Targeted supplementation of sheep to control gastrointestinal nematode populations

---

A thesis

submitted in partial fulfilment

of the requirements for the Degree of

Doctor of Philosophy

at

Lincoln University

by

Reny Debora Tambunan

Lincoln University

2024

Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy

Targeted supplementation of sheep to control gastrointestinal nematode populations

by

Reny Debora Tambunan

One potential approach to control GIN parasite and to improve ewe's immunity during the periparturient period is nutritional supplementation. However, there is limited data regarding the long-term effects of nutritional supplementation on grazing periparturient ewes that have a naturally acquired GIN infection where two or more parasite species are infecting ewes and lambs. This thesis describes a series of experiments designed to analyse the effects of targeted supplementation to twin-bearing ewes on pasture and determine whether the supplementation can provide long-term benefits to their lambs after weaning. Three experiments were carried out at LincolnSheep, Canterbury, New Zealand, over three sequential years (August 2015-April 2018).

Before lambing, twin-bearing crossbred ewes (n=140 in 2015, n=128 in 2016, and n=160 in 2017) were randomly allocated to one of two treatments, *viz*. supplemented and unsupplemented. Ewes on the supplemented paddocks were introduced to an advantage feeder with approximately 50 g/head/d of sheep pellets starting three weeks before lambing and then increased to 0.5 kg/head/d during the first four weeks of lactation. All sheep were allowed to graze on perennial ryegrass/white clover pasture. After weaning, all lambs were drenched and then exposed to a targeted selective treatment (TST) regime while grazing the areas in which ewes had been or not supplemented to determine if any epidemiological benefit of supplementation existed. Weaned lambs (n=240 in 2015, n=210 in 2016, and n=180 in 2017) originating from each treatment were stratified across the treatment area. For each lamb replicate, growth potential was assessed using sentinel lambs that were treated with a long-acting anthelmintic. The remaining lambs in each replicate were subjected to a TST regime where the need for anthelmintic was based on animals achieving acceptable growth rates. Within each farmlet, lambs were rotationally grazed for the remainder of the grazing season, with lambs grazing freshly grown forages, followed by the ewes.

In the first study (year 2015 and 2016, Chapter 4), the ewes were undrenched, consequently allowing the development of pre-existing parasite infections. The results in both years showed that supplementation of ewes did not affect ewe LW and BCS or the weight of lamb weaned per ewe ($P>0.05$ for all). However, supplementation was successful in reducing ewe's FEC to 50% reduction during lactation periods. However, in 2015, the effect was transient, as FEC of supplemented ewes increased and was not different at week 12 of lactation. In 2015, climatic conditions were not favourable for pasture growth, resulting in low pasture availability, with mean pasture mass declining to less than 700 kg DM/ha in all paddocks, and may have resulted in unintentional nutritional stress and increased the concentration of eggs in the faeces. In the 2016 trial, the reduction in ewe FEC was more consistent throughout lactation, indicating a longer-term benefit to the ewe as this extended beyond the pre-patent period of any larvae that would have been ingested after supplementation. The declining number of eggs observed in 2016 at late pregnancy until the 8th week of lactation agrees with the increase of IgA at the same time, which may be due to an improvement in immunity. However, unexpectedly, the *T. colubriformis*-specific L3 IgA absorbances in supplemented ewes were significantly lower than unsupplemented ewes throughout the experiment in 2016. The reasons for this are unclear but may reflect differences in the larval challenge. Moreover, supplementation also did not have any significant effects on serum proteins, serum urea, and serum phosphorus of the ewes. Before weaning, there was no difference between the mean LW of lambs reared by supplemented or unsupplemented ewes in 2015 ($P=0.27$), while in 2016, lambs reared by unsupplemented ewes were significantly heavier than those raised by supplemented ewes ($P=0.02$). After weaning, there were no differences in the performance of lambs between the two treatments in all years ($P>0.05$ for all), except for a higher number of drenches administered per lamb ($P=0.04$) of TST lambs in areas where ewes had not been supplemented in 2015. These findings allowed the conclusion that supplementation of ewes during the first four weeks of lactation had no effect on ewe performance but was successful in temporarily reducing faecal eggs by 50%, presumably reflecting better maintenance of immune function through higher nutrient supply, even though this was not detected in parasite specific-immunoglobulin. However, the reduction in parasite contamination was insufficient to provide a measurable and consistent epidemiological benefit to the grazing lambs after weaning.

