Figure 3.1b). Moreover, while weaned lambs receiving the lower infection rate (250 L_3 larvae d^{-1}) tended to have highest FEC on day 77, by 84 days they had fallen to similar values to those in their suckled contemporaries (Figure 3.1b). On the other hand, the net establishment of larvae, calculated as the number of L_3, L_4 and adult worms at necropsy relative to total larval challenge, was 26 and 28 % in weaned lambs infected with 250 and 1000 larvae d^{-1}, triple and double the 8 and 16 % observed at these rates of infection in suckled lambs, respectively. These rates of establishment are generally in the same range of 13 – 17 % and 20 % observed by Coop *et al.* (1977) and Smith *et al.* (1985), respectively, though in older sheep and which had experienced greater opportunity (time) to develop immunity. Leathwick *et al.* (1999) similarly observed a *T. circumcincta* larval establishment rate of 25 % in weaned parasite-naïve lambs. Although the differences in worm population numbers in the present study were not in themselves statistically significant, the large differences

in back-transformed worm counts and the trend for greater worm length and significantly greater number of eggs *'in utero'* of worms from suckled than from weaned lambs (Table 3.2) provide strong evidence of reduced worm crowding in a smaller worm population, or greater egg-laying by a numerically smaller but physiologically better developed nematode population. This is further evident in the negative correlations (Table 3.6) between total worm count, and female worm length and fecundity (eggs *'in utero'*). This intra-population regulation seems likely, in part at least, to be responsible for the lack of difference in FEC between suckled and weaned lambs during the later stages of the trial or between lambs subjected to the different rates of infection. The regulation of FEC, in terms of the decline in all groups as early as 63 – 70 days of age (21 – 28 days of infection), occurred much more rapidly, and completely than has been seen in studies with this nematode in older animals (Coop *et al.* 1982; Houdijk *et al.* 2006). Moreover, this earlier regulation, and the fact that it tended to occur earlier in the groups of lambs that were given the higher larval dose (Figure 3.1a and 3.1b), further suggests more of a parasite population-, than host immunity-induced phenomenon at play. This is also supported by the much larger proportion of immature worms from the lambs that received the higher larval dose (Table 1). A similar density-dependent influence of worm number on female worm length and fecundity in *T. circumcincta* nematode has been reported (Coop *et al.* 1977; Waller & Thomas 1978; Stear & Bishop 1999). The worm length in the present lambs infected with 250 larvae d^{-1} – ranging from 10.7 - 11.8 mm – was at the larger end of the range in the literature. Waller and Thomas (1978) observed worms of similar length – 11.5 mm – also in young suckling lambs that fell into the range (7.2 – 9.05 mm) observed in adult sheep exposed to similar rates of infection (Coop *et al.* 1977;

Donaldson *et al.* 2001) when the same lambs were weaned, older and carrying greater worm burdens.

The numbers of eggs observed in the *uteri* of female worms from the S250 and TS250 lambs (56 and 75epu, respectively), for example, are very much greater than previous observations though there are relatively few observations in the literature. The numbers were much greater than the 33 epu observed in females of this nematode from adult sheep given comparable rates of infection - 14 vs.21 L_3 larvae kg LW^{-1} d^{-1} (Donaldson *et al.* 2001). These data, together, allow the conclusion that the nematode population was subject to minimal pressure from the host immune system in this study. The 2 – 3.5 fold reduction in number of worms found at necropsy in suckled compared to weaned lambs seems likely therefore, to be attributable to a direct effect of continuing milk intake on worm establishment. The findings support those of Zeng *et al.* (2001; 2003) and suggest that milk could have a direct effect on larval establishment in the abomasum. Zeng *et al.* (2003) speculated that the effect was due to milk proteins or components associated with the proteins that reduced the motility of both sheathed and exsheathed larvae of *T. circumcincta*. On the assumption that the oesophageal groove reflex was operating to deliver milk beyond the rumen, we can further speculate that the effect would have occurred in the abomasum. It would therefore appear most likely to operate on exsheathed larvae as one would have anticipated exsheathment to occur predominantly in the rumen (Soulsby 1982; DeRosa *et al.* 2005).

In older immune sheep, shorter and less fecund adult worms have been associated with increased local IgA activity against 4^{th}-stage larvae (Stear *et al.* 1995). Also,

parasite-specific increases in serum IgA to L_3 larvae have been observed in previously naïve sheep during the period of acquisition of immunity to nematodes, though only after six weeks of infection (Greer *et al.* 2005). Despite significant differences in worm length between groups, there were no differences in serum IgA against L_3 larvae or any evidence for variation in any of the immunoglobulin isotypes with variation in larval challenge, worm burden or worm size in the present study. While circulating concentrations do not necessarily closely reflect production of IgA at the site of infection (Sinski *et al.* 1995), transient increases in the peripheral levels have been observed in older animals in association with the acquisition of immunity (Greer *et al.* 2005) and the subsequent reduction in FEC (Stear *et al.* 1995; Stear *et al.* 2003). Lack of change in these parameters of the immune response in association with the reduction in FEC in the present study adds further support to the hypothesis that the differences in worm size were worm population induced. Moreover, the correlations between weekly serum IgA and the parasitological parameters, in the present lambs (Table 3.5) showed very weak and inconsistent relationships, which further support the inference above.

The effect of infection on the performance of the lambs was smaller than anticipated and suggests either low pathogenicity of infection, as a result of the low rates of infection (14 and 54 L_3 larvae kg LW^{-1} d^{-1}), or that the lambs were relatively resilient to the pathogenic effects of infection. As expected, suckling enhanced the growth of lambs, but a greater effect of the imposed infection on performance of the weaned lambs than on the suckled lambs had been anticipated. Indeed, the decision to have two rates of larval infection in this study – 250 and 1000 larvae d^{-1} – was because I was unsure from literature as to whether lambs could withstand the higher rate of

infection without losses due to mortality, which would have confounded this experiment. However, weaned and suckled lambs infected with 1000 larvae d^{-1} suffered a similar proportional reduction in live-weight, relative to the weaned or suckled uninfected controls (20 and 15 %, respectively), despite the former harbouring more worms. Moreover, the weekly average daily weight gains of weaned and suckled lambs were weakly correlated (Table 3.4) with the parasitological indices in this study.

Several factors could indicate the possibility of low pathogenicity. First, serum pepsinogen levels though directly related to larval dosing (ranging from 0.11 – 0.18 and 0.13 – 0.37 U tyrosine l^{-1} in 250 and 1000 L_3-infected lambs, respectively), were lower than levels observed in older lambs exposed to higher rates of infection *viz.* 0.24 – 1.20 U tyrosine l^{-1} at 130 L_3 kg LW^{-1} d^{-1} (Sykes & Coop 1977); 0.30 – 2.50 U tyrosine l^{-1} at 300 L_3 kg LW^{-1} d^{-1}(Lawton *et al.* 1996); and 0.10 – 1.50 U tyrosine l^{-1} at 133 L_3 kg LW^{-1} d^{-1} (Barrett *et al.* 1998). Elevated pepsinogen was only observed in the lambs infected with 1000 L_3 d^{-1} (Figure 3.9a and 3.9b). This was surprising, especially in the later stages of the trial when worm masses of the W250 and S1000 groups were similar, since circulating pepsinogen concentrations have been associated with the presence of adult worms and not early infective larval stages or invasion of the mucosa (Lawton *et al.* 1996; Stear *et al.* 1999a; Scott *et al.* 2000).

Similarly, changes in plasma albumin concentration were small and more related to nutritional status than rate of infection, values dropping as a consequence of weaning rather than as an effect of infection, as had been anticipated on the basis of the literature (Coop *et al.* 1977; Stear *et al.* 2000). This suggests little competition existed, for example from an immune response, for amino acid needed for synthesis of albumin

to replace that leaked as a consequence of infection in the present work as has been suggested by reduction, due to supplementation, of hypoalbuminaemia in sheep exposed to 3750 L_3 larvae d^{-1} (Stear *et al.* 2000).

On the other hand, the changes in the pH of abomasal contents were comparable to those reported previously in older lambs, when exposed to higher rates of infection [3.3 – 3.9; Sykes and Coop (1977), Simcock *et al.* (1999), Scott *et al.* (2000)]. Interestingly, lambs with very different rates of infection and burdens at slaughter (48 and 2650 worms in W0 and W250 lambs, respectively) had comparable abomasal pH (3.11 and 3.16, respectively), and suckled lambs had higher abomasal pH than their weaned contemporaries at the same rate of larval dosing (250 larvae d^{-1}) while carrying lower worm burdens at slaughter (766 vs. 2650 worms). These observations may well be in line with those of Lawton *et al.* (1996) - that in sheep, abomasal pH often decreases to near normal levels around patency after a single larval infection despite adult worms still being present. In addition, young parietal cells mature within two days (Karam 1993) and then become very active (Karam *et al.* 1997), these cells could account for the abomasum regaining the ability to acidify its contents despite continued exposure to the parasites (Lawton *et al.* 1996) or presence of large worm burdens as observed in some of the lambs in the present study. It can therefore be inferred that the pH of abomasal contents at necropsy may not be a good indicator of damage to the abomasum.

An apparently low pathogenicity is supported by observations at necropsy of a relative absence of abomasal lesions, an observation made previously in very young lambs by Zeng *et al.* (2001). The resilience to infection may therefore reflect the

possibility that pathogenicity is a reflection of the strength of the host immune response *per se*. Greer *et al.* (2005) have demonstrated, by immune suppression, that a significant part of the reduction in animal performance during nematode parasitic infection is a consequence of the host's own immune response to the parasite, which includes reduction in voluntary food intake, particularly during the acquisition phase of immunity. Moreover, they observed lack of hypoalbuminaemia during infection when immune responses were suppressed with corticosteroids. The lack of evidence for the benefit of milk feeding on resilience, therefore, could simply mean that the lambs had not yet invoked an immune response to infection sufficient to trigger its associated nutritional costs (Hein *et al.* 2001; Colditz 2002) at this young age. Support for such a hypothesis is evident in the lack of changes in immune system parameters such as serum IgA. Competition for nutrients between immune and other body functions appeared only to occur during the acquisition phase of immunity as demonstrated in immune suppression studies and in association with an IgA response in serum (Greer 2005; Greer *et al.* 2005). The coincidence of impaired production parameters such as weight gain and nitrogen metabolism in association with the commencement of reductions in FEC and in blood eosinophil concentrations in long term studies of infection of naïve lambs with *Trichostrongylus colubriformis* (Steel *et al.* 1982; Kimambo *et al.* 1988) supports this conclusion. Moreover, FEC of lambs in the present study was weakly correlated with the corresponding weekly serum IgA (Table 3.3).

3.5. Conclusions

Several conclusions can be drawn from the results of this study. First, the very strong intra-population regulation of worm size and fecundity may render FEC an

insensitive quantitative measure of the magnitude and pathogenicity of parasitic infections in very young lambs. Secondly, the effects of infection on performance in very young lambs may have been overestimated. Thirdly, the weak relationship between FEC and worm burden, or between FEC and growth performance of lambs in this study argues for care in the interpretation of FEC in assessing the need to drench very young lambs. Finally, while milk may have some anti-parasitic properties, its benefit seems to lie more in providing nutrients for enhanced growth rate rather than in improving the resilience of the lamb to infection.

Chapter 4

Effects of suckling, and infection with the abomasal nematode parasite, *Teladorsagia circumcincta*, on the parasite status, immune development, and resilience of young lambs during the six to twenty-week post-lambing period

4.1. Introduction

The lower worm burden of suckled lambs in chapter three was attributed to a direct adverse effect of milk on larval establishment (Zeng *et al.* 2001; Zeng *et al.* 2003). Contrary to expectation, however, weaned lambs showed comparable resilience to their suckled contemporaries in terms of weight gain and carcass weight, despite harbouring almost twice the worm burden of the suckled lambs (Chapter 3). It was argued they may have been too young to exhibit an immune response (Smith *et al.* 1985; Kambara *et al.* 1993; Colditz *et al.* 1996) sufficient enough to affect performance, either through reduced intake (Greer *et al.* 2005) or through diversion of nutrients to immune functions (Coop & Kyriazakis 1999). The lack of an IgA response typical of the phase of acquisition of immunity in these lambs supported this argument. If this was the case, the chance of seeing a beneficial effect of milk on resilience would have been reduced.

Immunity to nematode infection has been considered to be a function that competes for nutrient resources against the requirement of the host to maintain other body functions (Coop & Kyriazakis 1999; Colditz 2002). In young growing lambs, the high protein requirement, relative to energy, for the deposition of lean tissue (Orskov 1992) may create a situation of competition for protein between immune function and growth. Indeed, post-ruminal protein supplementation of young lambs has been shown

to result in decreased faecal egg count and worm burden (Bown *et al.* 1991), and an increased concentration of mast cell protease in the abomasal mucosa of young lambs (Coop *et al.* 1995).

Milk contains a ratio of 10.7 g amino acid per megajoule of metabolisable energy (Geenty & Sykes 1983), which escapes microbial degradation in the rumen of suckling young ruminants via the oesophageal groove reflex. This will enhance protein nutrition of the lamb, and could theoretically ensure an ample supply of this nutrient that may facilitate immunological development with little diversion of nutrients to productive functions such as growth. It is anticipated that commencement of immunological responses by lambs at an older age (Gibson & Parfitt 1972; Smith *et al.* 1985; Kambara *et al.* 1993) might increase the protein demand, and therefore elicit the nutritional advantage of suckling over weaned lambs in development of immune response and resilience to infection.

This chapter describes a follow-up study that was designed to investigate the possible role of suckling for the resistance and resilience of lambs to *T. circumcincta* infection but was extended to the six to 20-week period after lambing when the immune response was anticipated to be strengthening. An *in vitro* direct larval challenge (IVDC) study, to monitor the tissue association phase of larval establishment, was also introduced.

4.2. Materials and methods

4.2.1. Management of experimental animals

Forty twin-bearing Coopworth ewes were selected, after scanning for number of lambs carried, and subjected to the same management conditions as with the ewes in Chapter 3. These included dosing with a controlled-release anthelmintic capsule 21 days prior to lambing (Extender^{TM} 100; 3.85 g/capsule of Albendazole @ a minimum dose of 0.5 mg/kg LW per day; Captec New Zealand Ltd, Auckland, New Zealand), and grazing on perennial ryegrass (*Lolium perenne L.*)-white clover (*Trifolium repens L.*) swards. Prior to commencement of the experiment lambs were tagged for identification along with recording of lambing date and were weighed fortnightly.

4.2.2. Experimental design

The resultant 80 twin-born lambs were allocated, along with their dams, to one of two infection (larval dose) groups *viz.* zero (-0) or 1000 (-I) L_3 *T. circumcincta* larvae d^{-1} from 42 days of age (day zero of experiment). One member of each set of twins was weaned (W-) on day 39 (approximately six weeks after median lambing date) while its twin was allowed to continue suckling (S-) until necropsy day. This created four treatment groups that were balanced for live weight (LW; mean ± SEM: 15.4 ± 0.38 kg on day 39 post-lambing), *viz.* weaned and not infected (W0; n = 16), suckled and not infected (S0; n = 16), weaned and infected (WI; n = 24), and suckled and infected (SI; n = 24). Suckled lambs and their dams were maintained on the ryegrass-white clover sward, while weaned lambs were reared in a separate area of the same paddock. A three-paddock rotational grazing of the two flocks was adopted to enable the nursing ewes, which had earlier been dosed with the controlled-release worm capsule, help reduce pasture larval contamination. The rotation also ensured an equivalent pasture

contamination, if any, for weaned and suckled groups. Ewes and their suckling lamb were made to follow the weaned lamb flock, which were moved to the third and fresh paddock, in the rotation.

Lamb live weight was recorded weekly. Faecal and blood samples were collected weekly as described in Chapter 3. Lambs were allocated hierarchically by lambing date to slaughter groups. Necropsy was carried out on groups of five and six lambs from each of the uninfected and infected treatments, respectively, on days 84 (week 12), 112 (week 16), and on six lambs from each group on day 140 (week 20) after lambing. In addition, six 'parasite-naïve' lambs were reared indoors, two of which were necropsied along with the outdoor-reared lambs at each slaughter date. Slaughter and worm recovery were carried out as described in Chapter 3. The ability of abomasal mucosa to prevent larval establishment was measured using an *in vitro* direct challenge (IVDC) technique, as described by Jackson *et al.* (2004), details of which are given in the next section.

4.2.3. Parasitology and necropsy procedure

The experimental procedures described in Chapter 3 were followed in the present study with respect to larval dosing, determination of faecal egg count, post-mortem worm counts, and measurement of worm length and carcass weight. Lambs to be necropsied were given the last larval dose five days prior to the necropsy date.

4.2.3.1. *In vitro* direct larval challenge technique: This was carried out on all lambs at the respective necropsies as described by Jackson *et al.* (2004). Immediately after the lambs were eviscerated at slaughter, a mid-section of the abomasum was clipped with forceps and a small fold, wide enough to cut six pieces of 20mm-diameter tissue

explants, was cut off from the rest of the abomasum. A purpose-made, sharp-edged, 20mm-diameter cutter was used to excise the six pieces of tissue explants (Plate 4.1), which were then rinsed gently in warm physiological saline (0.85%) to remove adherent abomasal digesta.

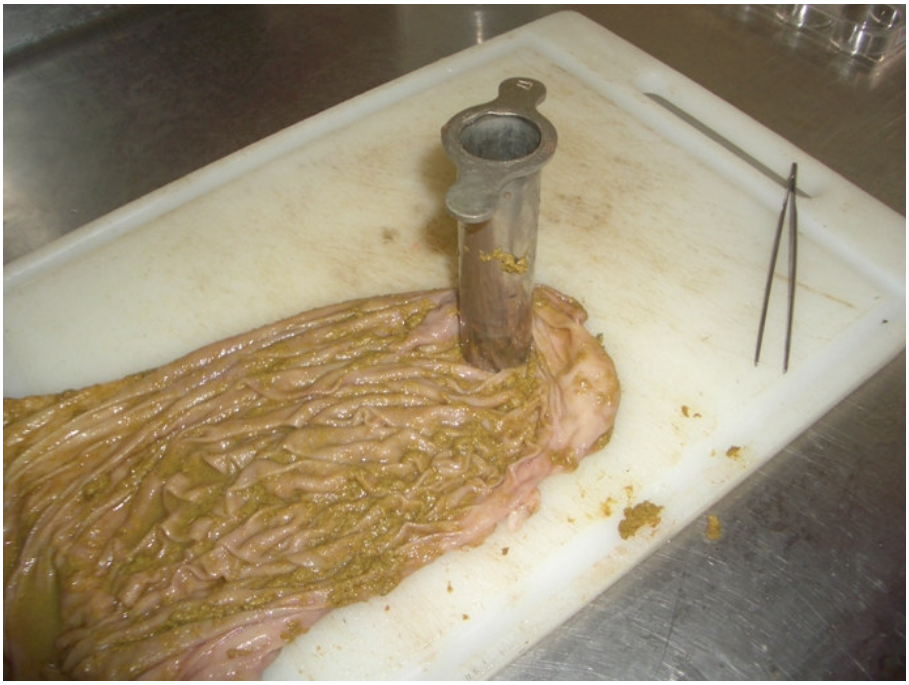


Plate 4.1: Excising an abomasal tissue explant with a 20-mm cork cutter. Photo courtesy of R. W. McAnulty.

The tissue explants were placed, one per well, into a six-well Corning plate. Warm Hank's medium (Sigma Chemical Company, St. Louis, MI, USA) with 20 mmol L^{-1} Hepes (Boehringer Mannheim GmbH, Germany) with 2 mL phenol red added to 1 L of sterile H_2O and brought to pH of 7.6 with NaOH, was added to surround but not submerge the tissues (Plate 4.2).

Plate 4.2: Abomasal tissue explant in Hank's medium. Photo courtesy of R. W. McAnulty.

Varying doses of exsheathed larvae were added into three of the six isolation cylinders, which were formed with the barrel of 5mL syringes and contained the larvae within a given area on the abomasal mucosal surface (Plate 4.3).

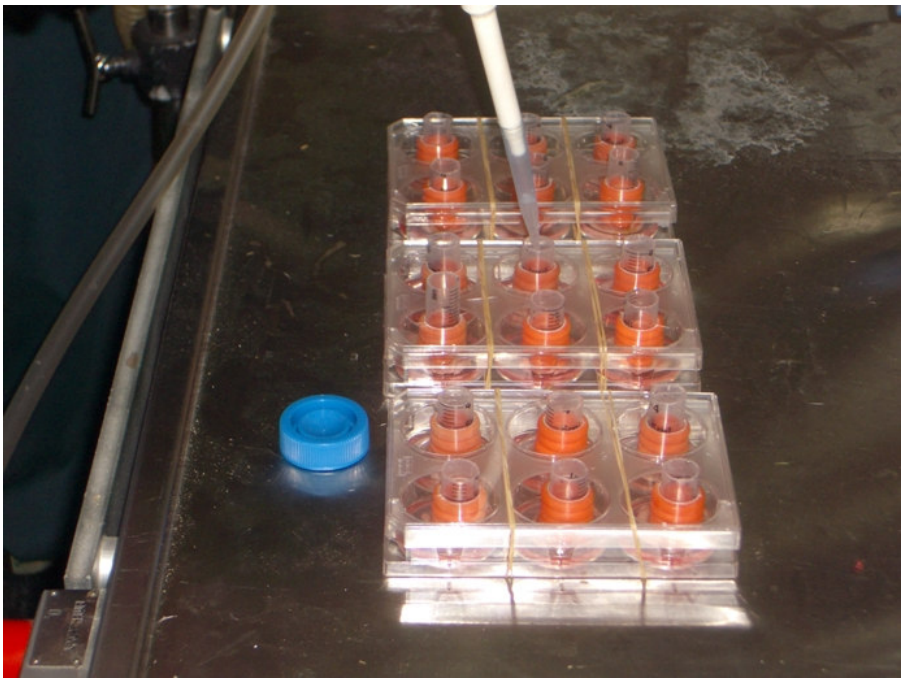


Plate 4.3: Exsheathed larvae being added on tissue explant inside isolation cylinder. Photo courtesy of R. W. McAnulty.

The remaining three tissue explants did not receive any larvae, effectively acting as controls. The plates were then flushed with oxygen (Plate 4.4) and incubated for 3 h at 37^{o}C.