The second trial (year 2017, Chapter 5) aimed to evaluate the effect of supplementation to reduce the establishment of larvae following short-acting drench pre-lambing. The results showed the benefits of ewe supplementation when the existing population is removed, such as the difference in FEC, the greater serum albumin concentrations, and the consistently higher milk protein contents. Similar to the previous studies, supplementation of ewes in 2017 also did not affect ewe LW and BCS or the weight of lamb weaned per ewe ($P>0.05$ for all). The reduction in FEC following

supplementation was not accompanied by the increase in *T. colubriformis*-specific L3 IgA absorbance; the values decreased over time (P<0.001), possibly indicating that the immune response to ingested larvae was unaffected. The level of serum IgG of supplemented ewes was similar to unsupplemented ewes (P=0.83). However, there was treatment and time interaction (P=0.001) on serum IgG concentrations, reflecting high IgG responses at week 4 of lactation, which then decreased with time in all groups; however, the decline was higher in unsupplemented ewes than their supplemented counterparts at week 8 of lactation. Similarly, supplementation on ewes did not affect milk production and composition (P>0.05 for all). There were extremely weak associations between milk production and lamb growth rate during the lactation period, except for the unsupplemented group in week 4 (P<0.001). Moreover, pastures grazed by supplemented ewes tended to have threefold lower pasture larval than unsupplemented pastures, which can be seen from the tendency of fewer L4 in the tracer lambs grazed areas where ewes had been supplemented. This lower pasture contamination, therefore, resulted in a slight difference in the FEC profile of lambs, effectively delaying the increase in FEC in the lambs throughout the summer. FEC of lambs after weaning increased significantly throughout the grazing season for both groups, although tended to be delayed in TST lambs grazing the areas where ewes had been supplemented, which reached a peak of 65 days after weaning compared with 51 days after weaning in TST lambs grazed unsupplemented areas. The difference in the growth rate of the sentinel lambs and TST lambs clearly shows that there was a substantial larval challenge, with the benefits of supplementation possibly masked due to the TST regime, even though there was no difference in the number of anthelmintic treatments administered. The reduction in the FEC of lactating ewes drenched pre-lambing was not sufficient to result in an epidemiological advantage to their lamb, as shown by results on FEC, worm burden, and growth performance from birth to reach slaughter weight while grazing pastures that were infected naturally by GIN parasites.

The third trial (year 2017, in conjunction with the second trial) indicated that supplementation and treatment with moxidectin at the end of supplementation (week four of lactation) appeared to assist the ability of the ewes to limit egg excretion. FEC of supplemented-undrenched and unsupplemented-undrenched groups was significantly increased throughout the lactation period, except at week eight of lactation, where FEC of all groups decreased. Surprisingly, the FEC of supplemented-undrenched ewes rapidly elevated to 1,400 epg at week 12 of lactation, compared to 650 epg in unsupplemented-undrenched ewes. The reason for the speedy elevation of FEC in the first group was probably associated with a decrease in ewes' immunity, as indicated by lower *T. colubriformis*-specific L3 IgA and *T. colubriformis*-specific L3 IgG levels, even though the effect on these values was not significant. Additionally, both drenced and undrenched groups had significantly lower serum albumin concentrations, indicating there was damage to the mucosa of the GI tract of

ewes by GIN parasites, which resulted in body protein loss. As expected, long-acting injections of moxidectin at the end of the supplementation period resulted in a consistent trend of higher LW than undrenched groups due to lower parasitic load. Similar to the results from previous trials, supplementation of ewes and administration of long-acting anthelmintic at the end of the supplementation period did not provide clear benefits to their lambs before weaning. However, lambs raised by supplemented and drenched ewes tended to have higher LW, LWG, and weight of lamb weaned per ewe (WLWE) than those raised by unsupplemented groups.

It was concluded from the series of experiments from this study that supplementation of ewes during the first four weeks of lactation, whether undrenched, drenched pre-lambing or drenched at the end of the supplementation period, did not affect ewe performance. However, it was temporarily successful in reducing faecal egg counts, presumably reflecting better maintenance of immune function through higher nutrient supply, although this was not detected in parasite-specific immunoglobulins. However, the reduction in parasite contamination was insufficient to provide a measurable and consistent epidemiological benefit to the grazing lambs that may assist with parasite control. If the supplementation of ewes during this time is to be employed as a means of reducing the need for anthelmintics to control parasitism, then the refinement, including an understanding of the specific amino acid requirement and any interaction with BCS, needs to be investigated.

Keywords: *supplementation, ewes, lamb, gastrointestinal nematode parasite, epidemiology, faecal egg count, lactation, weaning, pasture, Trichostrongylus colubriformis*, *IgA, IgG*.

Acknowledgements

This study would not have been possible without the mercy and grace from God and from the help and support of many persons and organizations who in one way or another contributed and extended their valuable assistance in bringing this study to completion.

First and foremost, I wish to express my sincere and deep sense of gratitude to my main supervisor Dr Andrew W. Greer, for his support and guidance from the start to the finish of this study, including providing much-needed assistance during my field works, data analysis, write up of the thesis, proof reading the manuscript, and financial support. I also would like to thank my associate supervisor Chris Logan for his assistance during my field works, advice and valuable input to my thesis.

I am grateful to the head of Indonesian Agency for Agricultural Research and Development (IAARD) through the Sustainable Management of Agricultural Research and Technology Dissemination (SMARTD) Project, for granting me a scholarship; to the head of Lampung Assessment Institute for Agricultural Technology for giving me a study leave; and to the staff of IAARD/SMARTD (Bu Nurjayanti, Bu Arini and pak Nardi). I also want to thank the Chairman of National Research and Innovation Agency (BRIN) and the Head of Research Center for Animal Husbandry BRIN, for the extension to my study permit while waiting for my final exam.

I would like to thank Robin McAnulty, who guided me through the techniques used in parasitology laboratory. To Rebecca Johnson, Caleb Sixtus, Ivan Barnett, James Meyer, Martin Ridgeway, Rosemary McAnulty, and Anabel McAnulty for their collaboration and help during the field works and parasitology works; to Andrea Hogan, Rosy Tung, and Shuang Jiang for their assistance during my laboratory works at Riddolls Laboratory; and to biometricians for statistical advice.

Big thanks to my fellow postgraduates Dr Sara Lundberg and Dr Joseph Hamie for their help with ELISA and Genstat; to Dr Kelly, Marsha Martin, and Olla Yusuf, for their assistance during my field works; to Dr Yingluck Moonsan, Dr Rizqi, IFGF fellowship, and the Briners ex-Kementan, for always being there as family and friends and providing much-needed support and prayers.

I am grateful to my dear family from both sides, whose encouragement and prayers allowed me to complete this study. In particular to my husband Sarman Manihuruk and our son Jeremia Manihuruk for love, prayers, and unconditional support. Lastly, I am much indebted to whoever participated in the accomplishment of this study. *Terima kasih*. Thank you so much.

Publications during the course of the study

Tambunan, R. D., Logan, C. M., Bywater, A. C., & Greer, A. W. (2018). Supplementation of ewes on pasture to provide an epidemiological benefit for gastrointestinal parasitism. *New Zealand Journal of Animal Science and Production, 78*, 45-50.

Table of Content

List of Tables

List of Figures

List of Plates

List of Abbreviations

AA	amino acid
ADF	acid detergent fibre
ANOVA	analysis of variance
BCS	body condition score
CP	crude protein
CRC	controlled release capsules
CSIRO	the Commonwealth Scientific and Industrial Research Organisation
d	day
DOMD	digestible organic matter
DM	dry matter
ELISA	enzyme-linked immunosorbent assay
epg	eggs per gram
FEC	faecal egg count
g	grams
GI	gastrointestinal
GIN	gastrointestinal nematode
g/l	gram per litre
h	hour(s)
H. contortus	*Haemonchus contortus*
Ig	immunoglobulin
IgA	immunoglobulin A
IgG	immunoglobulin G
IgE	immunoglobulin E
kg	kilogram
l	litre
L1	first stage of larval development
L2	second stage of larval development
L3	third stage of larval development
L4	fourth stage of larval development
L5	fifth stage of larval development
LDR	larval dosing regimes
LW	live weight
LWG	liveweight gain