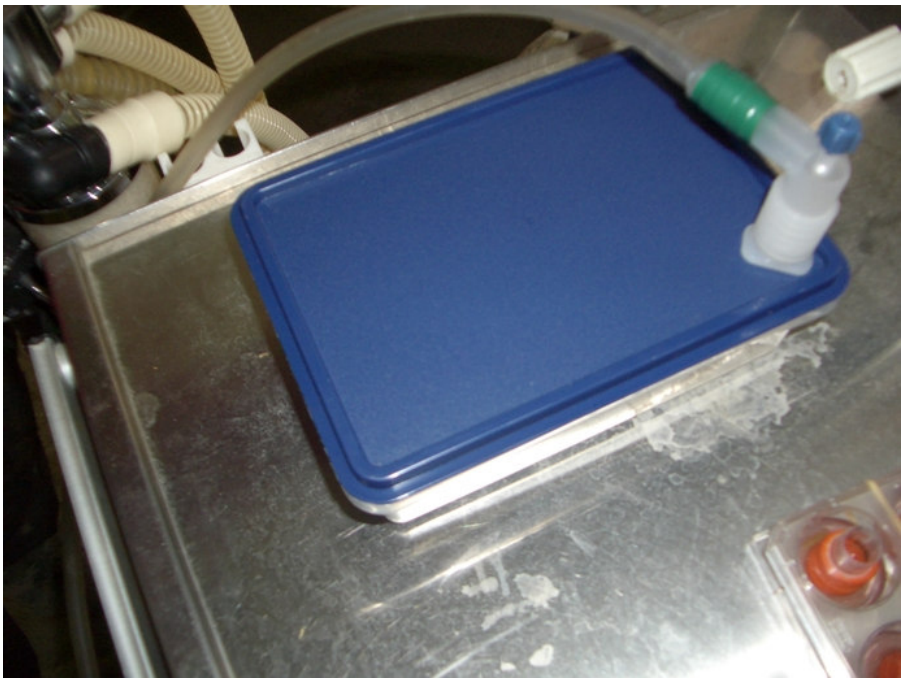


Plate 4.4: Tissue explant-fitted plates inside incubation chamber being flushed with Oxygen gas. Photo courtesy of R. W. McAnulty.

Tissues and isolation cylinders were then 'washed' by repeated plunging in warm physiological saline to recover any larvae that were loosely associated with tissues while the larvae that had penetrated the tissues were recovered after digestion of the tissue with 1% HCL pepsin. Larvae found in the saline wash and pepsin digest for each well were counted separately. Larval rejection was calculated as the number of larvae recovered in the wash divided by the total number recovered (washings and digest) for each well and expressed as a percentage.

4.2.4. Blood sampling and analyses

Blood samples were obtained weekly from lambs by jugular venipuncture as described in Chapter 3. Sample handling and analyses were carried out as described in

Chapter 3 with respect to plasma protein, plasma albumin, total antibody (Ig), immunoglobulin A (IgA), and immunoglobulin M (IgM). These parameters were measured on a weekly basis.

4.2.5. Statistical analyses

All data were analysed using the Genstat suite of statistical packages (GenStat Release 8.2 Copyright 2005, Lawes Agricultural Trust, Rothamsted Experimental Station). Abomasal worm burdens and FEC were log-transformed [LOG_{10}(count+1)] while percentage *in vitro* larval rejection underwent angular transformation before being analysed. All transformed data were later back-transformed to present group geometric means. Abomasal worm burdens, male and female worm length, percentage *in vitro* larval rejection, and carcass weight were analysed as an unbalanced design using ANOVA, with slaughter age, suckling (weaned or suckled), and infection as factors. However, the data for each necropsy were presented in separate Tables (Tables 4.1, 4.2, and 4.3, respectively). Percentage *in vitro* larval rejections for the naïve lambs (two lambs per necropsy date) were analysed using a two-way ANOVA with necropsy date and animal effect as factors.

Parameters measured on a weekly basis were subjected to sequential comparison of antedependence structures for repeated measures before being analysed using Restricted Maximum Likelihood (REML), with time, suckling (weaned or suckled), and infection (zero or 1000 L_3 d^{-1}) as factors. Data of repeated measures (weekly data) were analysed separately for the 80 lambs (with time period from onset of infection on day 42 up to first slaughter on day 84 of lamb age; phase 1), the remaining 58 lambs (from day 91 to second slaughter on day 112; phase 2), and then for 36 lambs (from

day 119 to last slaughter on day 140; phase 3), respectively. However, the graphical representations are given from onset of infection up to the time of necropsy, respectively (i.e. day 42 – 84 for the 80 lambs, day 42 – 112 for the 58 lambs, and day 42 – 140 for the 36 lambs).

The experiment was carried out under the authority of the Lincoln University Animal Ethics Committee (Approval No. 58).

4.3. Results

4.3.1. Clinical observations

None of the lambs showed any overt sign of infection, such as breech soiling, and no mortality was recorded during the course of the trial.

4.3.2. Faecal egg count

The mean faecal egg counts (FEC) of the lambs, according to the necropsy phases, are given in Figure 4.1. For the 80 lambs during the first phase (days 42 – 84), there was a time × infection interaction ($P < 0.001$) reflected in a greater FEC of the infected than the non-infected lambs from day 63 (week 3) until first slaughter on day 84 (week 6) and a trend for a suckling × infection interaction ($P = 0.089$) reflected in a smaller FEC in the SI compared with the WI group, which was most marked around the peak period of FEC on day 70 (Figure 4.1a). Faecal egg counts peaked at 218 e.p.g. on day 84 for WN, 251 e.p.g. on day 63 for SN, 1428 e.p.g. on day 70 for WI, and 633 e.p.g. on day 77 for SI. These were followed by a decline in FEC of both infected groups (WI and SI) by day 84 (Figure 4.1a).

For the 58 lambs remaining after the first slaughter, and covering the period – day 84 to 112 (second phase), a time × suckling interaction ($P = 0.015$) reflected FEC being smaller in suckled than in weaned lambs on days 98, 105, and 112, respectively (Figure 4.1b). Overall, FECs were at lower peaks during this phase compare with the first phase, except in the WN lambs, in which FEC rose to 480 e.p.g. on day 112 (Figure 4.1b), greater than the peak - 218 e.p.g. of this same group in the first phase.

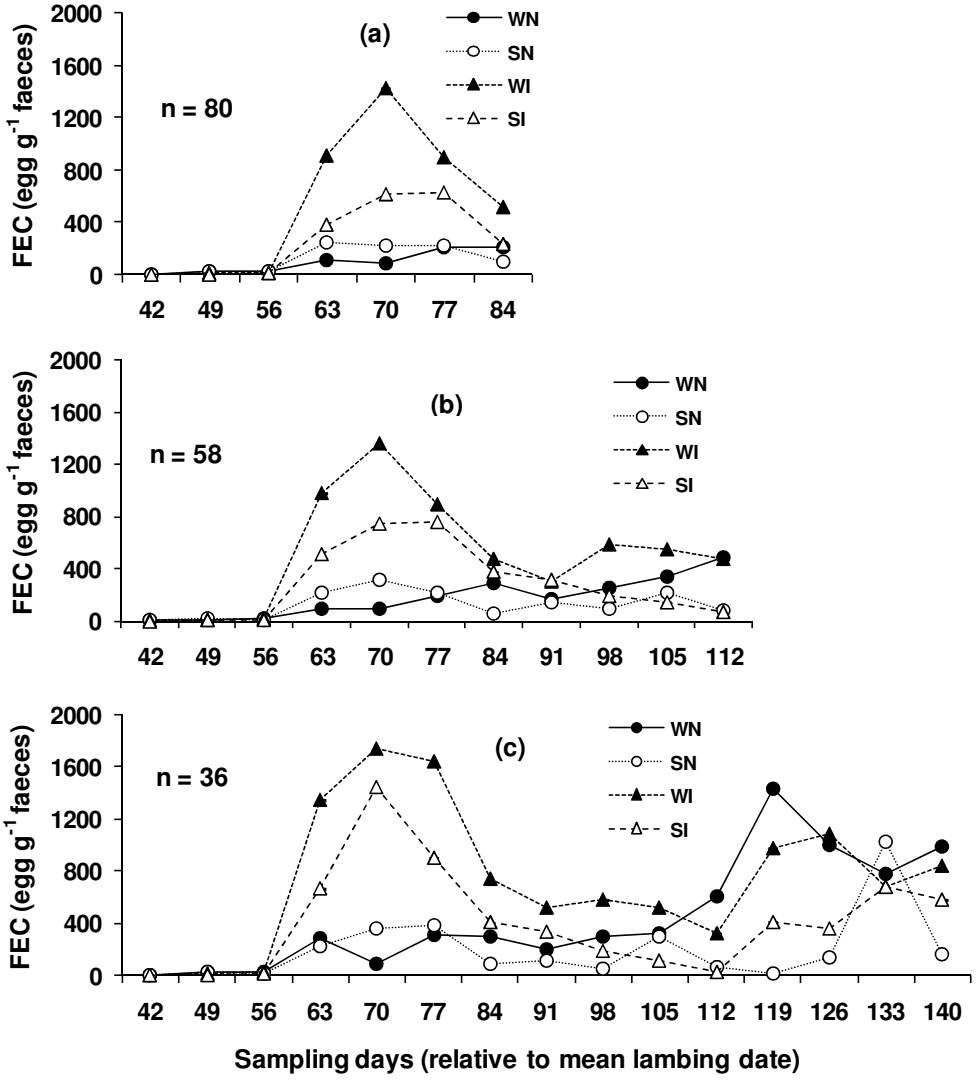


Figure 4.1: Geometric FEC means (eggs g^{-1} faeces) of lambs either weaned (W-) at 39 days of age, or continually suckled (S-) until necropsy, and dosed with either zero (-N) or 1000 (-I) L_3 *T. circumcincta* larvae d^{-1} from day 42 onwards, with necropsies of selected lambs on days 84, 112, and 140, respectively; 'n' represents the number of lambs carried on after each necropsy.

For the 36 lambs remaining after the second slaughter, and covering days 119 – 140 (third phase), a time × suckling × infection interaction (P = 0.006) reflected FEC being smaller in suckled than in weaned lambs of both the infected and uninfected groups on days 119 and 126, but only significantly so for the uninfected groups on day 140 (Figure 4.1c).

4.3.3. Abomasal worm burdens, male and female worm length, and in vitro larval rejection

Abomasal worm burdens, male and female worm length, and percent *in vitro* larval rejection are given in Tables 4.1, 4.2, and 4.3, showing these parameters in the first, second and third necropsies, respectively.

Parasitological data at the first necropsy on day 84 (12th week after lambing and six weeks of infection) are given in Table 4.1.

Table 4.1: Abomasal worm counts (Log_{10}-transformed values, with back-transformed means in parenthesis), female and male worm lengths (mm), and percentage in vitro larval rejection (angular-transformed values, with back-transformed means in parenthesis) in lambs either weaned (W-) at 39 days of age or suckled (S-) until necropsy on day 84 after lambing, and dosed with either zero (-N) or 1000 (-I) L_3 *T. circumcincta* larvae d^{-1} from day 42 onwards.

Lamb groups (n)	* Worm Counts				Female Worm Length (mm)	Male Worm Length (mm)	* % *in vitro* larval rejection
		Worm developmental stages					
	Total worm	L_3	L_4	Adults			
WN (5)	2.91^{c} (818)	0.21^{a} (1)	0.77^{c} (5)	2.91^{b} (806)	12.1^{a}	8.6^{a}	47^{b} (53)
SN (5)	2.83^{c} (669)	0.26^{a} (1)	0.72^{c} (4)	2.82^{b} (655)	11.2^{b}	8.2^{a}	49^{b} (58)
WI (6)	4.10^{a} (12722)	0.25^{a} (1)	3.30^{a} (2003)	4.02^{a} (10567)	11.0^{b}	8.6^{a}	64^{a} (81)
SI (6)	3.99^{b} (9850)	0.00^{a} (0)	2.72^{b} (521)	3.94^{a} (8769)	10.9^{b}	8.2^{a}	52^{b} (61)
SEM	0.088	0.180	0.280	0.100	0.24	0.16	5.7

^{abc} - Column means with similar superscript are not significantly different ($P > 0.05$).
SEM – Standard error of means.

* - Geometric (back-transformed) means in parenthesis.

Total worm burden, and numbers of L_4 and adult worm recovered from the infected groups were significantly greater ($P < 0.001$ in all cases) than those recovered from

the non-infected groups. Suckling significantly ($P < 0.05$) reduced worm burdens among the infected groups. Overall, the number of L_3-stage larvae was not influenced ($P > 0.05$) by suckling or infection in the first necropsy. The female worms recovered on the first necropsy were longer ($P < 0.05$) in the non-infected than in the infected lambs; however, suckling had no significant effect on female worm length except for a trend for longer worms ($P = 0.127$) in weaned than in suckled lambs, which was significant among the non-infected lambs. The male worms tended to be longer ($P = 0.064$) in weaned than in suckled lambs. The percentage *in vitro* larval rejection was not influenced ($P > 0.05$) by suckling or infection at this stage.

Parasitological data at the second necropsy on day 112 (16^{th} week after lambing and 12 weeks of infection) are given in Table 4.2 (next page). The total worm burden, number of adult worms and L_4-stage larvae were significantly reduced by suckling ($P < 0.01$, $P < 0.02$, and $P < 0.03$, respectively). Also, a suckling × infection interaction ($P < 0.05$) on the number of L_4-stage larvae reflected a significantly smaller number of L_4 worms in the SN compared with the WN lambs, but only a trend for smaller number of L_4 worms in the SI compared with the WI lambs. However, infection did not influence the total worm burden or number of adult worms at the second necropsy. In addition, the number of L_3- stage larvae was not influenced ($P > 0.05$) by suckling or infection as similarly observed at the first necropsy. A suckling × infection interaction on female worm length was reflected in shorter worms in SI compared with WI lambs but longer worms in SN compared with WN lambs. Male worms remained similar ($P > 0.05$) in length across groups except for significantly shorter worms from

Table 4.2: Abomasal worm counts (Log_{10}-transformed values, with back-transformed means in parenthesis), female and male worm lengths (mm), and percentage in vitro larval rejection (angular-transformed values, with back-transformed means in parenthesis) in lambs either weaned (W-) at 39 days of age or suckled (S-) until necropsy on day 112 after lambing, and dosed with either zero (-N) or 1000 (-I) L_3 *T. circumcincta* larvae d^{-1} from day 42 onwards.

Lamb groups (n)	*Worm counts				Female Worm Length (mm)	Male Worm Length (mm)	* % *in vitro* larval rejection
	Total worm	Worm developmental stages					
		L_3	L_4	Adults			
WN (5)	4.58^{a} (37669)	2.29^{a} (196)	4.29^{a} (19542)	4.21^{a} (16254)	8.5^{bc}	6.7^{a}	56^{b} (68)
SN (5)	3.61^{c} (4035)	2.58^{a} (377)	3.30^{c} (1985)	2.37^{c} (236)	9.8^{a}	7.1^{a}	51^{b} (60)
WI (6)	4.17^{b} (14927)	2.41^{a} (255)	3.83^{b} (6713)	3.84^{ab} (6981)	8.9^{ab}	6.6^{a}	63^{ab} (79)
SI (6)	3.78^{c} (5969)	2.50^{a} (314)	3.69^{b} (4886)	2.46^{bc} (289)	7.5^{c}	5.3^{b}	75^{a} (93)
SEM	0.187	0.273	0.178	0.479	0.58	0.41	9.1

^{abc} - Column means with similar superscript are not significantly different ($P > 0.05$).
SEM – Standard error of means.
* - Geometric (back-transformed) means in parenthesis.

suckled compared with weaned lambs of the infected group. Similarly, the percentage *in vitro* larval rejection was not influenced by suckling ($P > 0.05$) at the second necropsy but there was a trend for greater rejection of larvae by the mucosa explants of the infected lambs compared with those of the non-infected lambs ($P = 0.112$).

Parasitological data at the last necropsy on day 140 (20th week after lambing and 16 weeks of infection) are given in Table 4.3.

Table 4.3: Abomasal worm counts (Log_{10}-transformed values, with back-transformed means in parenthesis), female and male worm lengths (mm), and percentage *in vitro* larval rejection (angular-transformed values, with back-transformed means in parenthesis) in lambs either weaned (W-) at 39 days of age or suckled (S-) until necropsy on day 140 after lambing, and dosed with either zero (-N) or 1000 (-I) L_3 *T. circumcincta* larvae d^{-1} from day 42 onwards.

Lamb groups (n)	*Worm counts				Female Worm Length (mm)	Male Worm Length (mm)	* % *in vitro* larval rejection
	Total worm	Worm developmental stages					
		L_3	L_4	Adults			
WN (6)	4.37^{a} (23495)	2.41^{b} (254)	3.87^{a} (7412)	4.11^{a} (12822)	9.0^{a}	6.8^{a}	79^{a} (96)
SN (6)	4.25^{a} (17864)	2.13^{c} (132)	4.10^{a} (12588)	3.59^{ab} (3889)	7.2^{c}	6.0^{b}	78^{a} (96)
WI (6)	4.34^{a} (21826)	2.72^{a} (529)	3.95^{a} (8809)	4.07^{a} (11802)	8.4^{b}	6.6^{a}	73^{a} (91)
SI (6)	3.77^{b} (5860)	2.27^{bc} (186)	3.34^{b} (2177)	3.14^{b} (1386)	8.4^{b}	6.6^{a}	81^{a} (98)
SEM	0.179	0.102	0.192	0.311	0.26	0.15	5.1

^{abc} - Column means with similar superscript are not significantly different ($P > 0.05$).
SEM – Standard error of means.
* - Geometric (back-transformed) means in parenthesis.

A trend for a suckling × infection interaction ($P = 0.088$) on total worm burden reflected a significantly smaller worm burden due to suckling in the infected lambs, but only a trend for smaller worm burden due to suckling in the non-infected lambs. In addition, suckling tended to result in smaller numbers of adult worms ($P = 0.083$). A trend for a suckling × infection interaction ($P = 0.095$) in the number of L_4-stage larvae was reflected in greater numbers of L_4 worms in the WI than in the SI lambs while there was a trend for greater numbers of L_4 worms in the SN than in the WN

lambs. The number of L_3 larvae was greater ($P = 0.006$) in the weaned lambs than in suckled lambs, and tended to be greater ($P = 0.067$) in the infected compared with the non-infected lambs. A suckling × infection interaction resulted in shorter female ($P = 0.008$) and male ($P = 0.009$) worms due to suckling among the non-infected lambs while the infected groups of lambs harboured female worms of similar length. The percentage *in vitro* larval rejection was not influenced ($P > 0.05$) by suckling or infection at the last necropsy.

Generally, the total abomasal worm burdens were lower ($P < 0.001$) in the first necropsy compared with the second and third necropsies, which had similar worm burdens. The adult worm population was not influenced by lamb age at necropsy ($P > 0.05$). However, the numbers of immature worms (L_3 and L_4 larvae) were lesser ($P < 0.001$) in the first necropsy than in the second and third necropsies. On the other hand, the female worms were longer ($P < 0.001$) in length at the first necropsy (11.2 ± 0.16 mm) compared with those recovered at the second (8.8 ± 0.32 mm) and third necropsies (8.3 ± 0.20 mm), when worm length was similar. The same trend was observed for the length of male worms ($P < 0.001$; 8.4 ± 0.08, 6.7 ± 0.25, and 6.5 ± 0.10 mm, respectively). The percentage *in vitro* larval rejection was also influenced by lamb age at necropsy ($P < 0.001$); larval rejection by the mucosa explants increased with lamb slaughter age, averaging 64 ± 7, 77 ± 7, and 95 ± 4 % on necropsy days 84, 112, and 140, respectively. No L_3 larvae were recovered from either the wash or digest of the control tissue explants from any of the treatment groups at any necropsy i.e. tissues that did not received larvae *in vitro* prior to incubation. Percentage *in vitro* larval rejection in naïve animals was not influenced by slaughter date nor individual animal effect ($P > 0.05$), means being 67 (day 84), 46 (day 112), and 62 % (day 140).

Overall, a small intestinal worm population was made up of an average of 2400 *Trichostrongylus colubriformis* (46%), 1800 *Nematodirus species* (33%), 990 *Ostertagia species* (19%), and 90 *Cooperia species* worms (2%). Details of small intestinal worm populations are given in Appendices 4.1, 4.2, 4.3, and 4.4, respectively.

4.3.4. Immunology

The changes in total antibody (optical density; Ig) to L_3 *T. circumcincta* in plasma of lambs are given in Figure 4.2. Total antibody was influenced by time ($P < 0.001$) in the 80 lambs, reflecting an increase in total antibody from day 70 until the first slaughter on day 84 (Figure 4.2a). There were no significant effects ($P > 0.05$) of suckling or infection. For the 58 lambs during the second phase (day 91 – 112), a significant effect of time ($P < 0.001$) on total plasma Ig was reflected in a progressive increase of total plasma Ig in all groups from day 91 onwards (Figure 4.2b). There was no significant effect ($P > 0.05$) of suckling; however, infection resulted in greater total antibody ($P < 0.01$). In the third phase, a slight time × suckling × infection interaction ($P = 0.063$) reflected a trend for suckling to result in a rise in total antibody only of non-infected lambs on days 133 and 140 while infection resulted in a rise in both weaned and suckled lambs on day 119 but only in weaned lambs on days 133 and 140. Overall, total antibody increased with slaughter age ($P < 0.001$), being an average of 0.18 ± 0.001 OD during the first phase, 0.21 ± 0.002 OD during the second phase, and 0.25 ± 0.003 OD during the third phase.

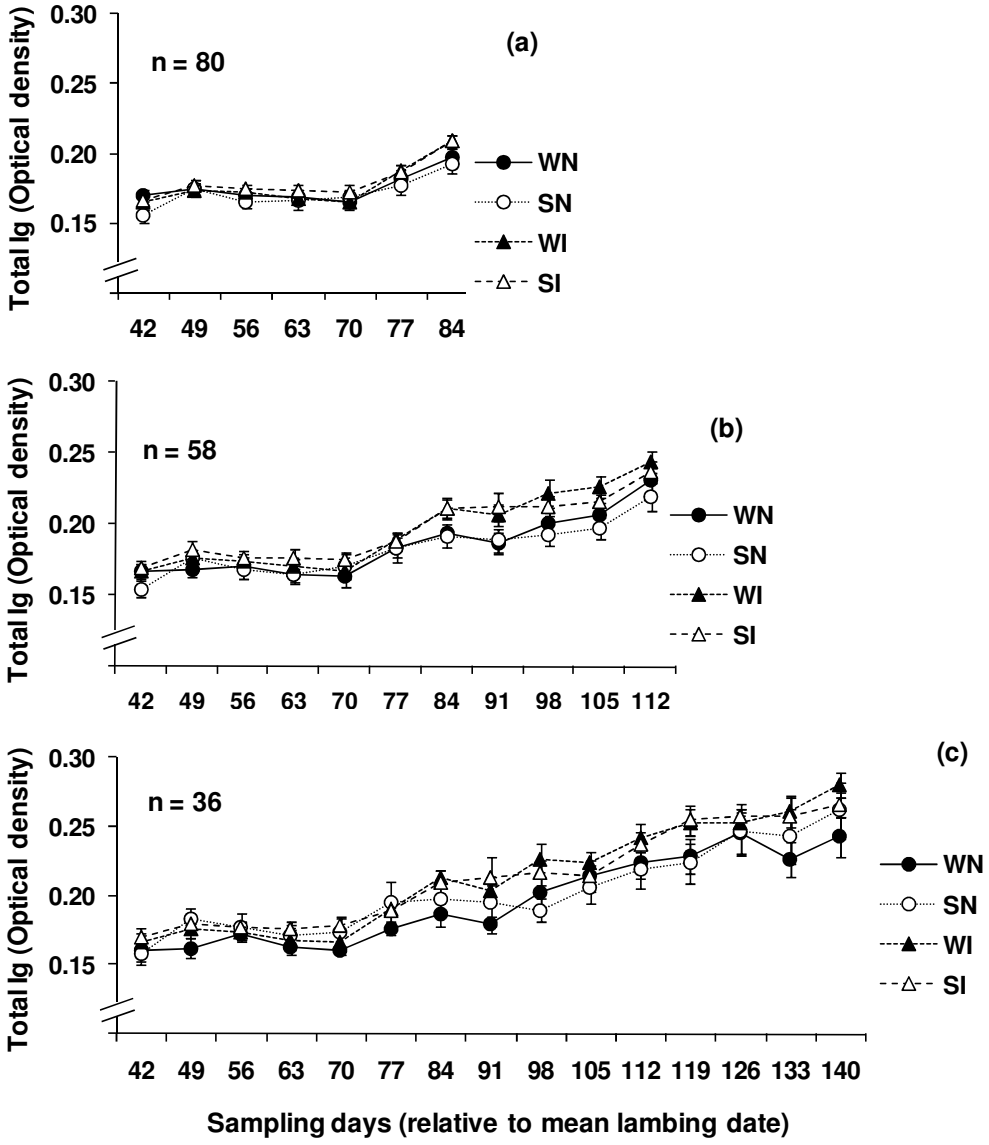


Figure 4.2: Total antibody (optical density ± SE) to L_3 *T. circumcincta* in plasma of lambs either weaned (W-) at 39 days of age, or continually suckled (S-) until necropsy, and dosed with either zero (-N) or 1000 (-I) L_3 *T. circumcincta* larvae d^{-1} from day 42 onwards, with necropsies of selected lambs on days 84, 112, and 140, respectively. 'n' represents the number of lambs carried on after each necropsy.

The changes in IgA response to L_3 *T. circumcincta* in plasma of lambs are given in Figure 4.3.

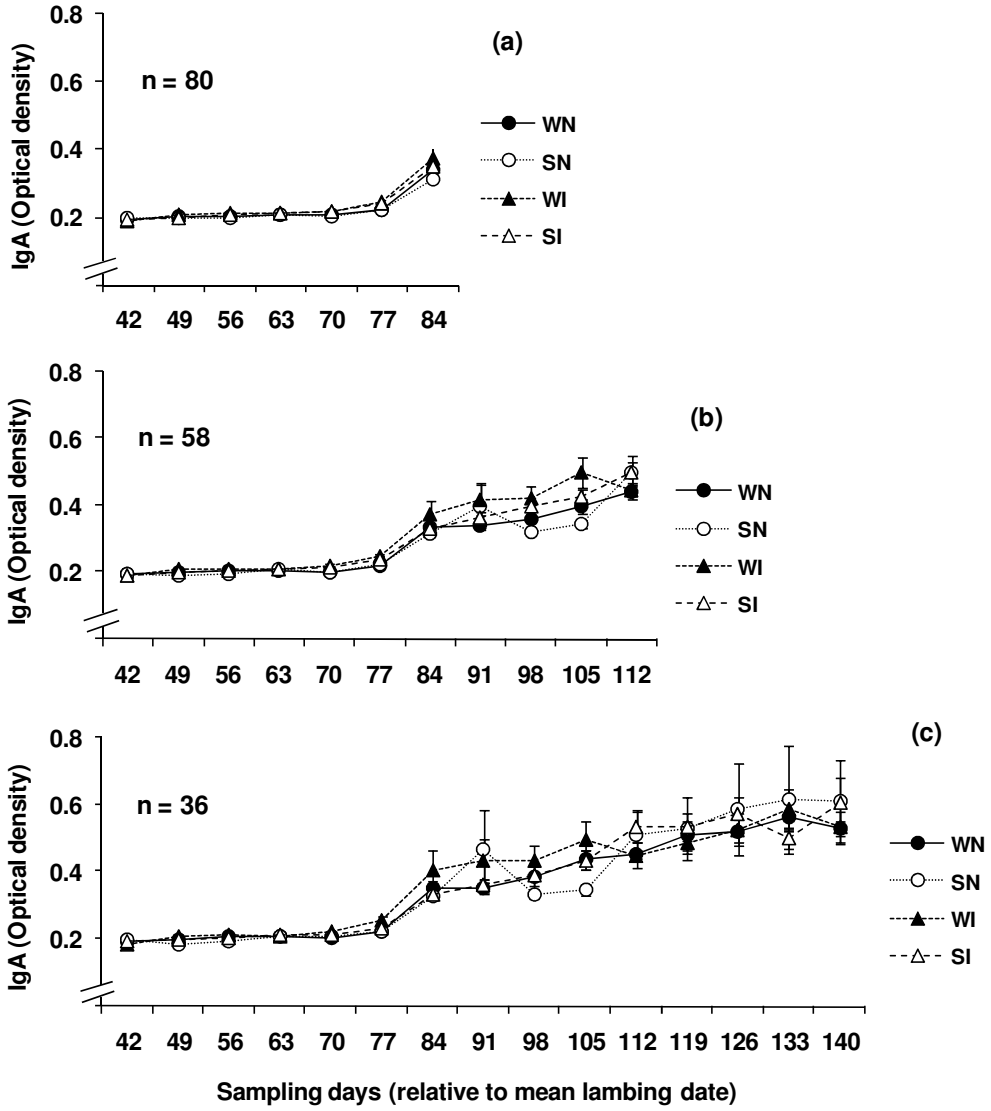


Figure 4.3: IgA (optical density ± SE) to L_3 *T. circumcincta* in plasma of lambs either weaned (W-) at 39 days of age, or continually suckled (S-) till necropsy and infected with either zero (-N) or 1000 (-I) L_3 *T. circumcincta* larvae d^{-1} from day 42 onwards, with necropsies of selected lambs on days 84, 112, and 140, respectively. 'n' represents the number of lambs carried on after each necropsy.

Plasma IgA was influenced by time ($P < 0.001$) in the 80 lambs, reflecting a significant increase in plasma IgA during the last week of the first phase, from day 70 until day 84 in all groups (Figure 4.3a). In addition, a time × infection interaction ($P <$

0.01) was reflected in greater plasma IgA of infected lambs compared with their uninfected contemporaries from day 70 onwards (Figure 4.3a). For the 58 lambs, a time × suckling interaction ($P < 0.01$), and a time × infection interaction ($P < 0.05$), on plasma IgA during the second phase (day 91 – 112) was reflected in similar plasma IgA concentrations for weaned and suckled, and infected and non-infected lamb after day 91. These were followed by greater titres in weaned lambs and infected lambs in both categories from day 98 (Figure 4.3b). In the third phase (from day 119 – 140), a trend for a time × suckling interaction ($P = 0.083$) was reflected in similar concentrations of plasma IgA in the weaned and suckled lambs from day 119 to day 133 but greater concentrations in suckled lambs on day 140 (Figure 4.3c). Overall, plasma IgA increased with slaughter age ($P < 0.001$), being an average of 0.23 ± 0.003 OD during the first phase, 0.42 ± 0.009 OD during the second phase, and 0.55 ± 0.016 OD during the third phase.

The changes in IgM response to L_3 *T. circumcincta* in plasma of lambs is given in Figure 4.4.

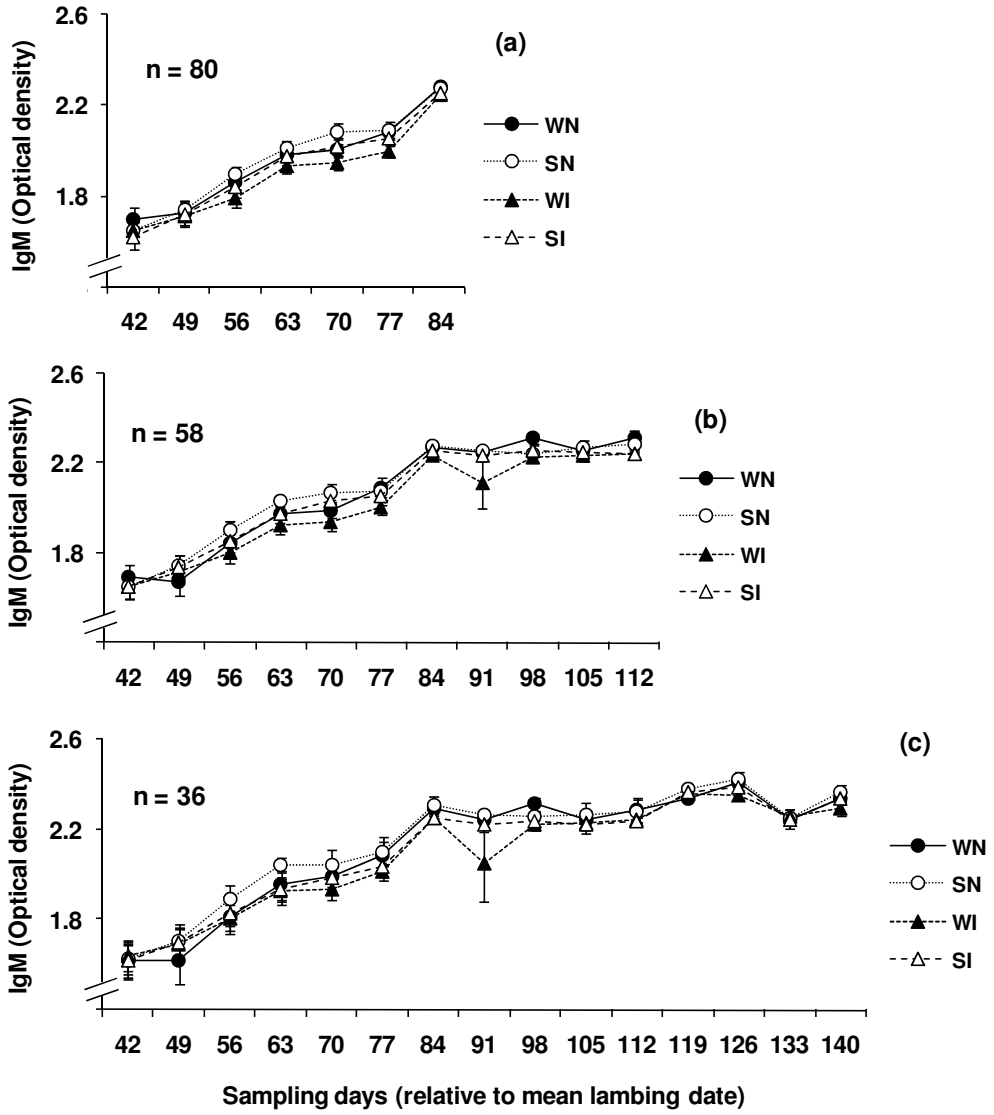


Figure 4.4: IgM (optical density ± SE) to L_3 *T. circumcincta* in plasma of lambs either weaned (W-) at 39 days of age, or continually suckled (S-) till necropsy, and infected with either zero (-N) or 1000 (-I) L_3 *T. circumcincta* larvae d^{-1} from day 42 onwards, with necropsies of selected lambs on days 84, 112, and 140, respectively. 'n' represents the number of lambs carried on after each necropsy.

A time × suckling interaction ($P < 0.01$) on plasma IgM in the 80 lambs was reflected in similar increase of plasma IgM of weaned and suckled lambs from day 42 to day 49, followed by a greater increase for suckled lambs from day 56 to day 70;

levels were similar for both groups on days 77 and 84 (Figure 4.4a). In addition, plasma IgM increased with time ($P < 0.001$) in the 80 lambs until the first necropsy on day 84 but there was no significant effect of infection ($P > 0.05$). For the 58 lambs during the second phase (day 91 – 112), a significant effect of infection ($P < 0.01$) was reflected in higher IgM levels of the zero-infected lambs compared with their infected contemporaries (Figure 4.4b). Also, a slight suckling × infection interaction ($P = 0.086$) reflected a trend for infection to result in reduced plasma IgM in the weaned groups but not in the suckled groups. There were no significant effects ($P > 0.05$) of time or suckling during this period. In the last phase (day 119 – 140), plasma IgM increased ($P < 0.001$) between day 119 and 126 in all groups of the remaining 36 lambs, followed by a significant reduction on day 133 (Figure 4.4c). Overall, plasma IgM increased with slaughter age ($P < 0.001$), being an average of 1.93 ± 0.011 OD in the first phase, 2.24 ± 0.011 OD in the second phase, and 2.33 ± 0.008 OD in the third phase.

4.3.5. Weekly live weight and lamb carcass weight

The changes in weekly live weights of the lambs are given in Figure 4.5. During the first phase (Figure 4.5a), there were effects of time ($P < 0.001$) and a time × suckling interaction ($P < 0.001$) was reflected in suckled lambs being heavier than their weaned contemporaries from day 42 onwards. In addition, a time × infection interaction ($P < 0.05$) was reflected in greater weight of infected lambs than their non-infected contemporaries from day 42 until day 70. In the second phase (Figure 4.5b), lamb weight continued to increase with time ($P < 0.001$) and at greater rate in the suckled lambs ($P < 0.001$). The sudden reduction in the live weight of all lamb groups on day

84 was probably due to an inclement weather condition of extreme cold and wetness during that period; a number of 0.0mm rainfall-days were followed by three days of up

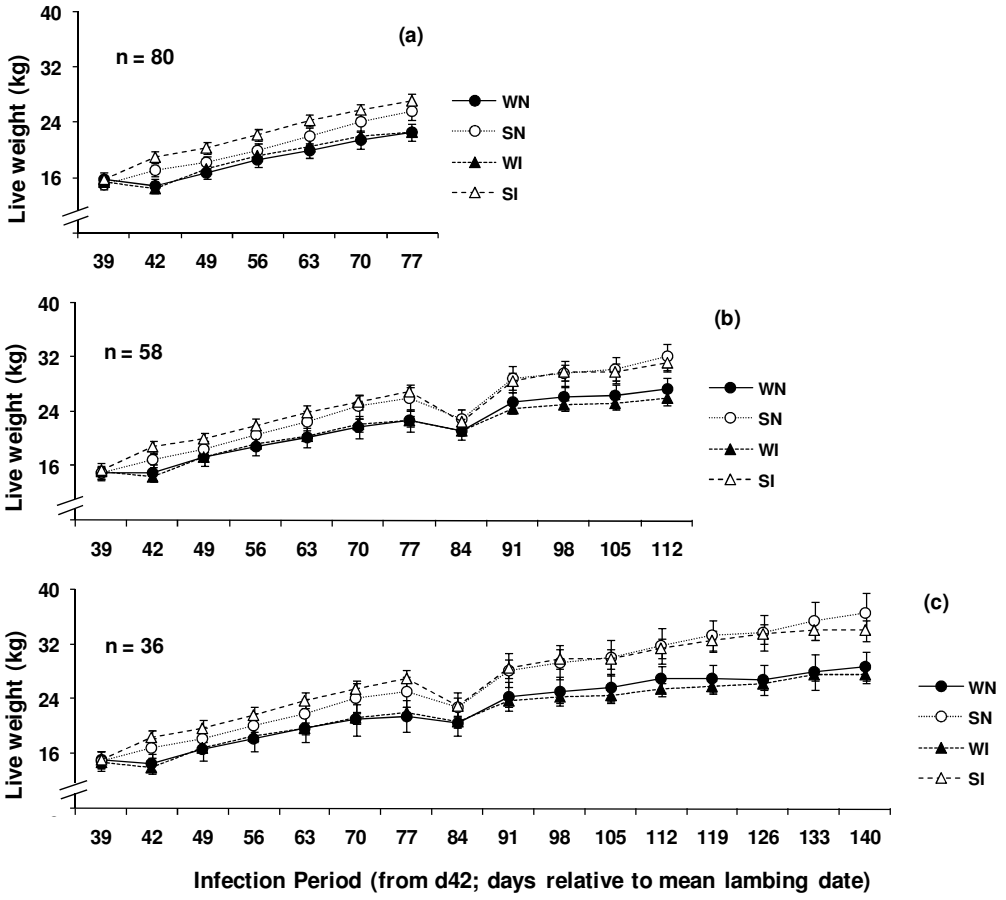


Figure 4.5: Mean live weight (± SE) of lambs either weaned (W-) at 39 days of age, or continually suckled (S-) till necropsy, and infected with either zero (-N) or 1000 (-I) L_3 *T. circumcincta* larvae d^{-1} from day 42 onwards, with necropsies of selected lambs on days 84, 112, and 140, respectively. 'n' represents the number of lambs carried on after each necropsy.

to 24 mm rainfall and cold draught (METEOR Weather Analysis Program, Lincoln University). By the end of the second phase (day 112), the WI lambs had suffered a 5 % and the SI lambs, a 3 % reduction in live weight relative to their non-infected contemporaries, respectively. The increment in lamb weight continued into, and during the third phase (Figure 4.5c) with suckled lambs still heavier than their weaned counterparts ($P < 0.001$). The remaining WI lambs suffered a 3 % and the SI lambs, a

7 % loss in live weight relative to their non-infected contemporaries, respectively, by day 140. However, there was no significant effect of infection on live weight change ($P > 0.05$) during the second and third phases of the trial.

The mean carcass weight of lambs is given in figure 4.6. The suckled lambs had a greater carcass weight than their weaned contemporaries in the first ($P < 0.05$) and third ($P < 0.001$) necropsies, respectively, while there was a trend for heavier carcass from suckled than from weaned lambs in the second phase ($P = 0.171$).

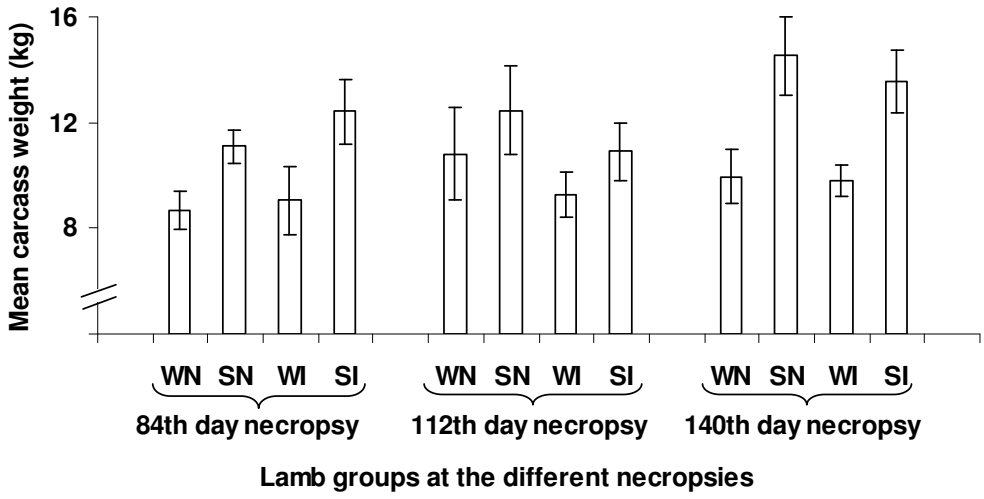


Figure 4.6: Mean carcass weight (± SE) of lambs either weaned (W-) at 39 days of age, or continually suckled (S-) till necropsy, and infected with either zero (-N) or 1000 (-I) L_3 *T. circumcincta* larvae d^{-1} from day 42 onwards, with necropsies of selected lambs on days 84, 112, and 140, respectively. 'n' represents the number of lambs carried on after each necropsy.

Overall, there was a trend for greater average carcass weight ($P = 0.121$) at the third necropsy (mean: 11.9 ± 0.55 kg) than at the second (mean: 10.8 ± 0.66 kg) and first necropsies (mean: 10.3 ± 0.58 kg), when average carcass weight was similar. Although, infection had no significant effect ($P > 0.05$) on lamb carcass weight, the weaned- and suckled-infected lambs (WI and SI) suffered a 14% and 12 % loss in carcass weight, respectively, relative to their non-infected contemporaries (WN and

SN) at the second necropsy and a 2 % and 7 % loss, respectively at the third necropsy. Surprisingly, carcass weight of infected lambs tended to be greater than that of their non-infected contemporaries in the first phase. On the other hand, weaning resulted in similar loss in carcass weight of non-infected and infected lambs, percentage losses relative to the suckled lambs being 22 vs. 27 %, 13 vs. 15 %, and 31 vs. 28 % for weaned-non-infected (WN) and -infected lambs (WI), respectively, at the first, second and third necropsies, respectively.

4.3.6. Plasma protein and plasma albumin

The changes in total plasma protein of the lambs are given in Figure 4.7. A time × suckling interaction ($P < 0.001$) on plasma protein in the 80 lambs reflected a reduction in plasma protein levels from day 42 to day 70, however, levels were greater in weaned than in suckled lambs during this period. This was followed by a rise in plasma protein till the first slaughter on day 84 but plasma protein was greater in suckled than in weaned lambs at these times (Figure 4.7a). In the second phase (Figure 4.7b), infection reduced plasma protein in the weaned lambs but not in their suckled contemporaries, which indicated a suckling × infection interaction ($P < 0.05$) among the 58 lambs. For the 36 lambs in the third phase (Figure 4.7c), a time × suckling interaction ($P < 0.01$) was reflected by a progressive reduction of plasma protein in weaned lambs while levels remain stable in the suckled lambs during the same period (Figure 4.7c), resulting in a 9 % reduction in plasma protein of the weaned lambs relative to their suckled contemporaries by day 140. Overall, plasma protein of the lambs was greater ($P < 0.001$) in the second phase (mean: 60.5 ± 0.30) than in the first (mean: 56.8 ± 0.21) and third (mean: 56.5 ± 0.34) phases, when plasma protein was similar.

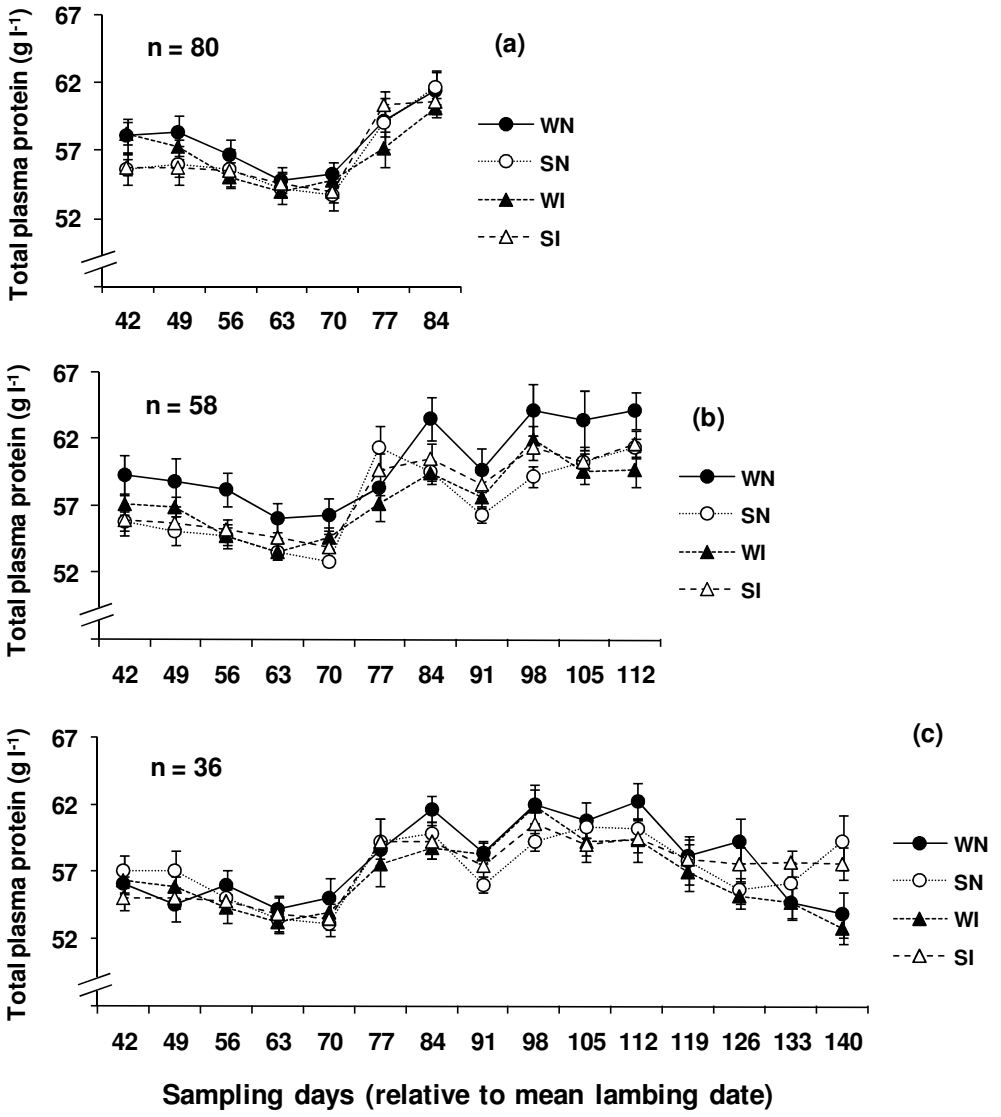


Figure 4.7: Total plasma protein (± SE g l^{-1}) of lambs either weaned (W-) at 39 days of age, or continually suckled (S-) till necropsy, and infected with either zero (-N) or 1000 (-I) L_3 *T. circumcincta* larvae d^{-1} from day 42 onwards, with necropsies of selected lambs on days 84, 112, and 140, respectively. 'n' represents the number of lambs carried on after each necropsy.