ME	metabolisable energy
MJ	mega joules
ml	millilitre
mmol/l	millimoles per litre
NDF	neutral-detergent fibre
NIRS	near infra-red spectrophotometer
MP	metabolisable protein
OD	optical density
OM	organic matter
PBS	phosphate buffered saline
PBST20	phosphate buffered saline + 0.05% Tween 20
PPR	periparturient rise
PPRI	periparturient relaxation in immunity
SCC	somatic cell counts
T. axei	*Trichostrongylus axei*
T. circumcincta	*Teladorsagia circumcincta* = *Ostertagia circumcincta*
T. colubriformis	*Trichostrongylus colubriformis*
TMB	tetramethylbenzidine dihydrochloride
TST	targeted selective treatment
WSC	water soluble carbohydrate
µl	microlitre
µm	micrometre

Chapter 1

General introduction

1.1 Introduction

Gastrointestinal nematodes constitute a significant animal health impediment for livestock around the world. They represent one of the primary sources of economic losses in farmed ruminants, especially sheep. In New Zealand, GIN parasitism in sheep is considered as one of the significant production-limiting factors for the farmers (Lawrence et al., 2007). Currently, of the $3.50/stock unit spent on sheep health by New Zealand farmers, $2 is for the drench cost; therefore, an effective nematode parasite control programme is vital to farm profit (Dalton, 2006).

The production losses caused by GIN mostly occur in growing lambs and periparturient ewes (Bassetto et al., 2018), as these are most susceptible to GIN infection (Bishop & Stear, 2001). During the periparturient period, a temporary reduction of immunity to GIN parasites was typically experienced by ewes (O'Sullivan & Donald, 1970), resulting in an elevated worm burden in ewe and consequently higher deposition of worm eggs on pasture (Houdijk, Kyriazakis, Jackson, & Coop, 2001). Throughout this period, naïve young lambs are exposed to infective larvae mostly originating from the eggs of nematodes eliminated in the faeces of their dams (Donald & Waller, 1973), thus supply the infection to the grazing lambs. The ewe plays a critical function in the epidemiology of GIN parasitism, therefore overcoming the breakdown in the expression of immunity may diminish the intake of infective larvae by lambs and hence potentially improve their performance (Coop, Sykes, & Angus, 1982).

Many control methods have been used to reduce the effect of parasites infection and thus increase levels of sheep production which includes the use of genetically resistant sheep (Bouix et al., 1998; Eady, Woolaston, & Barger, 2003; Gauly & Erhardt, 2001; Gray, 1997), anthelmintics (Köhler, 2001; Leathwick, Pomroy, & Heath, 2001), biological control (Chandrawathani et al., 2004; Knox & Faedo, 2001), vaccination (Bassetto et al., 2018; Jacobs, Wiltshire, Ashman, & Meeusen, 1999; Knox & Smith, 2001; Nisbet et al., 2016), grazing management (Burke, Miller, & Terrill, 2009; Colvin, Walkden-Brown, Knox, & Scott, 2008), and nutritional supplementation (Haile et al., 2002; Sebastiano, Sweeney, Keady, Hanrahan, & Good, 2017). Nevertheless, each method has disadvantages such as resistance to anthelmintics (Jackson & Coop, 2000; Leathwick et al., 2001), the increasing of consumer awareness about drug residues in food products and the environment (Ketzis et al., 2006), insufficient resources or not being feasible and cost-effective (Sayers & Sweeney, 2007). Hence,

control methods should not rely on only to a single approach but include selected combinations of various alternatives.