The changes in plasma albumin of the lambs are given in Figure 4.8. A time × suckling interaction ($P < 0.001$) on plasma albumin of the 80 lambs was reflected in a reduction in albumin levels from day 42 to day 70 in the weaned lambs only, followed by a small rise on day 77 and then a reduction on day 84. Plasma albumin levels

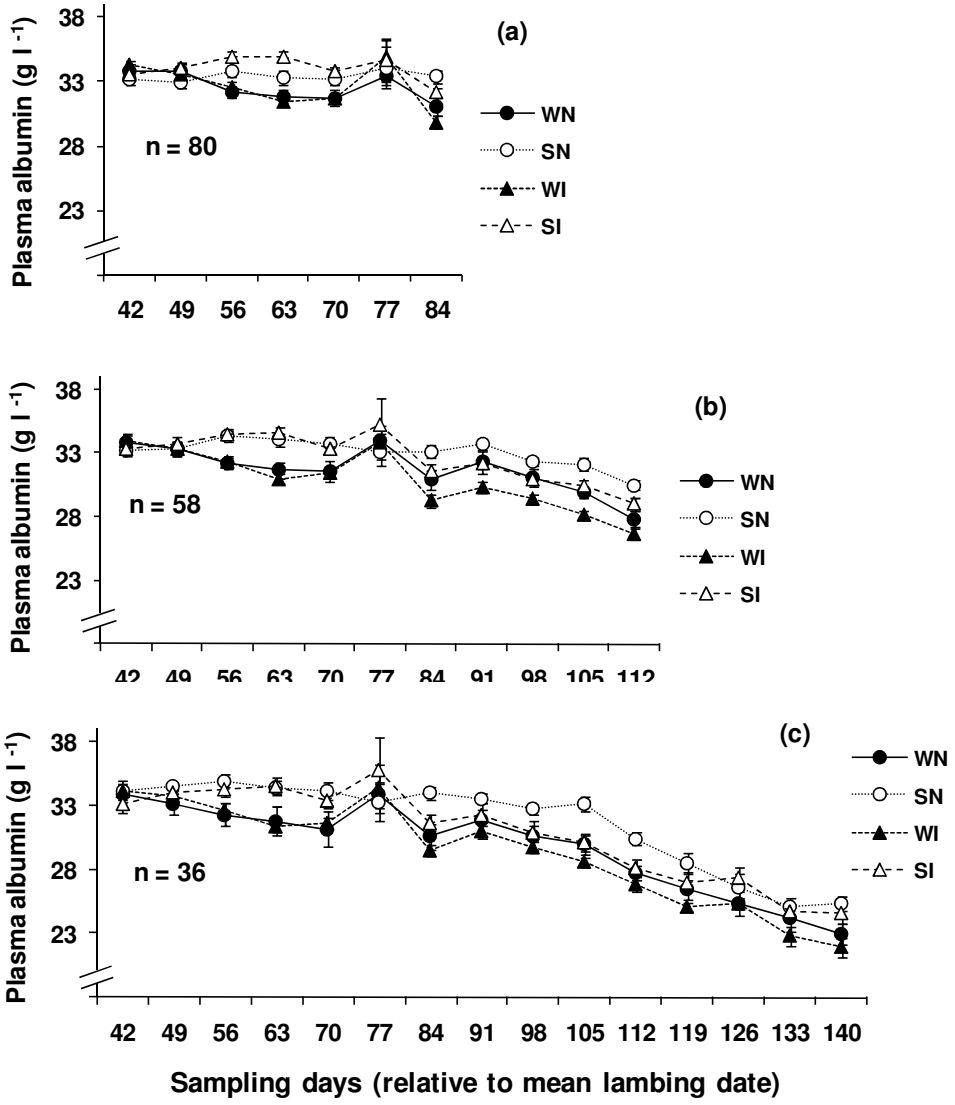


Figure 4.8: Plasma albumin (± SE g l^{-1}) of lambs either weaned (W-) at 39 days of age, or continually suckled (S-) till necropsy, and infected with either zero (-N) or 1000 (-I) L_3 *T. circumcincta* larvae d^{-1} from day 42 onwards, with necropsies of selected lambs on days 84, 112, and 140, respectively. 'n' represents the number of lambs carried on after each necropsy.

appeared to be stable in the suckled lambs until day 77 after which levels dropped until first slaughter on day 84 (Figure 4.8a). For the 58 lambs during the second phase (Figure 4.8b), the suckled lambs had a greater ($P < 0.001$) plasma albumin than their weaned contemporaries (31.4 vs. 29.5 g l^{-1}). In addition, the zero-infected lambs had greater ($P < 0.001$) plasma albumin than their 1000 L_3-infected contemporaries (31.3 vs. 29.6 g l^{-1}) while plasma albumin concentrations gradually declined with time ($P < 0.001$) in all groups until day 112. For the 36 lambs during the third phase (Figure 4.8c), the main effects of time ($P < 0.001$) and suckling ($P = 0.002$) were reflected in a general decline in plasma albumin with time and a significantly greater albumin levels in suckled (26.2 g l^{-1}) compared with weaned lambs (24.3 g l^{-1}). A time × infection interaction ($P = 0.023$) was reflected in greater plasma albumin in non-infected than infected lambs on days 119, 133, and 140 while levels were similar on day 126. Overall, plasma albumin of the lambs decreased with slaughter age ($P < 0.001$), being an average of 33.2 ± 0.14 g l^{-1} during the first phase, 30.3 ± 0.17 g l^{-1} during the second phase, and 25.1 ± 0.23 OD during the third phase.

4.4. Discussion

The present study was designed, first, to confirm if the direct effect of suckling (milk feeding) in reducing larval establishment of *Teladorsagia circumcincta* in young lambs, as found in Chapter three, is a consistent phenomenon. Secondly, to allow the investigation of the role of suckling in the acquisition of immune competence, and therefore resistance to *T. circumcincta* nematode infection, by lambs at an older age than that of the lambs in Chapter three. In addition, the design was premised on the hypothesis that an immune response at an older age could trigger competition for nutrients between growth and acquisition of immune competence (Coop & Kyriazakis 1999), and therefore increase nutrient demands amongst these competing end uses (Orskov 1992). This was expected to provide an opportunity to investigate the hypothesis that milk could offer a nutritional advantage by being beneficial for resilience to *T. circumcincta* infection; hence the 140-day period of the present study. However, the strategies adopted to minimize pasture larval contamination, which include the drenching of the ewes with a controlled-release anthelmintic and the grazing of ewe/suckling lambs and weaned lambs in rotation, were not as effective as expected. This is apparent in the early presence of nematode eggs, and large worm burdens at the 112^{th} and 140^{th} day necropsy, in the supposedly non-infected groups (WN and SN). This was a major limitation of this study, which, however, did not jeopardize the trial in its totality.

Of some consolation in allowing interpretation of the data was the similar pattern of response of the lambs in the successive phases, with respect to repeated measures (weekly data), despite the withdrawal of lambs for necropsy at the end of each phase. This was shown in FEC (Figure 4.1), antibody responses (Figures 4.2, 4.3 and 4.4),

bodyweight (Figure 4.5), and plasma proteins (Figure 4.6 and 4.7). These give the strong indication that the response of the lambs was uniform and the treatments were not confounded by the withdrawal of lambs for necropsy.

Overall, the results did provide evidence to support the hypotheses that – (a.) suckling or milk feeding can reduce worm burden and FEC, (b.) suckling may have long-term (indirect) benefit for the ability of the host to limit nematode populations, (c.) there may be beneficial effects of early exposure to nematode larvae while suckling for subsequent development of immunity, but (d.) suckling may have little effect on the resilience to *T. circumcincta* infection, in terms of weight gain and carcass weight, during the acquisition of immunity.

4.4.1. Direct effects of milk on nematode burdens

The responses of the 80 lambs in the first phase of this study (days 42 – 84 after lambing and six weeks of infection) replicated, to a large degree, the findings in Chapter three and therefore confirmed the possible direct beneficial effect of milk in reducing the establishment of *T. circumcincta* larvae in young suckling lambs. Within the infected groups there appeared to be a greater ability of suckled lambs to limit worm establishment, seen in significantly smaller numbers of L_4 larvae and lower total worm burdens in SI compared with the WI lambs at the 84^{th} day necropsy (Table 4.1). This was consistent with the findings in Chapter three, and in agreement with those of Zeng *et al.* (2001) in which worm burden was smaller in milk-fed lambs. The present work does not help explain the exact mechanism by which milk affects the establishment of incoming larvae. Zeng *et al.*(2001) speculated that the lower pH of the abomasal contents of the pre-ruminant lambs may have been a factor, as the

parasites have previously been shown to die more rapidly *in vitro* at low pH, and moreover, abomasal parasites are known to inhibit acid secretion (Anderson *et al.* 1985; Lawton *et al.* 1996), presumably because their survival is favoured at high pH. An *in vitro* trial (Zeng *et al.* 2003) had revealed the involvement of bovine proteins, or components associated with the proteins, in reducing the motility of both sheathed and exsheathed L_3 *T. circumcincta* larvae, which may reduce worm establishment. Studies with *Ostertagia ostertagi*, also an abomasal resident in milk-fed calves, had concluded that the degree of ruminal development was the important factor in reducing the worm burden, even though milk-fed calves given 25,000 exsheathed larvae had lower burdens than those given normal sheathed larvae (Satrija *et al.* 1991).

The worm burdens of the weaned and suckled lambs (12722 vs. 9850 worms, respectively) that were infected with 1000 L_3 *T. circumcincta* larvae d^{-1} (≈ 61 L_3 larvae kg LW^{-1} d^{-1}) and necropsied at day 84 corresponded with those of the similarly infected (≈ 54 L_3 larvae kg LW^{-1} d^{-1}) and necropsied weaned and suckled lambs in Chapter three (11333 vs. 6399 worms, respectively). Both sets of burdens are in the range of those reported by Waller & Thomas (1978) in suckling lambs that were exposed to natural infection and necropsied at 12 weeks of age (4258 – 8660 worms), but are greater than the average of 3500 worms from lambs dosed at ≈ 57 L_3 larvae kg LW^{-1} d^{-1} for 38 days by Zeng *et al.* (2001). Clearly, a significant infection was established in both suckled and weaned lambs.

Taking the trial of Chapter 3 and the first phase of the current trial together, the direct effect of milk on FEC was not so marked in the present trial (Figure 4.9).

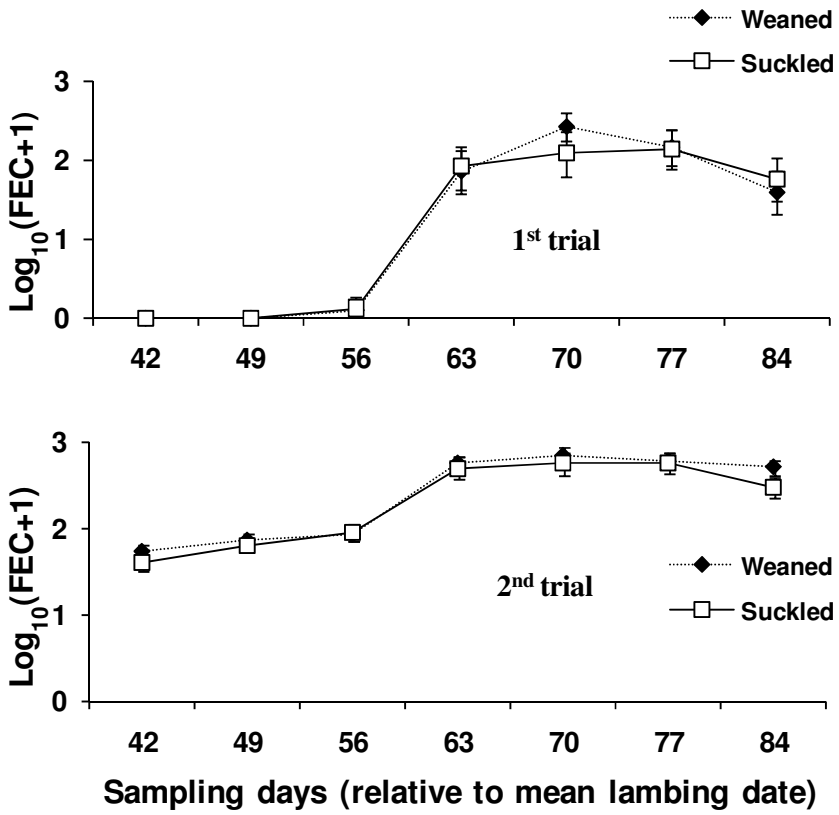


Figure 4.9: FEC of weaned and suckled lambs of the 1st trial (Chapter 3; n = 60) and 2nd trial (i.e. phase one of current study; n = 80).

This could be due to the lack of sensitivity of FEC to small differences in worm population and/or what appeared to be an intra-worm population dynamics effect on worm egg production as observed in Chapter three. Therefore, the conclusion could be that the direct effect of milk is probably quite small, or that FEC is not a good indicator of worm burdens. Invariably, a large difference in worm burdens needs to be realised, to offset the intra worm-population effects on worm fecundity, for the influence on FEC to be marked. Looked at from a larval establishment standpoint in the current study, moreover, 21 % of larvae established in suckled lambs compared with 36 % in weaned lambs, which compares with 16 vs. 28 %, respectively for the Chapter three study. These figures, particularly, those for the weaned lambs could theoretically be an overestimate of establishment because of contamination from

residual larvae on herbage. If these values are corrected for intake of larval from residual contamination on pasture, by assuming relatively equal exposure of lamb groups to larval contamination and therefore subtracting the burdens of the non-infected groups from those of their corresponding infected counterparts, the establishment rates are still 19 % for suckled lambs and 34 % for the weaned lambs. The lack of sensitivity of FEC in this study could also be related to differences in gut fill arising from intake of milk and herbage or herbage alone given that the weaned lambs would consume more herbage and therefore have greater gut fill than their suckled counterpart has. This may result in higher concentrations, and therefore overestimation of worm egg number in the faeces of the suckled lambs.

4.4.2. Indirect and long term effects of milk feeding on immune development

The greatest beneficial effect of suckling to the development of immunity, however, appeared to occur during the second and third phases (day 84 – 140), when suckled lambs maintained lower FEC (Figure 4.1c) and smaller worm burdens (Figure 4.10, next page) than their weaned counterparts. This would have occurred after peak milk production when the milk intake of suckling lambs would have reduced, and therefore the possible direct effect in the first six weeks may have been overridden by an indirect influence via improved nutrient supply from earlier milk consumption. Other evidence for a delayed effect of additional protein supply has been provided by several workers (Bown *et al.* 1991; Watson & Gill 1991b; Kambara *et al.* 1993; Coop *et al.* 1995; van Houtert *et al.* 1995; Datta *et al.* 1999b; Greer 2005). Previously, Watson & Gill (1991b) reported the mean FEC for weaned lambs to be twice that for suckled when lambs were experimentally infected with 5000 *Haemonchus contortus* and 10,000 *Trichostrongylus colubriformis*, and attributed this to the possibility that the

immune response was hastened in the suckled lambs as a result of milk feeding. Bown *et al.* (1991) gave abomasal infusion of 50 g d^{-1} Na caseinate to weaned lambs as crude protein supplement while infecting with 3000 L_3 *Trichostrongylus colubriformis* per day, and van Houtert *et al.* (1995) fed weaned lambs fishmeal as protected-protein

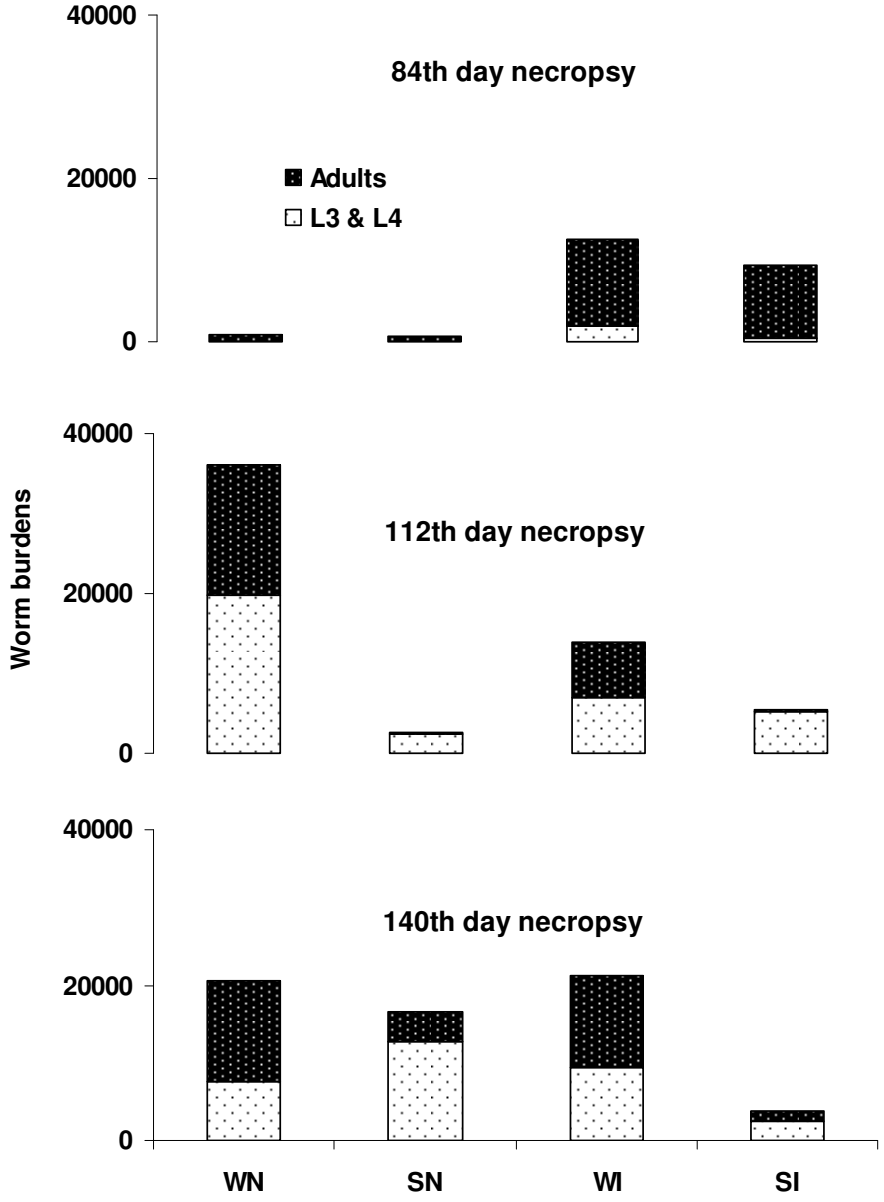


Figure 4.10: Adult and immature worm burdens in lambs from the 4 treatment groups at the 3 necropsy times.

supplement while infecting them with 0 or 1000 *Trichostrongylus colubriformis* thrice a week. Both studies observed that the beneficial effect of protein supplements occurred at least six weeks after the commencement of infection, and speculated that this represented a speeding up of the acquisition of immunity rather than an enhancement of innate immunity. Coop *et al.* (1995) found that the continuous abomasal infusion of Na caseinate, as protein supplement, to growing lambs experiencing vaccinating infection, increased mucosal mast cell proliferation and protease activity in the gut mucosa, thus providing direct evidence of the enhancement of development of local immune response by additional protein. Datta *et al.* (1999b) fed increasing concentrations of dietary CP (i.e. 10, 13, 16, 19 and 22 % of feed DM) to groups of weaner lambs for nine weeks, and then exposed them to the same environmental variables, including nematode larval challenge. It was observed that the short period of enhanced protein intake in the young lambs provided delayed and long-term beneficial effects in reducing FEC for up to one year, and was associated with enhanced antibody responses to both *Haemonchus contortus* and *Trichostrongylus colubriformis* antigenic *in vitro* challenge. These findings together suggest that the additional protein supplied by milk could have hastened immune development in the suckled groups during the period after the first necropsy (> 84^{th} day).

However, the increase in FEC (Figure 4.1b & 4.1c) and the much greater worm burdens in WN at day 112 and in SN at day 140 (Figure 4.11, above) suggests that these lambs had failed to regulate a considerable (not measured) natural infection from pasture in contrast to their WI and SI counterparts. The effect appeared to have been more severe in the weaned lambs as indicated by the greater worm burdens of the weaned- (WN) compared with the suckled-non-infected (SN) lambs at day 112 (Figure

4.10 & Table 4.2) and a trend for this at day 140 (Figure 4.10 and Table 4.3). This either suggests greater herbage and therefore larval intake in weaned lambs, or a possibly greater development of resistance in the suckled groups. Although, the milk intake of the suckled lambs was not measured, a range of 3.4 – 7.1 with a mean of 5.5 g milk g^{-1} lamb gain in weight has been estimated to be the efficiency of transformation of milk into lamb gain in previous studies on ewe lactation and lamb growth (Butterworth *et al.* 1968; Peart *et al.* 1972, 1975). In a review, Treacher & Caja (2002) reported studies indicating that lambs consuming only milk gain 160-170 g d^{-1} per kg of liquid milk, which is equivalent to about 6.0 kg of milk kg^{-1} of gain or about 1 kg gain kg^{-1} milk dry matter consumed. On this basis, and given that the average growth rate of suckled lambs in the present study was in excess of 200 g d^{-1}, it can be calculated that the milk production of their dams, which by day 100 was likely to be less than 500 g d^{-1} (Peart *et al.* 1975), would provide less than 20 % of total energy requirement for growth. Therefore, if grazing had supplemented milk intake to that extent, the difference in worm burdens between WN and SN lambs would seem less likely to be due to difference in herbage (and consequently larval) intake than a more rapid development of immunity in the suckled lambs.

Indeed, the pattern of worm burdens (Figure 4.10), and progressive shortening of worm length (Tables 4.1, 4.2 & 4.3), which appeared to be independent of adult worm populations and therefore ruled out the possibility of a crowding effect, tended to confirm that the lambs, particularly the SI group had entered the phase of acquisition of immunity during the period after the 84^{th} day necropsy. Evidence for this is shown in the progressive decline of worm burdens in the SI lambs, which may suggest any, or a combination of reduced larval establishment, arrested development at L_4 stage or

worm expulsion. The shortening of worm length, which has been closely associated with worm fecundity (Stear *et al.* 1995; Stear & Bishop 1999) could also be implicated for the lower peaks of FEC from the second phase onwards; a further evidence of immune development. While the adult worm population at each stage was reflected in the subsequent FEC pattern of the lambs (Figures 4.10 & 4.1, respectively), the greater proportion of the worm population at the L_4 stages in suckling lambs on days 112 and 140 suggests development of a capability to regulate worm development, which also may signal the onset of an immune response.

4.4.3. Vaccinating effects of early exposure to parasite larvae

Although not planned, a further outcome of exposure of the lambs to natural infection from pasture was an apparent vaccinating effect of the early imposed larval dosing of both infected groups of lambs (WI and SI). This was shown by the FEC profile and pattern of worm burdens in the 36 lambs that were carried to the 140th day (20 weeks) and experienced 14 weeks of larval dosing. A closer examination of the worm burdens as shown in Figure 4.10, and of the FEC profile in Figure 4.1c perhaps reveals the importance of subtle and judicious exposure of lambs to parasites in early life for augmenting natural immunity as observed in previous study and review, respectively (Watson & Gill 1991a; Colditz *et al.* 1996). Although all groups appeared to be infected from the second phase onwards, the WI and SI lambs that were experimentally infected from the first phase and therefore had the benefit of earlier exposure, showed declining worm burdens while their corresponding non-infected counterparts (WN and SN) showed sustained worm burdens (Figure 4.10). This was markedly so for the two suckled groups (SN and SI) compared with the weaned groups (WN and WI), which further indicated the benefit of milk feeding for hastened

acquisition of immunity. This finding also supported the view that the phenomenon of age-dependent immunity to nematode infection could be due to a relative protein deficiency in the young growing lamb as observed by Kambara *et al.* (1993) and Coop *et al.* (1995). Moreover, it could also reflect the concept explained in a nutrient partition framework by Coop & Kyriazakis (1999), if the demand of protein for acquisition of immunity is prioritized over the need for growth. The pattern of worm burdens was also reflected in the FEC profile of the 36 lambs (Figure 4.1c) where the WI and SI lambs were able to maintain relatively lower FEC compared with their non-infected counterparts at the later part of the trial. This could be an indication of a vaccinating effect of the earlier exposure (larval dosing) of the infected lambs to parasite larvae.

4.4.4. Immune development of the lambs

The inability to detect immune response during the first phase, in terms of peripheral IgA and total Ig, was consistent with those of Chapter three, and further confirmed the possible direct adverse effect of milk on the nematode population rather than an indirect effect via improved host protein nutrition and immune response during the first six weeks of infection. The length of the female worms recovered from the lambs that were necropsised at day 84, ranging from 9.9 – 12.8mm, were similar to those of the suckling lambs of similar age that were exposed to natural infection composed of 90 % *Ostertagia* (*Teladorsagia*) *circumcincta* in Waller and Thomas (1978) – 11.5 mm - but longer than those of the older and immunologically competent sheep in previous studies [Coop *et al.* (1977) – 9.05 mm; Seaton *et al.* (1989) – 8.5 mm; Donaldson *et al.* (2001) – 7.2 mm]. This may indicate the absence of a typical IgA-associated reduction of worm size during the first phase (Stear *et al.* 1996; Stear

et al. 1999b; Henderson & Stear 2006). These findings are in line with those of previous studies that have demonstrated the immunological unresponsiveness of very young lambs to nematode infection (Monsell *et al.* 1984; Smith *et al.* 1985; Watson & Gill 1991a; Kambara *et al.* 1993; Watson *et al.* 1994; Colditz *et al.* 1996). The role of the progressive increase in plasma IgM in the first phase, which appeared to be a replica of the IgM response of the lambs in Chapter three, and inconsistent with the pattern of IgA and total Ig responses in lambs of these studies, could not be explained other than that very weak and insignificant correlations between IgM and parasitological status of lambs have been reported previously (Bisset *et al.* 1996), and IgM response was reported to be less persistent in infected but previously naïve eight-month old lambs (Douch *et al.* 1994).

The onset of an immune response in the lambs during the second phase is evident by the significant rise in the total plasma Ig and plasma IgA of the lambs from that period onwards (Figures 4.2 & 4.3, respectively), which could also be associated with the shortening of worm length at the later phases. The fact that the IgA response was only noticeable from the second phase onwards appears likely to be attributable to ability of the immune system to respond to antigenic stimulation. The increase in percent larval rejection by abomasal explants, as the lambs aged, in the *in vitro* direct larval challenge study further confirmed a possible immune development at the tissue level. However, suckling did not appear to provide any benefit until the last phase when the tissue explants of the SI lambs showed greater rejection of larvae than of the WI lambs, which, as discussed earlier, could indicate a long-term benefit of improved protein nutrition as a result of suckling in enhancing immune response at that age. The similar larval rejection by the naïve controls that were reared indoors and the

experimental lambs at day 84 is consistent with the apparent absence of immune competence in the lambs during the first phase and in agreement with the findings in Chapter three. However, the relatively lower larval rejection by the naïve lambs compared with the infected lambs at second slaughter (day 112) and all lamb groups at the last slaughter (day 140) could mean the experimental lambs had acquired some immune competence by those slaughter ages. The percentage rejection in the third phase of this study (95 %) is similar to that reported in abomasal tissues of lambs with a fully established immunity by Jackson *et al* (2004). The potential of the IVDC as a means of examining the critical first phase of larval establishment (Jackson *et al.* 2004), however, needs to be further investigated as the present study showed some inconsistencies between the larval rejection and worm burdens of the lambs. For example, the large adult worm burdens of the weaned lambs in the second and last slaughters were not consistent with the high larval rejection in these lambs during IVDC in the last slaughter. On the other hand, this may be interpreted as indicating that the effect of suckling (milk) on immunological development, with respect to reduced worm burdens, may occur at a later stage than the immediate entry of incoming L_3 larvae.

4.4.5. Effects of suckling on lamb resilience

From the performance of the lambs, as shown by carcass (Figure 4.6) and weekly live weights (Figure 4.5), it is clear, contrary to expectation, that while suckling enhanced weight gain and carcass weight, weaning may not have been associated with reduced resilience of lambs to infection even during the second and third phases when acquisition of immunity was expected to be strengthening. Weaned and suckled lambs suffered a similar percentage loss in live and carcass weights as a result of the artificial

infection during the second and third phases of the experiment. Although the considerable natural infection from pasture in this trial may weaken this assertion, the findings are consistent with the data in Chapter three, in which pasture larval contamination was minimal. That trial also showed a similar much smaller difference in performance than anticipated between controls and infected-weaned compared to the difference between controls and infected-suckled lambs, indicating comparable resilience to infection in weaned and suckled lambs. One interpretation is that suckling, and the presumed improved protein nutrition, speeds up the development of immunity, for which there is evidence in the present work (Figures 4.10 and 4.11) and in earlier works (Bown *et al.* 1991; Watson & Gill 1991b; Coop *et al.* 1995; van Houtert *et al.* 1995), but that the consequences of such acquisition of immunity are increased metabolic costs (Greer *et al.* 2005). This could also explain, in the light of the conclusions of Greer *et al.* (2005), the absence of a reduced resilience to infection of the weaned lambs during the first phase of this trial as similarly observed in Chapter three. Greer *et al.*(2005) had demonstrated, in an immune suppression study, that the phase of acquisition of immunity in lambs is associated with a nutritional cost that impairs weight gain. In fact, and surprisingly, the infected lambs, regardless of suckling, had comparable carcass weight and live weight gains to their non-infected contemporaries for most of the first phase. This could be an indication that the lambs were able to cope with nematode infection, which may be explained by the absence of an immune response and its associated metabolic cost at this age of the lambs. Although the drastic fall in plasma proteins of the lambs appears unusual, and may be explained as being a consequence of either enteric plasma protein loss, or a reduced metabolic synthesis of albumin, it appeared to be more severe in the weaned lambs.

Interestingly, however, a negative impact of this on the resilience of the weaned lambs was not apparent. The total plasma protein and albumin of the 80 lambs were relatively stable in the first phase; indicating the resilience of the lambs at this stage, except for the weaned groups that suffered a slight reduction in plasma albumin, which was similarly observed in the weaned lambs of Chapter 3. Plasma albumin therefore appears to be the only parameter that indicated an advantage of milk feeding at this stage; the milk may have helped supply amino acid needed for synthesis of albumin to replace that leaked as a consequence of infection. However, infection resulted in similar proportional loss in plasma albumin of the weaned (0.04 and 0.05) and suckled (0.05 and 0.03) lambs by the 112^{th} and 140^{th} day, respectively in this study. These losses are far less than the 0.13 reported by Symons *et al.* (1981) and 0.26 observed by Coop *et al.* (1982), both in older lambs with comparable rates of larval dosing to the present study, which suggest tolerance of the present lambs in pathophysiological terms. The fact that the weaned lambs appeared to tolerate infection without metabolic cost may well indicate that they may still not have fully entered the phase of acquisition of immunity with its associated costs (Greer *et al.* 2005), or that the speed of immunity acquisition was slow enough for the lambs to cope in nutrient economy terms. I would, however, have been more confident in this hypothesis had there been lower residual infection on pasture. Nevertheless, the lack of effect of suckling on peripheral antibody levels may well explain the comparable resilience of weaned and suckled lambs.

4.5. Conclusions

In conclusion, the results of this study re-emphasized the possible direct beneficial effect of milk in reducing *T. circumcincta* larval establishment in the abomasum. It

was also clear that suckling could enhance protein nutrition of lambs and consequently hasten their acquisition of immunity, particularly when exposed to larvae early in life. Lastly, while milk feeding enhances weight gain weaning may not necessarily be associated with reduced resilience of lambs to *T. circumcincta* infection.

Chapter 5

Effects of suckling, and mixed infection with *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*, on the parasite status, immune development, and resilience of young lambs

5.1. Introduction

While studies in Chapters 3 and 4, and those of previous workers (Waller & Thomas 1978; Satrija *et al.* 1991; Zeng *et al.* 2001), have demonstrated there may be advantages of suckling in reducing abomasal worm burdens of lambs, the benefit of late over early weaning for resilience was still confounded in the present work (Chapter 4) by the nematode larval contamination of the pasture. Even though contrived infection had no significant effect on lamb live weight changes during the 98 days of the trial described in Chapter 4, the assertion that milk can be beneficial for lamb resilience remains to be clarified. However, the lack of evidence for effects of suckling on resilience suggests that either early weaning may not necessarily reduce resilience of lambs to infection, or that the rate of infection used was not sufficiently pathogenic to affect performance. The likelihood of immune unresponsiveness of the very young lambs to infection has also been advanced as a possible reason.

The reality of gastrointestinal nematode infection in the field is that it is invariably comprised of mixed-nematode species, the prevalence of any species being a function of host-parasite-environment interaction factors (Brunsdon 1982). In a study in which 4-month-old lambs were concurrently infected with *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*, the effects of mixed infection on performance were found to be greater than the effects of a single-species infection, and multiplicative rather than additive (Sykes *et al.* 1988). Steel *et al.*(1982) reported similar findings in

a 16-week infection trial that involved *T. circumcincta* and *T. colubriformis* and ascribed the greater loss of productivity in concurrent infection to reduced feed consumption, and a concomitant decrease in the efficiency of nitrogen retention.

It could be anticipated, therefore that a mixed species infection could trigger a circumstance of nutritional stress while mimicking the reality of field parasitic infection in the lambs, and therefore provide an opportunity for clarifying the benefits, if any, of late weaning for improved resilience.

I had the opportunity to be part of a larger trial run by AgResearch Scientists at Lincoln in a design, which was complementary to the work in previous Chapters, and investigated the role of suckling, this time in mixed nematode species infection. Swards were available that had been grazed previously for two years by cattle, which together with strategic worming of ewes with slow-release anthelmintic before lambing, as in Chapters 3 and 4, was expected to ensure minimal pasture larval contamination. In addition, it was possible to investigate the possibility, revealed in the unintentional pasture larval contamination in Chapter 4, of a benefit of early vaccinating exposure of lambs while suckling to infective larvae for subsequent resistance to larval intake.

While the running of the major trial was with AgResearch Scientists and Technical Staff, I had the personal practical responsibility of carrying out the processing of blood samples and the assays for plasma immunoglobulin, but had access to other data from the trial.

5.2. Materials and methods

5.2.1. Management of experimental animals

Experimental animals in this study comprised twin born and reared lambs from a single mob of Coopworth breed of sheep, which were identified to dams and tagged within 12 h of birth. Ewes and lambs were managed on mature perennial ryegrass (*Lolium perenne*) – white clover (*Trifolium repens*) swards that had been grazed by cattle for two years, purposely to ensure a minimal contamination of herbage by sheep nematode species. To minimise the possibility of cross-infection among treatment groups, the mob was strip-grazed at four-day intervals, and not allowed access to previously grazed strip areas. Strip size was determined by herbage mass and percentage dead material in the sward, providing an average of 12 kg DM/d feed allowance. Ewes were drenched with Moxidectin (Cydectin^{TM} - 0.2 mg Moxidectin / kg LW at 1 mL / 5 kg LW; Fort Dodge New Zealand Ltd) on day 120 of gestation.

5.2.2. Experimental design

Ninety three pairs of twinborn lambs were used. Ninety-four lambs commenced a vaccinating infection on day 36 of age, which ceased on day 103. This comprised 60 L_3 larvae/kg LW/d of *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* in ratio 40:60 and were termed group -.V.-. The remainder was uninfected (-.N.-). On day 51 equal numbers from each group were either weaned early (E.V.- and E.N.-) or continued to suckle but weaned later on day 108 (L.V.- and L.N.-) (see acronym description – Table 5.1). On day 108, twelve lambs from each of these groups were necropsied for estimation of worm burdens and carcass weight measurement, and the remainder treated with anthelmintic [Exhelm^{TM} (Morantel tartrate), with same mode of action as levamisole – 5.94 mg Morantel base / kg LW @ 1 mL / 5 kg LW; Pfizer New

Zealand Ltd]. During a second phase (day 116 – 218) all remaining lambs of the vaccinated groups (i.e. E.V.- and L.V.-) and 24 lambs of each of the non-vaccinated (i.e. E.N.- and L.N.-) received a challenge infection of 60 L_3 larvae/kg LW/d of *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* in ratio 40:60, which ceased on day 203, forming groups E.V.C, L.V.C. and E.N.C, L.N.C, respectively (see acronym description – Table 5.2). On day 184 another set of 12 lambs each from these groups were necropsied. The remainder of the non-vaccinated groups were not challenged forming E-N-N and L-N-N groups. These were necropsied on day 218, along with the remainder of the challenged lambs (E.V.C, L.V.C. and E.N.C, L.N.C) resulting in six treatment groups as shown in Table 5.2.

Table 5.1: Showing experimental treatments before the 108-day necropsy

Group	No. of lambs	Weaned	Infection*
E.N.-	46	Early	Not-vaccinated
E.V.-	47	Early	Vaccinated
L.N.-	46	Late	Not-vaccinated
L.V.-	47	Late	Vaccinated

Table 5.2: Showing experimental treatments before the 184-day necropsy

Group	No. of lambs	Weaned	Infection*
E.N.N	10	Early	Not-vaccinated; not-challenged
E.N.C	24	Early	Not-vaccinated; challenged
E.V.C	35	Early	Vaccinated; challenged
L.N.N	10	Late	Not-vaccinated; not-challenged
L.N.C	24	Late	Not-vaccinated; challenged
L.V.C	35	Late	Vaccinated; challenged

* Vaccinating and challenge infections were carried out at the rate of 60 L_3 larvae/kg LW/d of *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* at ratio 40:60, respectively.

5.2.3. Measurements

Lamb live weight was recorded weekly for all groups until respective necropsies. Faecal samples were collected as follows: on days 58, 73, 86, 108, 135, 149, 171, and 184 from the vaccinated and challenged groups (-.V.C); days 108, 135, 149, 171, and 184 from the non-vaccinated but challenged groups (-.N.C); and days 58, 66, 73, 86, and 108 from the non-vaccinated non-challenged groups (-.N.N). Faecal samples were processed and FEC determined as described in Chapter 3. Blood samples were collected fortnightly, and antibodies in plasma (Total antibody, IgA and IgM) assayed as described in Chapter 3. Carcass weight and worm burdens in the abomasum and small intestine were also measured at necropsy as described in Chapter 3.

5.2.4. Statistical Analyses

Data were analysed using the Genstat suite of statistical packages (GenStat Release 8.2 Copyright 2005, Lawes Agricultural Trust, Rothamsted Experimental Station). FEC and worm counts were subjected to log-transformation [LOG_{10} (count+1)] before analysis, and later back-transformed for geometric means. The generalized linear model was used to analyse worm burdens and carcass weight as unbalanced design, while the Restricted Maximum Likelihood (REML) was used for repeated measures after being subjected to sequential comparison of antedependence structures. The repeated observations in time were analysed in phases *viz.* periods before and up to the early weaning (i.e. start of infection on day 36 to weaning on day 51); periods between early weaning and the 108-day-necropsy (i.e. days 58 to 108); and the challenge infection period (i.e. days 121 to 218).

For the vaccinating period, which comprised the phases 1 and 2 (i.e. days 36 to 108), the weekly data were analysed by categorising lambs into one of four groups of a 2 × 2 factorial design that incorporated weaning or suckling and the presence or absence of a vaccinating nematode infection. During the challenge period, however, they were categorised into one of six groups of either early or late weaned [i.e. weaned on either days 51 (E.-.-) or 108 (L.-.-), respectively] and either not vaccinated and not challenged (-.N.N), not vaccinated but challenged (-.N.C), or vaccinated and challenged (-.V.C).

Faecal egg counts for all groups were taken to be strongyle egg counts while worm burdens were taken as abomasal *Teladorsagia* and intestinal *Trichostrongylus* populations, respectively, and analysed separately. The populations of *Trichostrongylus axei*, *Haemonchus*, *Cooperia*, and *Nematodirus* are presented in Appendices 5.1, 5.2, and 5.3.

5.3. Results

5.3.1. Faecal egg count

The changes in mean FEC of the lambs are given in Figure 5.1. During the vaccinating infection period, there were significant time × infection ($P < 0.001$) and time × weaning ($P < 0.05$) interactions. These reflected the increase in FEC in infected lambs, compared with virtually zero egg counts in unvaccinated lambs and greater FEC in weaned lambs from day 73.

During the challenge period there were significant time × vaccinating infection ($P < 0.001$) and weaning × vaccinating infection ($P < 0.01$) interactions reflecting lower increase in FEC in all vaccinated lambs and a trend for lower rates of increase in late

weaned lambs, especially in those that received the vaccinating infection. The non-vaccinated and non-challenged animals (E.N.N and L.N.N) had FEC peaks of less than 10 e.p.g. throughout the trial period.

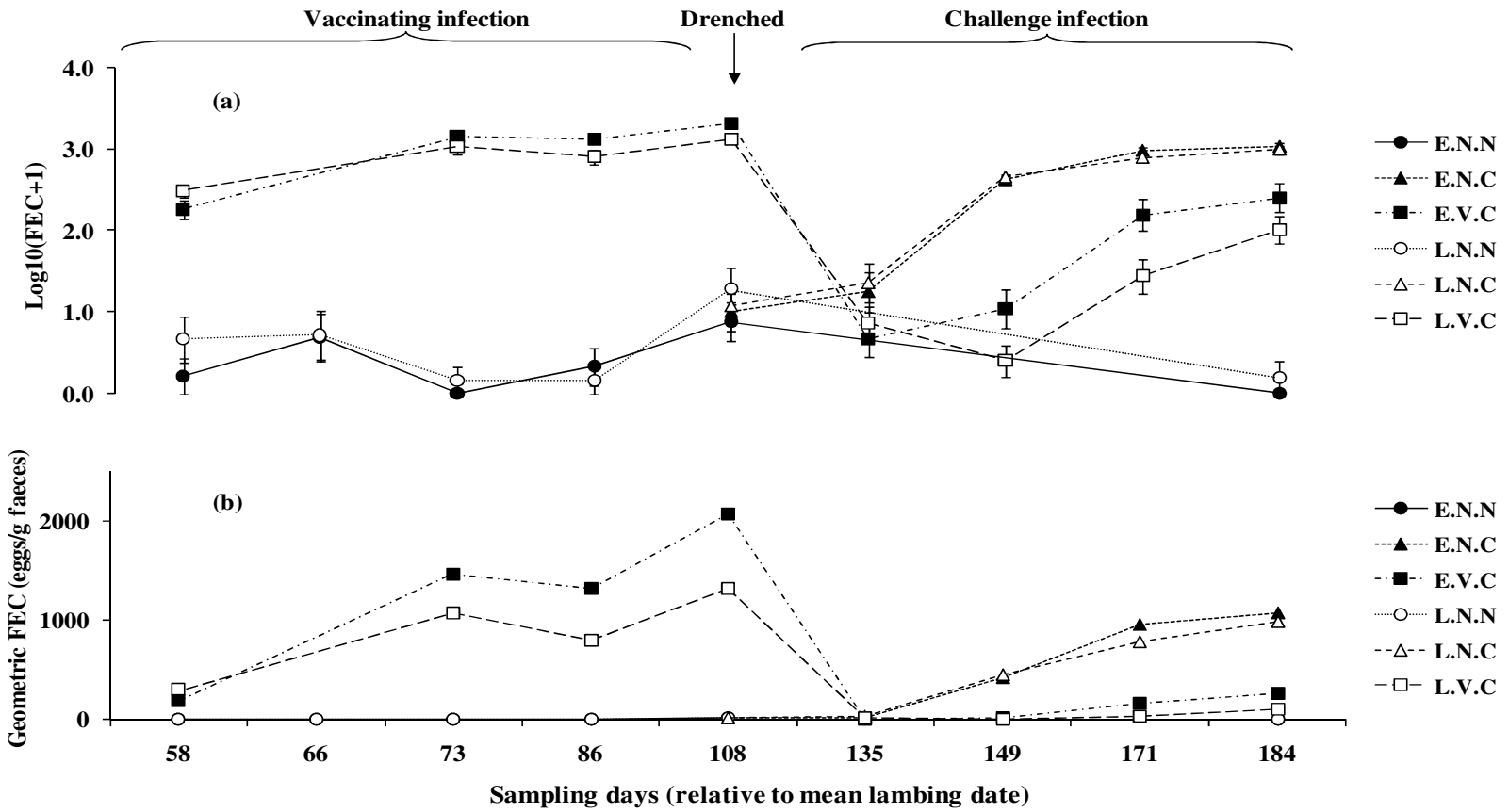


Figure 5.1: Mean FEC of lambs that were either weaned early at day 51 (E.-.-) or late at day 108 (L.-.-). Lambs received vaccinating infection with 60 L_3 larvae mixture/kg LW per day of *T. circumcincta* and *T. colubriformis* (-.V.-; ≈ 40:60, respectively) or not (-.N.-) during days 36 – 103 and challenge infection (-.-.C) or not (-.-.N) during days 116 – 179. All lambs were drenched on day 108 and necropsy groups were slaughtered on days 108, 184, and 218, respectively.

5.3.2. Worm burdens

Necropsy day 108: The mean worm burdens of the lambs slaughtered on day 108 are given in Table 5.3.

Table 5.3: Logarithm-transformed [Log_{10}(count+1)] worm burdens (with back-transformed means in parenthesis) of lambs on necropsy day 108 following vaccinating infection with 60 L_3 larvae mixture/kg LW/d of *T. circumcincta* and *T. colubriformis* (≈ 40:60, respectively; -.V.-) during days 36 – 103 or not (-.N.-). Lambs were either weaned on day 51(E.-.-) or suckled (L.-.-) until necropsy.

Lamb Groups	Worm counts					
	T. circumcincta			*T. colubriformis*		
	L_4	Adult	Total	L_4	Adult	Total
E.N.-	0.62^a (3)	1.50^b (30)	1.64^b (43)	0.00^c (0)	0.98^c (9)	0.98^c (9)
E.V.-	0.42^a (2)	3.05^a (1120)	3.05^a (1123)	2.65^a (447)	4.18^a (15178)	4.20^a (15787)
L.N.-	0.85^a (6)	1.65^b (44)	1.85^b (70)	0.00^c (0)	1.42^b (25)	1.42^b (25)
L.V.-	0.67^a (4)	2.74^a (543)	2.74^a (547)	2.19^b (155)	4.12^a (13222)	4.14^a (13847)
SEM	0.294	0.364	0.379	0.106	0.176	0.177

abc – means with same superscript within each column are statistically similar ($P > 0.05$).
SEM – standard error of means.

There was a highly significant effect of the vaccinating infection on the adult and total *T. circumcincta* populations ($P < 0.001$ in both cases) due to the greater burdens of worms recovered at slaughter from the vaccinated compared with the non-vaccinated groups.

A trend for a weaning × infection interaction ($P = 0.161$) in the *T. colubriformis* L_4 larvae population reflected the large number of L_4 larvae recovered at necropsy due to early weaning among the lambs that received the vaccinating infection (-.V.-) and

absence of an L_4 population in both groups of the non-vaccinated lambs. In addition, the number of *T. colubriformis* worms recovered were significantly influenced ($P < 0.001$) by larval dosing reflected in the greater number of worms (of all developmental stages) recovered from the vaccinated compared with the non-vaccinated lambs. There was no significant effect of weaning on worm burdens ($P > 0.05$) regardless of worm species, however, there were trends for smaller numbers of worms being recovered from the late weaned compared with the early-weaned lambs.

Necropsy day 184: The mean worm burdens of the lambs slaughtered on day 184 are given in Table 5.4.

Table 5.4: Logarithm-transformed [Log_{10} (count+1)] worm burdens (with back-transformed means in parenthesis) of lambs on necropsy day 184 following challenge infection (-.-.C) with 60 L_3 larvae mixture / kg LW/d of *T. circumcincta* and *T. colubriformis* (≈ 40:60, respectively) during days 116 – 179. Lambs were drenched on day 108 after receiving either a vaccinating infection as above (-.V.-) or not (-.N.-) during days 36 – 103, and had been weaned on day 51(E.-.-) or day 108 (L.-.-).

Lamb Groups	Worm counts					
	T. circumcincta			*T. colubriformis*		
	L_4	Adult	Total	L_4	Adult	Total
E.N.C	0.98^{a} (9)	2.87^{ab} (740)	2.88^{a} (765)	1.49^{a} (30)	3.85^{ab} (7007)	3.85^{ab} (7074)
E.V.C	0.91^{a} (7)	2.86^{b} (727)	2.87^{a} (747)	1.47^{a} (28)	3.78^{b} (5979)	3.78^{b} (6041)
L.N.C	0.72^{a} (4)	3.18^{a} (1511)	3.19^{a} (1552)	1.66^{a} (45)	4.21^{a} (16387)	4.22^{a} (16498)
L.V.C	0.92^{a} (7)	3.16^{ab} (1448)	3.17^{a} (1473)	0.92^{b} (7)	3.00^{c} (990)	3.01^{c} (1015)
SEM	0.267	0.158	0.159	0.266	0.191	0.191

abc – means with same superscript within each column are statistically similar ($P > 0.05$).
SEM – standard error of means.

There was a trend for greater numbers of adult ($P = 0.128$) and total ($P = 0.134$) *T. circumcincta* worms recovered from the late compared with the numbers recovered from the early weaned lambs. There were no significant effects of weaning or infection on the *T. circumcincta* L_4 populations ($P > 0.05$), which were very small.

There was a significant weaning × vaccinating infection interaction on the numbers of adult and total *T. colubriformis* worms ($P < 0.05$ in both cases) and a similar trend on L_4 larvae ($P = 0.184$) reflecting highest worm burdens in the late weaned non-vaccinated sheep (L.N.C) and particularly low burdens in their vaccinated contemporaries (L.V.C).

Necropsy day 218: The mean worm burdens of the lambs slaughtered on day 218 are given in Table 5.5 (next page). There was a significant weaning × vaccinating infection interaction ($P < 0.05$) on the numbers of *T. circumcincta* L_4 larvae and a similar trend on total *T. circumcincta* burdens ($P = 0.116$) reflecting the lowest burdens in early weaned lambs, which did not receive the vaccinating infection and in late weaned lambs, which did receive the vaccinating infection. Similarly, there was a significant weaning × vaccinating infection interaction on the adult and total *T. colubriformis* populations ($P < 0.05$ in both cases) reflecting the fact that late weaned lambs, which did not receive vaccinating infection had higher worm burdens than the other groups. In addition, late weaning resulted in a significantly greater ($P < 0.05$) *T. colubriformis* L_4 population.

Table 5.5: Logarithm-transformed [Log_{10} (count+1)] worm burdens (with back-transformed means in parenthesis) of lambs on necropsy day 218 following challenge infection (-.-.C) with 60 L_3 larvae mixture/kg LW/d of *T. circumcincta* and *T. colubriformis* (≈ 40:60, respectively) or not challenged (-.-.N) during days 116 – 213. All lambs were drenched on day 108 after receiving either a vaccinating infection as above (-.V.-) or not (-.N.-) during days 36 – 103, and had been weaned on day 51(E.-.-) or day 108 (L.-.-).

Lamb Groups	Worm counts					
	T. circumcincta			*T. colubriformis*		
	L_4	Adult	Total	L_4	Adult	Total
E.N.N	1.70^{bc} (49)	2.90^{a} (794)	2.97^{a} (942)	0.00^{c} (0)	1.67^{c} (45)	1.67^{c} (45)
E.N.C	1.29^{c} (19)	2.67^{b} (465)	2.73^{b} (536)	0.11^{bc} (0)	2.19^{bc} (153)	2.19^{bc} (153)
E.V.C	1.81^{abc} (63)	2.98^{a} (948)	3.04^{a} (1105)	0.00^{c} (0)	2.26^{b} (183)	2.26^{b} (183)
L.N.N	2.02^{ab} (104)	2.95^{a} (898)	3.02^{a} (1058)	0.35^{ab} (1)	3.03^{a} (1074)	3.04^{a} (1083)
L.N.C	2.33^{a} (214)	2.93^{a} (854)	3.11^{a} (1285)	0.54^{a} (2)	2.81^{a} (647)	2.82^{a} (662)
L.V.C	1.48^{bc} (29)	2.86^{ab} (716)	2.96^{a} (902)	0.11^{bc} (0)	1.93^{bc} (84)	1.93^{bc} (84)
SEM	0.275	0.104	0.108	0.155	0.265	0.265

abc – means with same superscript within each column are statistically similar ($P > 0.05$).

SEM – standard error of means

5.3.3. Live and carcass weights

The changes in mean live weight of the lambs are given in Figure 5.2. All groups progressively gained weight ($P < 0.001$) during the pre-weaning period.

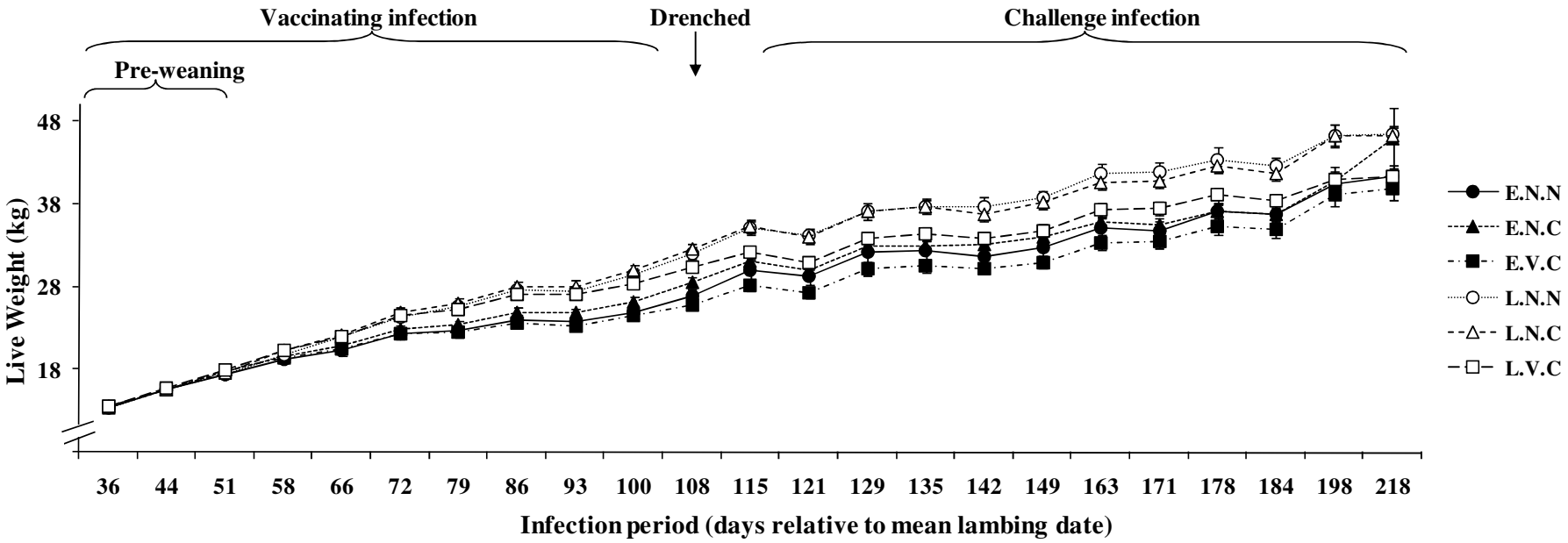


Figure 5.2: Mean live weight changes of lambs that were either weaned early at day 51 (E.-.-) or late at day 108 (L.-.-). Lambs either received vaccinating infection with 60 L_3 larvae mixture/kg LW per day of *T. circumcincta* and *T. colubriformis* (-.V.-; ≈ 40:60, respectively) or not (-.N.-) during days 36 – 103 and challenge infection (-.-.C) or not (-.-.N) during days 116 – 179. All lambs were drenched on day 108 and necropsy groups were slaughtered on days 108, 184, and 218, respectively.

During the vaccinating infection period, but after the weaning on day 51, there was a significant time × weaning interaction ($P < 0.001$) as a result of the late weaned lambs progressively gaining weight at a faster rate than their early weaned counterparts, and a decline in body weight (an average of 0.7 %) between day 86 and 93, which was significant only in the early weaned lambs. Similarly, a time × infection interaction ($P < 0.001$) was due to the non-infected lambs gaining weight faster than their infected counterparts and the greater decline in body weight between day 86 and 93 in the infected lambs. In addition, the late weaned lambs had significantly greater weight than their early-weaned counterparts at every time period of the vaccinating infection but after weaning on day 51, while the greater weight of the non-infected compared with the infected lambs only became significant from day 79 onwards, about 43 days after the commencement of infection. By the day 108 necropsy, infection had resulted in a 7 % lower body weight in the early weaned lambs and a 6 % lower body weight in the late weaned lambs.

During the challenge infection period, days 116 to 218, there was a significant time × weaning interaction ($P < 0.05$) as a result of a continuing greater weight gain in late weaned than in early-weaned lamb even after weaning of the former. There was also a significant effect of vaccinating infection ($P < 0.001$) reflecting the consequently lower body weight of the lambs that had received the vaccinating infection (-.V.C).

By the day 184 necropsy, the early-weaned lambs that received the vaccinating infection (E.V.C) had suffered a 5 % loss in body weight as a result of the challenge infection while their late weaned counterparts (L.V.C) suffered a 10 % weight loss. Likewise, the early-weaned lambs that did not receive the vaccinating infection

(E.N.C) suffered only a 0.3 % reduction in weight due to the challenged infection compared with a 2 % reduction in their late weaned counterparts (L.N.C).

By the day 218 necropsy, however, the early-weaned lambs that had not received the vaccinating infection (E.N.C) had an 11 % weight advantage despite the challenge infection while their late weaned counterparts (L.N.C.) suffered a 0.3 % weight loss. On the other hand, the early-weaned lambs that received the vaccinating infection (E.V.C) suffered a 3 % weight loss as a result of the challenge infection compared with an 11 % weight loss in their late weaned counterparts (L.V.C). The lambs that did not receive the vaccinating infection (-.N.C) appeared not to suffer any loss in performance due to the challenge infection.

The mean carcass weight of the lambs that were slaughtered on day 108 is given in Figure 5.3. Weaning significantly reduced carcass weight ($P < 0.001$), while the vaccinating infection tended to reduce carcass weight ($P = 0.161$), the extent of which appeared to be more marked among the late weaned lambs (L.-.-).

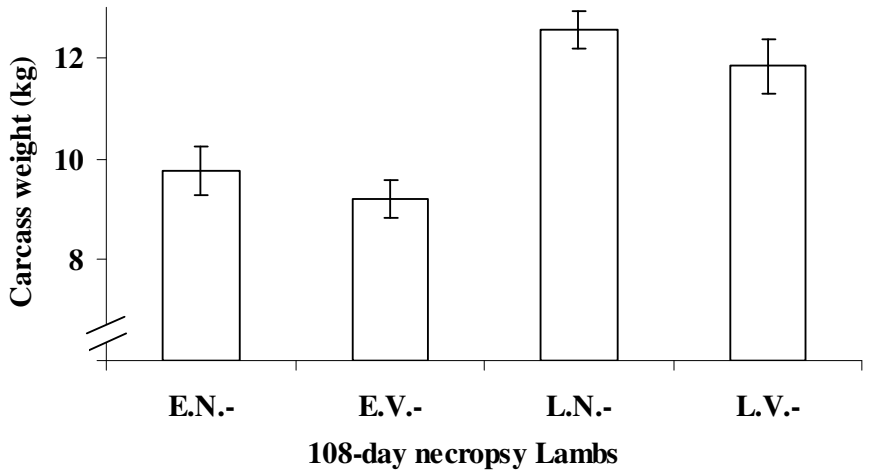


Figure 5.3: Mean carcass weight (± SE) of lambs on necropsy day 108 following vaccinating infection with 60 L_3 larvae mixture/kg LW/d of *T. circumcincta* and *T. colubriformis* (≈ 40:60, respectively; -.V.-) during days 36 – 103 or not (-.N.-). Lambs were either weaned on day 51(E.-.-) or suckled (L.-.-) until necropsy.

Similarly, early weaning significantly reduced ($P < 0.001$) the carcass weight at the 184 day necropsy (Figure 5.4), while the vaccinating infection reduced carcass weight ($P < 0.05$), the extent of which appeared to be more marked among the late weaned lambs (L.-.-).

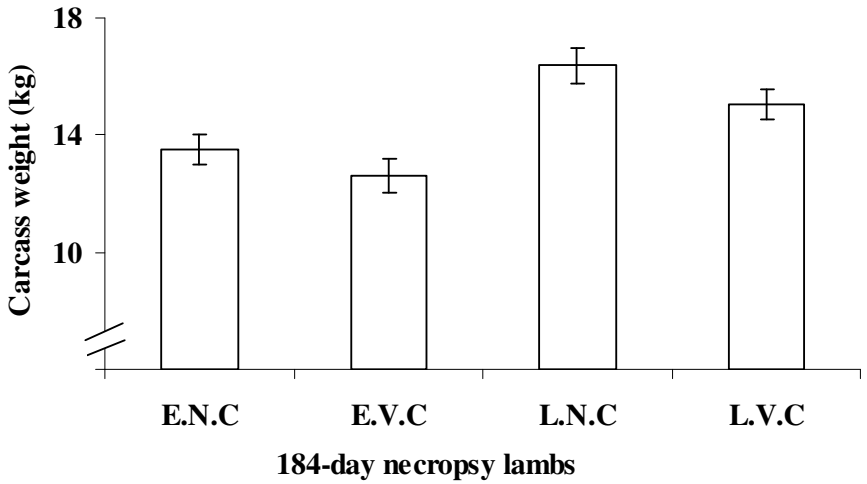


Figure 5.4: Mean carcass weight (± SE) of lambs on necropsy day 184 following challenge infection (-.-.C) with 60 L_3 larvae mixture/kg LW/d of *T. circumcincta* and *T. colubriformis* (≈ 40:60, respectively) during days 116 – 179. Lambs were drenched on day 108 after receiving either a vaccinating infection as above (-.V.-) or not (-.N.-) during days 36 – 103, and had been weaned on day 51(E.-.-) or day 108 (L.-.-).

The mean carcass weights of the lambs slaughtered on day 218 are given in Figure 5.9. The late weaned lambs had significantly greater carcass weight ($P < 0.001$) than their early-weaned counterparts did. In addition, vaccinating infection resulted in a reduced carcass weight ($P < 0.01$), an extent which, again, appeared to be more marked among the late weaned lambs (L.-.-).

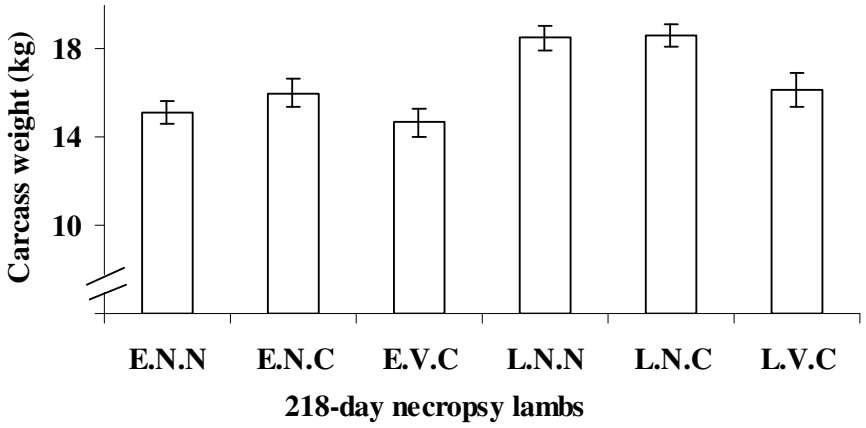


Figure 5.5: Mean carcass weights (± SE) of lambs on necropsy day 218 following challenge infections (-.-.C) with 60 L_3 larvae mixture/kg LW/d of *T. circumcincta* and *T. colubriformis* (≈ 40:60, respectively) or not challenged (-.-.N) during days 116 – 213. All lambs were drenched on day 108 after receiving either a vaccinating infection as above (-.V.-) or not (-.N.-) during days 36 – 103, and had been weaned on day 51(E.-.-) or day 108 (L.-.-).

5.3.4. Immunology

Total antibody response

The changes in total antibody (optical density, OD; Ig) response to L_3 larvae in plasma of the lambs are given in Figure 5.6. During the vaccinating infection there was a significant effect of time ($P < 0.001$) and infection ($P < 0.05$) reflecting a decrease in total antibody titre of all groups during the pre-weaning period and a greater Ig OD of the lambs that did not receive the vaccinating infection (-.N.-). During the challenge infection period there was a time × vaccinating infection interaction ($P < 0.001$) reflecting the higher titres in the previously vaccinated sheep (L.V.C and E.V.C)

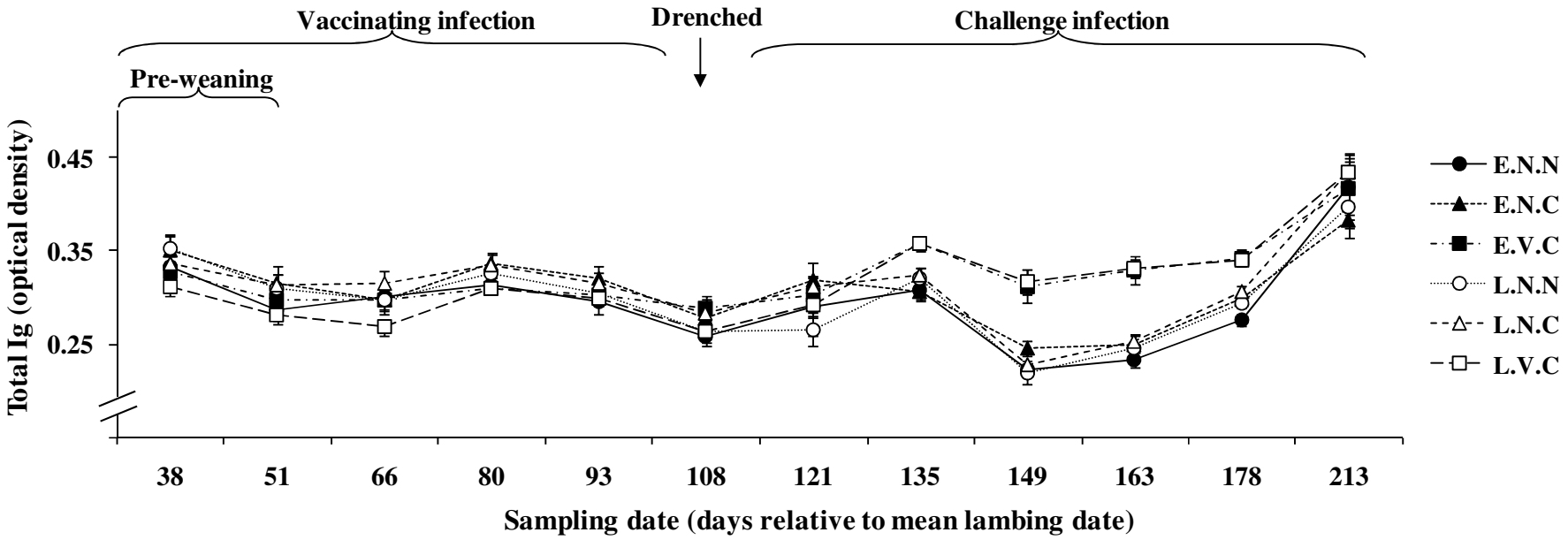


Figure 5.6: Changes in mean total plasma antibody (± SE) of lambs that were either weaned early at day 51 (E.-.-) or late at day 108 (L.-.-). Lambs either received vaccinating infection with 60 L_3 larvae mixture/kg LW per day of *T. circumcincta* and *T. colubriformis* (-.V.-; ≈ 40:60, respectively) or not (-.N.-) during days 36 – 103 and challenge infection (-.-.C) or not (-.-.N) during days 116 – 179. All lambs were drenched on day 108 and necropsy groups were slaughtered on days 108, 184, and 218, respectively.

Immunoglobulin A (IgA) response

The changes in IgA (optical density) response to L_3 larvae in plasma of the lambs are given in Figure 5.7. During the vaccination period there was a significant progressive decrease in IgA titre of all groups during the pre-weaning period ($P < 0.001$) but, after weaning on day 51, there was a significant time × infection interaction ($P < 0.01$) reflecting the greater rise in IgA titre in the vaccinated lambs. During the challenge infection period there were time × vaccinating infection ($P < 0.001$) and weaning × vaccinating infection ($P < 0.01$) interactions reflecting greater increases in titres in the non-vaccinated and non-vaccinated and non-challenged sheep than in previously vaccinated sheep.

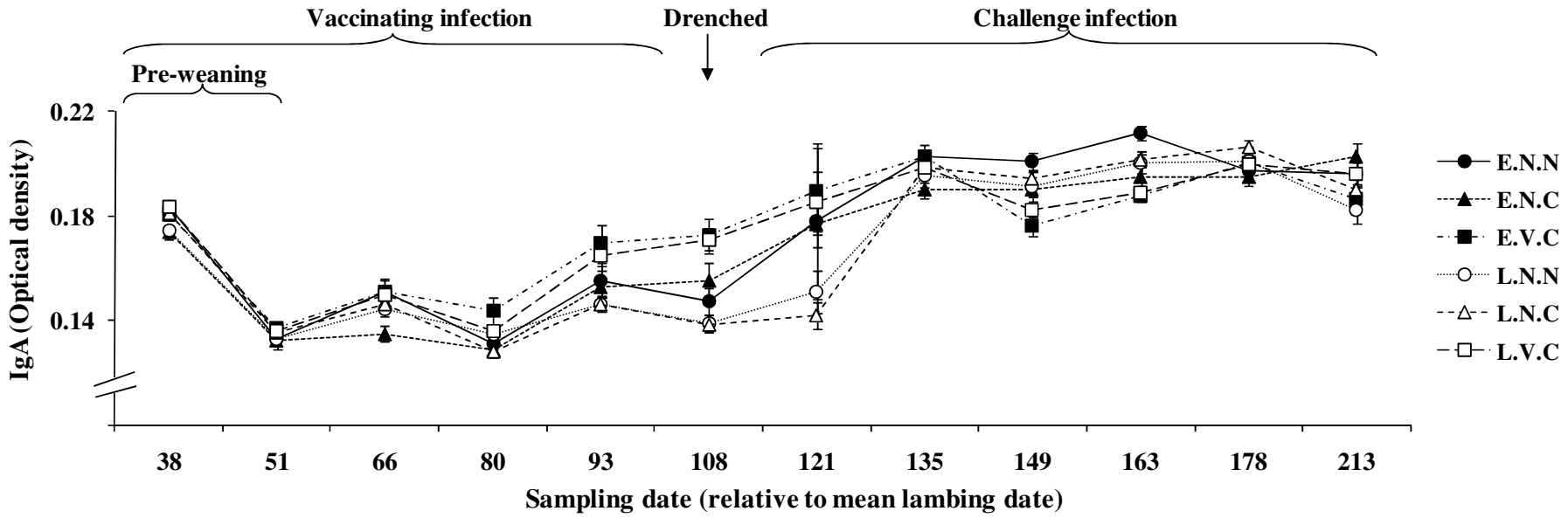


Figure 5.7: Changes in mean IgA (± SE) of lambs that were either weaned early at day 51 (E.-.-) or late at day 108 (L.-.-). Lambs either received vaccinating infection with 60 L_3 larvae mixture/kg LW per day of *T. circumcincta* and *T. colubriformis* (-.V.-; ≈ 40:60, respectively) or not (-.N.-) during days 36 – 103 and challenge infection (-.-.C) or not (-.-.N) during days 116 – 179. All lambs were drenched on day 108 and necropsy groups were slaughtered on days 108, 184, and 218, respectively.

Immunoglobulin M (IgM) response

The changes in IgM (optical density) response to L_3 larvae in plasma of the lambs are given in Figure 5.8. There was a significant decrease in IgM titre during the pre-weaning period ($P < 0.001$). During the vaccinating infection period but after weaning on day 51 a time × weaning × infection interaction ($P < 0.05$) reflected the significant decline in IgM titre due to the vaccinating infection among the late weaned but an insignificant rise among the early-weaned lambs by day 93. During the challenge infection period a significant time × vaccinating infection ($P < 0.001$) interaction was due to greater titres in the previously vaccinated groups during days 149 – 178.

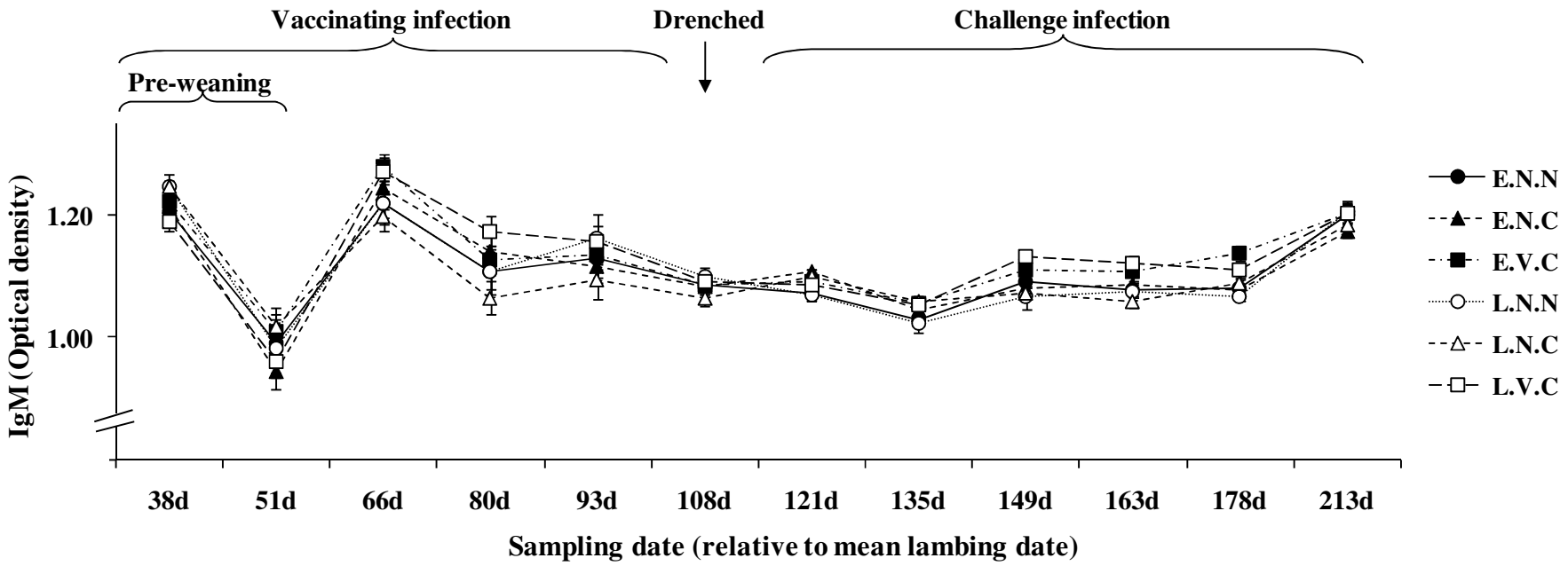


Figure 5.8: Changes in mean IgM (± SE) of lambs that were either weaned early at day 51 (E.-.-) or late at day 108 (L.-.-). Lambs either received vaccinating infection with 60 L_3 larvae mixture/kg LW per day of *T. circumcincta* and *T. colubriformis* (-.V.-; ≈ 40:60, respectively) or not (-.N.-) during days 36 – 103 and challenge infection (-.-.C) or not (-.-.N) during days 116 – 179. All lambs were drenched on day 108 and necropsy groups were slaughtered on days 108, 184, and 218, respectively.

5.4. Discussion

The results of this experiment confirm the earlier findings of some benefits of suckling for reducing worm burdens and FEC but also suggest that longer-term effects of larval experience during the suckling period may also occur. On the other hand, there was little evidence that continued suckling improved the resilience of the lambs to the effects of infection and indeed, such evidence as there was could be interpreted to the contrary. An important achievement of the current study also was the very low pasture larval contamination, reflected in the relatively low FEC (< 10 e.p.g; Figure 5.1) and the low worm burdens of the non-infected lambs at the day-108 necropsy (Figure 5.9), which had allow the opportunity of assessing the effect of suckling on resilience to infection of the present lambs.

The FEC profiles of the lambs during the vaccinating infection period suggest some benefits of extended suckling for the ability of lambs to shed fewer nematode eggs in the short term. These are consistent with the findings of the trials of Chapter 3 and the 42-day infection phase of the Chapter 4 trial, which further suggests a probable direct effect of milk on larval establishment as previously hypothesized by Zeng *et al.* (2001; 2003). Although peripheral titres are not a precise indication of IgA production at the site of infection, particularly at low rates of infection (Sinski *et al.* 1995), the inability to detect IgA response in plasma during the same period (Figure 5.7), as was the case in the previous Chapters, may rule out an immune response as the cause of the lower FEC in the suckled lambs at this stage and offer support for the direct effect theory.

Given that previous works have suggested that additional protein appears to enhance the development of acquired rather than innate immunity (Bown *et al.* 1991;

van Houtert & Sykes 1996; Donaldson *et al.* 2001), the possible indirect and long term benefit of improved protein nutrition as a result of suckling, which was also demonstrated in Chapter 4, appeared to have been featured in the FEC profiles of the present lambs during the challenge infection. However, that benefit appeared to be conditional on the vaccinating effect of early exposure of lambs to infective larvae. While late weaning appeared not to benefit the challenged lambs that did not experience the vaccinating infection (-.N.C), their vaccinated counterparts (-.V.C) appeared to benefit from the extended suckling, suggesting the strengthening of the development of immunity under larval challenge by the extended suckling. Previous workers have demonstrated the advantage of vaccinating infection (Downey 1968; Jackson & Christie 1979; Wagland *et al.* 1984; Seaton *et al.* 1989; Emery *et al.* 1999), and of enhanced protein nutrition (Downey *et al.* 1972; Wagland *et al.* 1984; Bown *et al.* 1991; Kambara *et al.* 1993; Coop *et al.* 1995; van Houtert & Sykes 1996; Kahn *et al.* 2000; Walkden-Brown & Kahn 2002; Houdijk *et al.* 2005; Louvandini *et al.* 2006) for development of resistance, but few appeared to have explicitly shown the interdependence between the two. Wagland *et al.*(1984) did find that lambs on a high plane of nutrition, based on pelleted lucerne hay (18 g CP / 100 g DM), had higher titres of antibodies to *T. colubriformis* following vaccination, and after challenge had lower worm egg outputs and lower worm burdens than lambs on the same diet but on a low plane of nutrition. In a similar experiment, in which groups of four-and-a-half-month-old worm-free lambs were either trickled infected with *T. circumcincta*, trickle infected and given abomasal infusion of casein, or not trickle infected but challenged (Coop *et al.* 1995), the trickle-infected lambs had higher concentrations of gastric mast cell protease than the challenged controls; an increase that was significantly greater in

the casein infused than in the unsupplemented lambs. They concluded that the provision of by-pass protein supplement (abomasal infusion of casein) might have accelerated the development of immunity to *T. circumcincta* in their experimental lambs. Emery *et al.*(1999) reached the same conclusion in a study where two age groups of lambs, 7-week old neonate and 4-month-old weaned lambs, were trickle-immunised or not for six weeks and then challenged. Lambs had similar worm counts 10 days after challenge, but from 25 days, significant reductions in mean FEC ($P < 0.01$) and reductions in worm counts in excess of 75 % were displayed by the immunised neonates; an effect that was greater than observed in the 4-month-old weaned animals. They attributed the differences in protection between the two age groups to a difference in nutrition as suckling lambs showed better protective response from the immunizing infection than 4-month-old lambs. A similar reduction in FEC due to trickle-immunisation in the current study occurred about 33 days after the commencement of the challenge infection. In an earlier study, Bown *et al.* (1991) found abomasal protein infusion reduced mean FEC and mean total parasite count, and concluded that the debilitating effect of intestinal parasites could be markedly reduced by increasing duodenal protein supply. With the mechanism of the oesophageal groove reflex, routing milk directly into the abomasum during sucking, milk feeding would be expected to be a good source of high quality duodenal protein supply. It may be significant that in all the studies that have shown some benefits of additional protein to the abomasum, either by direct infusion of casein (Bown *et al.* 1991; Coop *et al.* 1995); suckling [the present, (Wagland *et al.* 1984; Watson & Gill 1991b)]; or use of protected protein (van Houtert *et al.* 1995), improvement of aspects of immune development have been seen.

On the contrary, however, Datta *et al.* (1999b) could not find any interaction between previous infection and dietary protein concentration in a study with lambs that involved short period (9-weeks of uninfected, or infected with *H. contortus*) of protein supplementation (ranging from 10 – 22 % CP) followed by 69 weeks of pasture grazing without supplementation. However, they did find a long-term benefit of the supplementation for resistance to parasitism. Similarly, prior exposure to antigen could not induce anti-*H. contortus* antibody response or resistance to the parasite in neonatal lambs of Watson & Gill (1991a); they, however, concluded that post natal ontogeny of immune responses was different for the various antigens/pathogens used in their study. The conflicting evidence cited above could be as a result of differences in the nature of the protein fed; the benefit of improved protein nutrition may be more related to specific amino acids, which might be limiting in some supposed high protein experimental feed. Differences in results could also be due to the differences in the immunogenecity of the nematode species investigated in the various studies. It might be important that the development of resistance to *H. contortus* is little related to prior exposure to antigen judging by the findings of Watson & Gill (1991a) and Datta *et al.* (1999b).

The peak FEC values observed during the vaccinating period are comparable to those observed in the previous chapters but the trend did not show the evidence for intra-worm population regulation seen in the 84-day period of the previous Chapters. Faecal egg count rose to a peak and was maintained at a plateau up to day 108 in all groups compared with the abrupt drop in FEC as early as day 36 in the work described in previous Chapters. This may well be a consequence of the use of mixed species infection. Steel *et al.* (1982) and Sykes *et al.* (1988) observed a similar maintenance of

plateau values with mixed infection of these nematode species. This is probably a reflection of the dominance (in number) of the *T. colubriformis* population (Figure 5.9, next page). Egg numbers in faeces are known to be more closely related to the adult worm burdens of this nematode (Steel *et al.* 1980; Dobson *et al.* 1990) in contrast to *T. circumcincta* populations, which are considered to be both poorer egg layers and are recognised for their intra-worm population regulation of egg production (Symons *et al.* 1981; Callinan & Arundel 1982; Coop *et al.* 1985). The contrast between nematode species may well reflect the basic differences in worm population dynamics of the two species. Barger (1987) argued that adult worm populations of *T. circumcincta* are inversely related to larval intake of the host, i.e. adult worm numbers are regulated by the number of incoming larvae, whereas for *T. colubriformis*, an established adult population is considered to regulate establishment of incoming larvae.

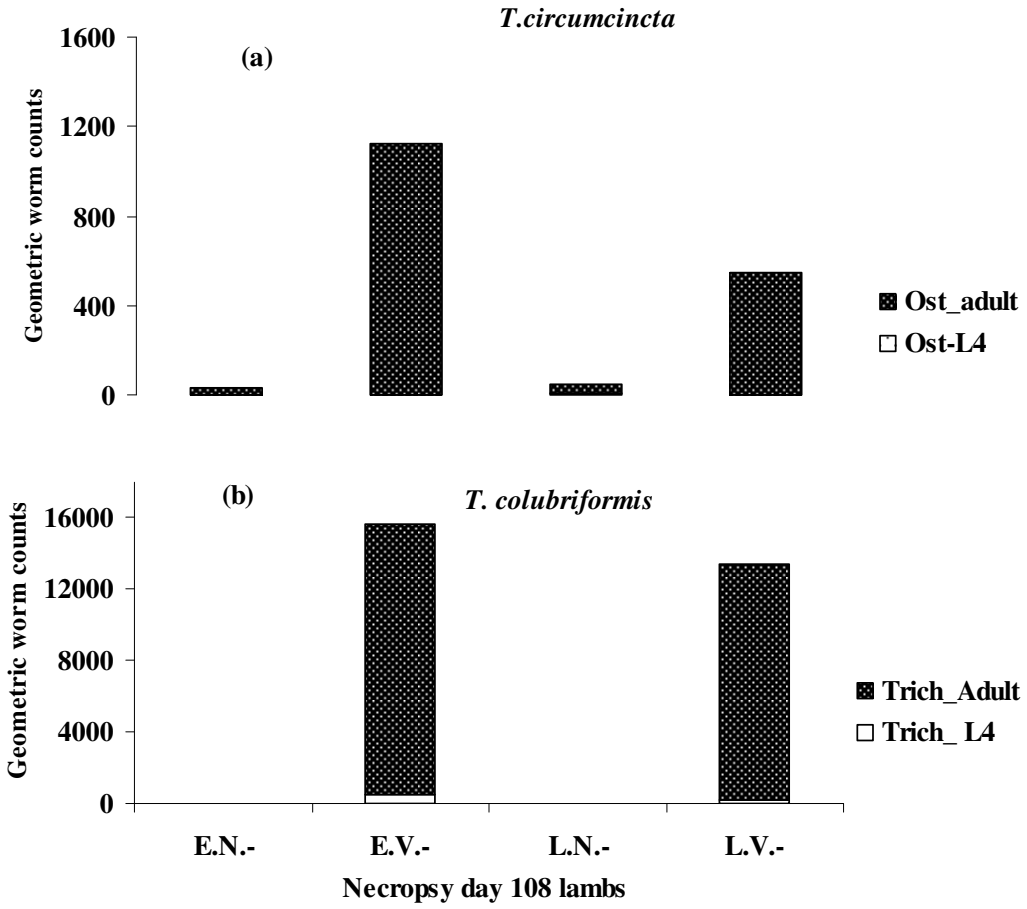


Figure 5.9: Adult and L_4 worm populations of *T. circumcincta* and *T. colubriformis* in lambs necropsied on day 108.

The peak and trend of FEC from the vaccinated groups, dosed at the rate of 60 larval mixture / kg LW / d, were comparable to those of Sykes *et al.* (1988) from lambs infected for 84 days with 1000 larvae each of *T. circumcincta* and *T. colubriformis*, which amounted to a similar rate of 62 larvae / kg LW/d, but in older lambs. Peak FEC were lower but the trend remained the same as with the lambs of Steel *et al.* (1982), which were infected at about double the rate and at older age than the present lambs.

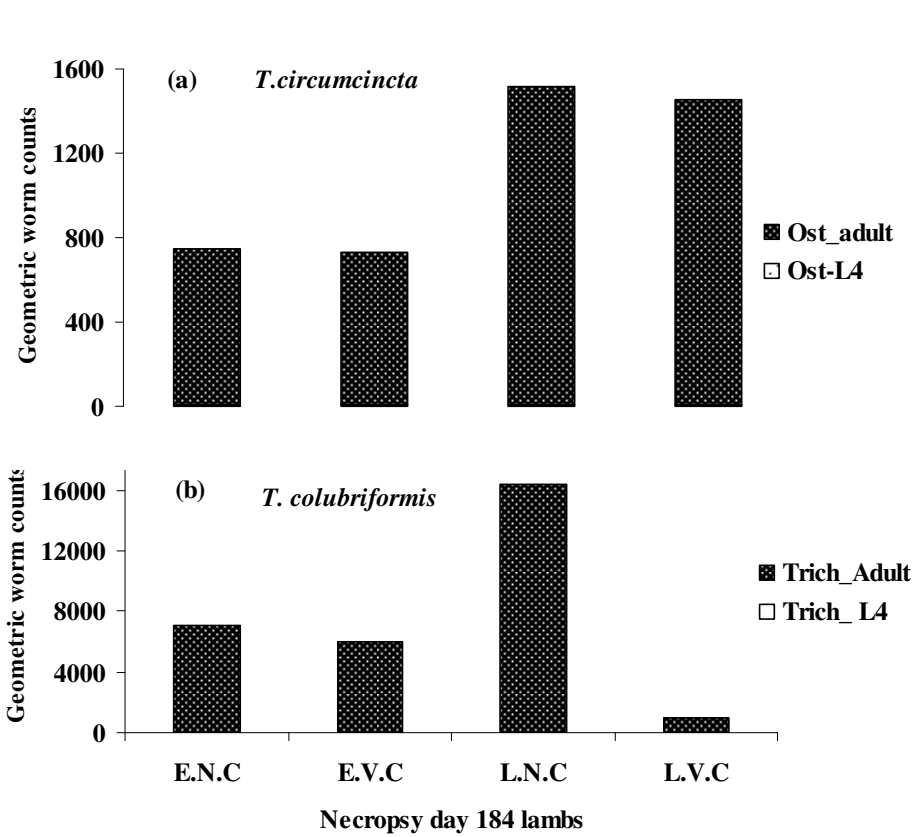
The worm burdens observed suggest a very low establishment rate of the contrived infection, which may indicate a low infectivity of the larvae given. The low *T. circumcincta* burdens compared with the previous Chapters, which could be explained

by the later necropsy on day 108 compared to day 84 of the previous Chapters, since lambs may have shed considerable numbers of established worms before necropsy. The small *T. circumcincta* population might, however, be consistent with an observation in mixed infection studies by Sykes *et al.* (1988) that infection with *T. colubriformis* reduced the number of adult worms of *T. circumcincta* present at necropsy.

The pattern of worm burdens observed in the day 108 necropsy (Figure 5.9a) did suggest a possible direct effect of milk on establishment of *T. circumcincta* in the abomasum as similarly observed in the previous Chapters and in the works of Zeng (2001; 2003). The effect was, however, not as obvious with the *T. colubriformis* population at this stage (Figure 5.9b). It would be pure speculation to suggest that components of the milk, which could influence larval motility, would have been partly digested before reaching the small intestine, and therefore fail to deter larval establishment of *T. colubriformis*. If exsheathment is a factor, and both species of worms are similar in their sensitivity, the establishment of *T. colubriformis* larvae would not have been negatively affected by milk medium in the abomasum, since failure to exsheath was not found to be a hindrance to the establishment *T. circumcincta* larvae in the milk-fed lambs of Zeng *et al.* (2001). The conditions in the small intestine are, however, very different to those of the abomasum, for example, the differences in pH would be expected to affect the nature of proteins and many other chemical factors, which could influence exsheathment and motility of larvae. It is interesting in the same vein, in relation to the adverse effects of supplementation with copper wire particles, which release copper in the abomasum, that only the abomasal dwellers, *T. circumcincta* and *H. contortus*, were reduced by copper wire

supplementation but not *T. colubriformis* living only a few metre further down the gut (Bang *et al.* 1990a). The conditions at the site of predilection of the particular nematode could therefore have a major bearing on the response to components of the diet.

The burdens at the necropsy days 184 and 218 did reflect the long-term benefit of suckling, but only when the lambs were exposed to the vaccinating infection. This appeared to be more pronounced in the reduction of *T. colubriformis* burdens on day 184 (Figure 5.10, next page) and day 218 (Figure 5.11), and to a lesser extent for *T. circumcincta* on day 218. The long-term benefit of suckling thus appeared to be more pronounced against the *T. colubriformis* burdens than against the *T. circumcincta* burdens. This is more likely to be due to an enhanced immune response rather than as a direct effect of milk on larval establishment, and might be a reflection of a more immunologically active small intestine than abomasum. The presence of clusters of immunologically important cells of the immune armoury in Peyers patches and the greater numbers of globule leukocytes in intestinal compared with abomasal mucosa of both immunized and genetically resistant sheep (Stankiewicz *et al.* 1995; Douch *et al.* 1996b) support this conclusion. Therefore, the immune response in the small intestine may, in reducing the *T. colubriformis* population, be more sensitive to protein nutrition with consequences beyond weaning. The fact that lowest burdens of *T. colubriformis* were observed in the late weaned and vaccinated lambs (L.V.C on



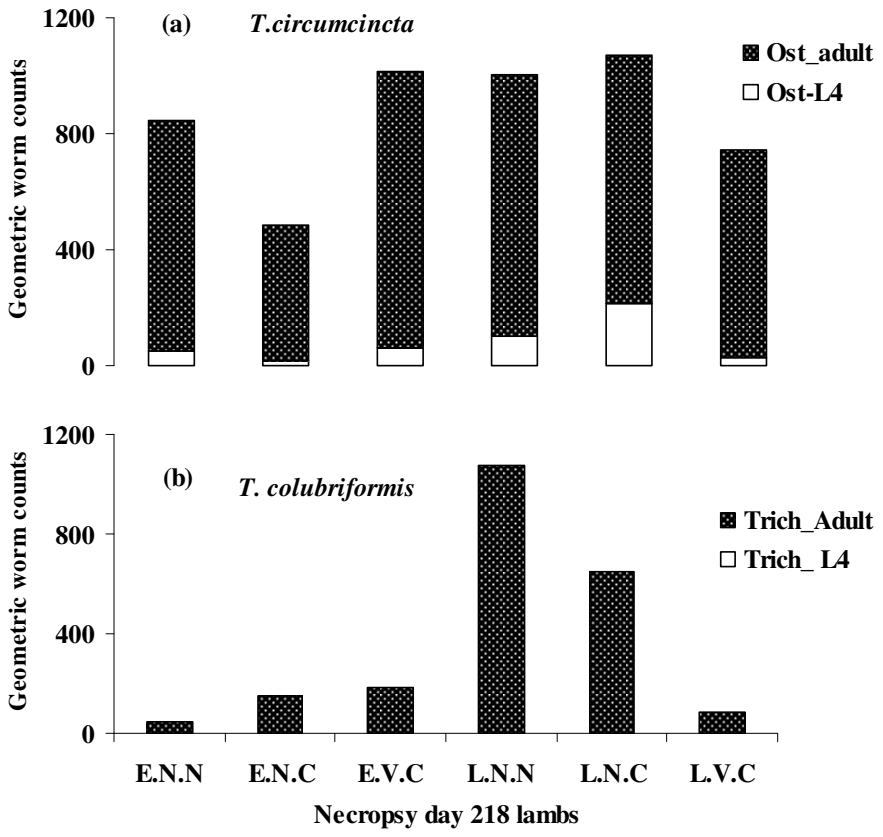


Figure 5.10 and 5.11: Adult and L_4 worm populations of *T. circumcincta* and *T. colubriformis* in lambs necropsied on day 184 and day 218, respectively.

day 184 and day 218 slaughters) argues for the importance of nutrition, and probably the protein supply (Bown *et al.* 1991) for the development of immunity to the nematode, even on the long term (Datta *et al.* 1999b). This was consistent with the lower FEC profile during the challenge infection of lambs previously vaccinated while suckling.

A feature of the worm burdens at day 184 and 218 was the fact that highest worm burdens were observed in lambs weaned late without receiving a vaccinating infection. This was the case in group L.N.C on day 184 (Figure 5.10) and groups L.N.N and L.N.C on day 218 (Figure 5.11). This appeared to be at odd with the concept of benefit of suckling for early development of resistance. Looked at from the standpoint that vaccinating infection had enabled the observation of a benefit of suckling for resistance, it seems plausible to suggest that in the absence of a sensitising infection suckling had resulted in a prioritising of metabolic resources away from the development of the immune armoury, probably towards growth. The surprising issue is that it occurred under circumstances of relatively small difference in growth rate and, therefore presumable small difference in total nutrient intake. This suggests that specific nutrients, possibly amino acids, or lack of physiological signalling associated with the response to the vaccinating infection at a critical time of development may be involved. This is an important and possible fruitful area for research if, in the future, methods of parasite control other than chemicals are to be used.

The effect of infection (vaccinating) on performance - 6 to 7 % reduction in growth rate - of early weaned and suckled lambs, respectively, was very small. The rate of infection used (60 L_3 larvae / kg W / d) was at the low end of the range of trickle

infection used in the literature with these nematode species. Sykes *et al.* (1988) using a 50:50 mixture of these nematodes at the rate of 71 L_3 larvae / kg W / d observed a 43 % reduction in growth rate and abolition of growth at a rate of 142 L_3 larvae / kg W / d. On this basis, I had anticipated a reduction in growth rate of at least 30 – 40 %. The fact that both the present work and that of Sykes *et al.* (1988) used fresh herbage diets suggests that diet was unlikely to be responsible for the variation in pathogenicity. A low establishment rate, in which the incoming larvae can be presumed to have failed to penetrate tissues of the host (Jackson *et al.* 2004) seems likely to have resulted in the low pathogenicity of infection (Colditz *et al.* 1996). This makes interpretation of effects of suckling on resilience more difficult to ascertain. On the other hand, the low pasture larval contamination did provide maximum opportunity to detect any resilience. In agreement with the findings in earlier Chapters, early weaning was not associated with reduced resilience to nematode infection. Furthermore, the vaccinated lambs of the suckled groups tended to suffer a greater reduction in carcass weight than early weaned and vaccinated counterparts. While the effect was small, it does nevertheless suggest a possible negative consequence, for productivity, of the early development of resistance as shown in carcass weights (Figures 5.4 and 5.5). This was consistent with the findings in Chapter 4. It further supports the argument that while improved protein nutrition could speed up the development of resistance (Bown *et al.* 1991; Coop *et al.* 1995; van Houtert *et al.* 1995), the consequences of increased resistance could be the increased metabolic cost of the immune system. Such increased costs and evidence for appetite depressing effects associated with the developing immune response have been recently observed (Greer *et al.* 2005). Reduction of food intake during nematode infections of young sheep has been found to vary from less

than 0.1 to complete anorexia (Sykes 1983; Dynes *et al.* 1990), and has been calculated to be responsible for 0.6 – 0.9 of the loss in performance during nematode infection (Coop & Holmes 1996; Kyriazakis *et al.* 1996; van Houtert & Sykes 1996). It would have been logistically difficult to measure feed intake in a trial of this magnitude but a reduction of food intake may also have been part of the metabolic cost of the resistance to parasites in this study.

The immunological response of the lambs appears to be consistent with the low establishment of infection. While a rise in plasma IgA was observed as early as day 77 (i.e. 35 days of infection) in the previous Chapters, such rise was delayed in the present lambs until day 122, about 82 days from the commencement of vaccinating infection (Figure 5.7). This coincided with a slight depression in live weight gain of the lambs about the same time (Figure 5.2), perhaps suggesting the metabolic cost of immune development and possible reduction in feed intake which has been reported by Greer (2005) in an immune suppression study. The responses of the lambs in the post challenge-infection period showed greater plasma levels of total antibody in the vaccinated lambs compared with their challenged counterparts (Figure 5.6), which further suggests the vaccinating effect of their earlier exposure to infective larvae during the vaccination period. This was consistent with the more rapid IgA response to initiation of infection in the vaccinated compared with the non-vaccinated lambs (Figure 5.7).

5.5: Conclusions

In conclusion, the work provides support to the hypothesis that: a. suckling may reduce the establishment of nematode larvae through the direct effect of milk, b. may

promote the more rapid development of host immunity to infection, and c. it further suggests that lack of larval experience during suckling may have long-term negative implications for host resistance. Finally, it suggests that milk may play little role in the enhancement of host resilience to infection and, on the contrary, that additional metabolic cost may be associated with a more rapid development of immunity resulting from larval challenge while suckling.

Chapter 6

General Summary, Conclusions, and Future Research Prospects

The results of the experiments reported in this work have unequivocally demonstrated the role extended suckling could play in the welfare of young lambs during nematode parasite infection, both in the short and long term beyond weaning. The approach was to investigate the various means by which milk could influence parasite infection in lambs *viz.* the possibility of a direct effect of milk on larval establishment in the short term, the role of milk as a good source of nutrient for development of immune system and therefore *Resistance*, and the performance of lambs in the face of infection, often refer to as *Resilience*.

In particular, the hypothesis by Zeng *et al.* (2001) and some earlier workers (Waller & Thomas 1978; Satrija *et al.* 1991) that milk could have a direct effect on the establishment of infective larvae of *Teladorsagia circumcincta* and *Ostertagia ostertagi* in the abomasum and consequently result in a reduction of adult worms and number of eggs shed in faeces was confirmed in all the three experiments. Although the interpretation of the faecal egg counts was sometimes made difficult by the peculiar nature of the *T. circumcincta* worm, as a poor egg producer and capable of intra worm - population regulation, the two to three fold reduction in worm burdens due to suckling in the Chapter 3 lambs suggests milk could help reduce parasite population in the short term. The design of the Chapter 3 trial was short termed, purposely to separate the possibility of an enhanced host immune mechanism, due to improved nutrient supply from milk, from the direct effect of milk *per se* on larval establishment. This was premised on the knowledge of the facts in literature, though

with older hosts, that host immunity could be anticipated to begin to limit the worm population after about six weeks of infection (Gibson & Parfitt 1976; Coop *et al.* 1977, 1982). This was confirmed in the present work by the inability to detect peripheral titres of immunoglobulin isotypes, specifically IgA, IgM and total Ig, although histological measurements of local immunity would have clarified this better. In addition, the lack of effect on some parameters of pathogenicity, notably plasma pepsinogen and abomasal pH, allowed for the conclusion that the lower worm burdens in suckled lambs at slaughter could have been a consequence of a possible direct effect of milk on larval establishment at that stage rather than a more systemic process of enhanced immunity as a result of improved nutrient supply. The exact mechanisms by which milk may hinder larval establishment are yet to be clarified, and therefore remained a prospect for future research. A knowledge of these mechanisms could avail the opportunity of exploiting a natural phenomenon of neonate nutrition (act of suckling) in controlling abomasal parasites at a reduced or almost costless investment. Arguments to support the involvement of various components in milk have been adduced in some previous work, for example, milk proteins and components associated with milk proteins on motility of nematode larvae (Zeng *et al.* 2003) and of oligosaccharides on the adhesion of pathogens to host mucosa (Hakkarainen *et al.* 2005).

The potential of the milk diet as a superior source of nutrient for the development of resistance and resilience to infection by lamb was also tested and confirmed in the second and third experiments. The suckled lambs consistently harboured lower worm burdens at necropsy dates beyond the period when the earlier direct effect of milk on larval establishment would have waned. At this point, the acquisition of immune

competence by the growing lambs was observed to be enhanced by suckling judging by the findings in earlier works on the role of improved protein diet in enhancing the resistance to nematode infection (Sykes 1987; Bown *et al.* 1991; Coop *et al.* 1995)

The absence of an effect of improved nutrient supply from milk on resilience of lambs to infection in the 42-day infection trials of Chapter 3 and 4 was not totally unexpected, and was explained by the likelihood that lambs may have been spared the metabolic cost of an immune response at that age, based on the conclusions of Greer (2005). However, it was surprising that early weaning could not be associated with reduced resilience of lambs during the longer-term trials of Chapters 4 and 5 when the immune response by the lambs was anticipated to be developing. In fact, the evidence found during those trials suggests that the speeding up of acquisition of resistance in the lambs by suckling or improved nutrient supply had some metabolic costs, which affected the performance of the suckled lambs. By and large, however, suckling enhanced weight gain in the lambs but there were no evidence to suggest early weaning could be associated with reduced resilience.

The phenomenon of the vaccinating effect of early exposure of animals to nematode, which has been demonstrated in older animals (Downey 1968; Jackson & Christie 1979; Wagland *et al.* 1984; Seaton *et al.* 1989; Emery *et al.* 1999) was incidentally demonstrated in the longer term design of the Chapter 4 trial in neonate lambs and was confirmed in the design of the Chapter 5 trial. Of interest was the interdependence between the vaccinating infection and suckling for the ability of lambs to show resistance to nematodes, which, again, was accompanied by the metabolic cost of acquiring immune competence as observed in the vaccinated lambs

during the challenge infection period of the Chapter 5 trial. A corollary to this in the same trial was the high FEC and worm burdens observed in the groups that had not received the vaccinating infection which, however, showed better weight gains and carcass weight than their vaccinated counterparts. The results in Chapter 5 also revealed some differences in the immunopathology of the abomasum and small intestine in relation to infections with *T. circumcincta* and *T. colubriformis*, respectively. Judging by the greater reduction of *T. colubriformis* populations in the small intestine than of the *T. circumcincta* populations of the abomasum in the mixed infection trial of Chapter 5, it was suggested that the immune response in the small intestine may be more sensitive to protein nutrition with consequences beyond weaning.

The series of experiments conducted in this work were set out to contribute to the on-going search for alternatives to the current conventional and widely practised chemical-based control of nematode parasites, which is becoming unpopular. While several studies of this kind have focused mainly on nutritional intervention to nematode control in weaned lambs and breeding ewes, the present work was targeted to address the same issue in very young suckling lambs, an age group that has not received the attention deserved given their importance in sheep meat production and for replacement of breeding stock. As much as possible, these experiments were designed to mimic the popular pastoral sheep farming system, which could ease the adoption of the outcomes in commercial lamb production. While some knowledge of lamb response to nematodes during the neonatal stage has evolved from this series of experiments, there are more prospects for research into the mechanisms involved in the lamb response to infection and the feasibility of adopting the evolved technologies.

Notably, a. the mechanism of direct effect of milk on larval establishment, b. the histopathology and mechanism of the immune armoury of young lambs that are favoured by the improved nutrient supply of suckling and the role of specific micronutrients such as amino acids, c. estimating the body composition of lambs at the various stages of immune development to determine the level of body protein reserve d. investigation of the economics of adopting late weaning in farming systems in relation to feed supply for nursing ewes and sustenance of milk production, and many more.

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Appendices

Appendix 3.1: *Trichostrongylus colubriformis* counts (Back-transformed means) recovered from the Chapter 3 lambs

Lamb groups (n)	Worm Counts			
	Worm developmental stages			Total worm
	L_3	L_4	Adults	
W0 (7)	0	0	0	0
S0 (7)	0	0	0	0
W250 (8)	0	0	1	1
S250 (8)	0	0	0	0
W1000 (8)	0	0	0	0
S1000 (8)	0	0	1	1
TS250 (6)	0	0	1	1
TS1000 (7)	0	0	0	0

Appendix 3.2: *Nemadirus* species counts (Back-transformed means) recovered from the Chapter 3 lambs

Lamb groups (n)	Worm Counts			
	Worm developmental stages			Total worm
	L_3	L_4	Adults	
W0 (7)	0	0	0	0
S0 (7)	0	0	0	0
W250 (8)	0	0	0	0
S250 (8)	0	0	0	0
W1000 (8)	0	0	0	0
S1000 (8)	0	0	0	0
TS250 (6)	0	0	0	0
TS1000 (7)	0	0	0	0

Appendix 3.3: *Ostertagia* species counts (Back-transformed means) recovered from the Chapter 3 lambs

Lamb groups (n)	Worm Counts			
	Worm developmental stages			Total worm
	L_3	L_4	Adults	
W0 (7)	0	0	0	0
S0 (7)	0	0	0	0
W250 (8)	0	0	0	0
S250 (8)	0	0	0	0
W1000 (8)	0	0	1	1
S1000 (8)	0	0	1	1
TS250 (6)	0	0	0	0
TS1000 (7)	0	0	1	1

Appendix 3.4: *Cooperia* species counts (Back-transformed means) recovered from the Chapter 3 lambs

Lamb groups (n)	Worm Counts			
	Worm developmental stages			Total worm
	L_3	L_4	Adults	
W0 (7)	0	0	0	0
S0 (7)	0	0	0	0
W250 (8)	0	0	0	0
S250 (8)	0	0	0	0
W1000 (8)	0	0	0	0
S1000 (8)	0	0	0	0
TS250 (6)	0	0	0	0
TS1000 (7)	0	0	0	0

Appendix 4.1: *Trichostrongylus colubriformis* counts (Back-transformed means) recovered from the Chapter 4 lambs

Age at necropsy (Days)	Lamb groups (n)	Worm Counts			
		Worm developmental stages			Total worm
		L_3	L_4	Adults	
84	WN (5)	1	0	213	215
	SN (5)	0	2	31	33
	WI (6)	0	73	825	906
	SI (6)	1	71	647	739
112	WN (5)	0	200	1942	2587
	SN (5)	1	150	312	539
	WI (6)	0	122	1268	1482
	SI (6)	0	316	1439	1879
140	WN (6)	0	187	4117	4355
	SN (6)	0	142	5735	5935
	WI (6)	0	361	3699	4132
	SI (6)	0	182	4379	4653

Appendix 4.2: *Nematodirus* species counts (Back-transformed means) recovered from the Chapter 4 lambs

Age at necropsy (Days)	Lamb groups (n)	Worm Counts			
		Worm developmental stages			Total worm
		L_3	L_4	Adults	
84	WN (5)	0	139	454	827
	SN (5)	0	56	53	230
	WI (6)	0	173	577	950
	SI (6)	0	36	32	56
112	WN (5)	0	336	1492	2120
	SN (5)	1	30	256	306
	WI (6)	0	123	1994	2191
	SI (6)	0	79	875	1038
140	WN (6)	0	93	3010	3289
	SN (6)	0	298	1646	2048
	WI (6)	0	100	2346	2585
	SI (6)	0	3	1164	1216

Appendix 4.3: *Ostertagia* species counts (Back-transformed means) recovered from the Chapter 4 lambs

Age at necropsy (Days)	Lamb groups (n)	Worm Counts			
		Worm developmental stages			Total worm
		L_3	L_4	Adults	
84	WN (5)	0	30	6	54
	SN (5)	0	2	2	2
	WI (6)	0	5	235	268
	SI (6)	0	4	16	38
112	WN (5)	0	1017	642	1690
	SN (5)	0	259	7	329
	WI (6)	0	34	240	346
	SI (6)	0	2	4	15
140	WN (6)	0	79	462	629
	SN (6)	0	98	91	302
	WI (6)	0	421	969	1424
	SI (6)	0	80	323	411

Appendix 4.4: *Cooperia* species counts (Back-transformed means) recovered from the Chapter 4 lambs

Age at necropsy (Days)	Lamb groups (n)	Worm Counts			
		Worm developmental stages			Total worm
		L_3	L_4	Adults	
84	WN (5)	0	2	2	6
	SN (5)	0	18	7	27
	WI (6)	0	12	14	38
	SI (6)	0	1	11	13
112	WN (5)	0	19	7	27
	SN (5)	0	0	1	1
	WI (6)	0	4	2	5
	SI (6)	0	0	10	10
140	WN (6)	0	0	11	11
	SN (6)	0	0	1	1
	WI (6)	0	0	6	6
	SI (6)	0	0	0	0

Appendix 5.1: Other Nematode species (Log_{10} back-transformed means) recovered from lambs on the day 108 necropsy of Chapter 5

Lamb	*Nematodirus*		*Cooperia*		*Haemonchus*		*Ostertagia*		*T. axei*	
groups	Adult	L_4	Adult	L_4	Adult	L_4	Adult	L_4	Adult	L_4
E.N.-	334	103	2298	5	0	0	33	2	0	0
E.V.-	121	1	164	1	0	0	1120	2	4	0
L.N.-	229	20	2319	9	0	0	140	7	1	0
L.V.-	384	0	36	2	0	0	543	4	17	0

Appendix 5.2: Other Nematode species (Log_{10} back-transformed means) recovered from lambs on the day 184 necropsy of Chapter 5

Lamb	*Nematodirus*		*Cooperia*		*Haemonchus*		*Ostertagia*		*T. axei*	
groups	Adult	L_4	Adult	L_4	Adult	L_4	Adult	L_4	Adult	L_4
E.N.C	9	0	8	1	0	0	179	29	0	0
E.V.C	82	1	137	4	0	0	103	41	0	0
L.N.C	176	0	41	58	0	0	65	29	0	0
L.V.C	357	0	307	23	0	0	38	19	0	0

Appendix 5.3: Other Nematode species (Log_{10} back-transformed means) recovered from lambs on the day 218 necropsy of Chapter 5

Lamb groups	*Nematodirus*		*Cooperia*		*Haemonchus*		*Ostertagia*		*T. Axei*	
	Adult	L_4	Adult	L_4	Adult	L_4	Adult	L_4	Adult	L_4
E.N.N	9	0	8	1	0	0	179	29	0	0
E.N.C	82	1	137	4	0	0	103	41	0	0
E.V.C	176	0	41	58	0	0	65	29	0	0
L.N.N	357	0	307	23	0	0	38	19	0	0
L.N.C	313	2	26	3	0	0	90	48	1	0
L.V.C	128	3	53	2	0	0	45	8	0	0

Publications in the course of study

Iposu, S. O.; Greer, A. W.; McAnulty, R. W.; Stankiewicz, M.; & A. R. Sykes (2005). The role of suckling on the parasite status of very young lambs infected with *Teladorsagia circumcincta.* ***Proceedings of the New Zealand Society of Animal Production*** 65: 182 – 185**.**

Iposu, S. O.; Greer, A. W.; McAnulty, R. W.; Stankiewicz, M.; & A. R. Sykes (2006) Further studies on the role of suckling in the parasite status of very young lambs infected with *Teladorsagia circumcincta****. Proceedings of the New Zealand Society of Animal Production*** 66: 187-192.

Iposu, S. O. (2006). Does suckling offer a protection to the lamb against *Teladorsagia circumcincta* parasitism in the period – six to 20 weeks after lambing? In : ***Ideas on the egde***. Lincoln University Postgraduate Conference held on August 15 - 16, 2006 at Stewart 2 lecture theatre, Lincoln University, Canterbury, New Zealand.

Iposu, S. O.; McAnulty, R. W., Greer, A. W., Xie, H. L., Green, R. S., Stankiewicz, M., & A. R. Sykes (Under Peer Review) Does suckling offer a protection to the lamb against *Teladorsagia circumcincta* infection? ***Veterinary Parasitology***