One control method that has been extensively investigated is nutritional supplementation. Many studies have investigated the significance of nutrition on GIN infection in sheep (Athanasiadou, Houdijk, & Kyriazakis, 2008; Donaldson, van Houtert, & Sykes, 1998; Houdijk, Kyriazakis, Jackson, et al., 2001; Houdijk, Kyriazakis, Jackson, Huntley, & Coop, 2003, 2005; Sebastiano et al., 2017). These studies have supported the view that protein is expected to be more important in cases of parasitism than other nutrients. Several controlled pen trials in lambs indicated that nutritional supplementation had been shown to enhance host resistance and resilience to GIN infection (Bricarello et al., 2005; Coop, Huntley, & Smith, 1995; Haile et al., 2002; Kahn, Kyriazakis, Jackson, & Coop, 2000). However, most of these experiments used artificial infection, with a single helminth species. There are limited studies on the interactions between host nutrition and gastrointestinal parasitism in grazing periparturient ewes.

Previous nutritional supplementation studies on ewes during the periparturient period suggested that supplementation provides a substantial benefit through reducing the periparturient rise (PPR) in faecal egg count (FEC) (Donaldson et al., 1998; Donaldson, van Houtert, & Sykes, 2001; Houdijk et al., 2005) and hence decreasing the contamination laid down onto pasture by the ewe. However, these studies only evaluated the effects on the ewe in either indoor study with trickle infection or for a relatively short term while on pasture. They have fallen short of examining the epidemiological advantage for the grazing lambs for the remainder of the grazing season. The series of experiments reported in this thesis was designed to examine the previously shown benefits of nutritional supplementation on pasture during periparturient period in the long-term and determine whether targeted supplementation of ewes can provide long-term benefits, providing to more options available for the control of GIN populations.

1.2. Thesis aims and objectives

The aim of this study was to evaluate the benefits of supplementing twin-bearing ewes infected naturally by GIN on pasture and its provision of an epidemiological benefit to grazing lambs after weaning. These studies also aimed to obtain more information in the immunological changes associated with the periparturient rise in FEC.

The specific objectives were:

1. To examine the effect of supplementing lactating ewes on pasture and its epidemiological benefit to weaned grazing lambs after weaning.

2. To evaluate the effect of supplementation of ewes following a short-term drench pre-lambing and its epidemiological benefit to grazing lambs after weaning.
3. To determine the effect of supplementation and a long-acting anthelmintic treatment to the performance of lactating ewes.

Chapter 2

Literature review

2.1 Introduction

Gastrointestinal nematode parasitism is a significant factor affecting sheep production system around the world, including New Zealand. Currently, nematode populations in New Zealand are controlled by the management strategies that rely heavily on the administration of anthelmintics (Lawrence et al., 2007; Leathwick et al., 2001). However, the development of anthelmintic resistance by the parasites as well as increasing demand of consumers for more organic animal products makes the existing approaches for the control of GIN parasites unsustainable. As a consequence, alternative methods of nematode control must be developed.

Effective control relies on knowledge of parasite epidemiology, including their life cycle, and the interaction with the host immune system. Vlassoff, Leathwick, and Heath (2001) suggested that proper knowledge of the parasite epidemiology and nutritional requirements and the immune response of sheep will sufficiently empower the management of the problem. Thus, targeting approaches to epidemiologically critical periods has the potential to obtain maximum cost-effectiveness of control. Evaluation of control options is needed to be undertaken to examine the entire production system. Therefore, this literature review presents a background of the epidemiology of nematode parasite infections in sheep, the effects of GIN, the mechanism of immunity to GIN, economic consequences of parasitism, host nutrition, and dietary supplementation.

2.2 Parasite and epidemiology of nematode infections in sheep

Sheep are infected with numerous genera of gastrointestinal nematodes. In New Zealand, the most important genera that are associated with clinical disease and production losses in sheep are *Haemonchus contortus*, *Teladorsagia (Ostertagia)* spp., and *Trichostrongylus axei* in the abomasum and *Nematodirus* spp., *Trichostrongylus* spp., and *Cooperia* spp. in the small intestine (Vlassoff et al., 2001; West, Bruere, & Ridler, 2002). Others, such as *Oesophagostomum* and *Chabertia,* can be influential in some cases; however, they are generally as part of a combined burden (Morgan & van Dijk, 2012).

2.2.1 The life cycle of parasite nematode

The life cycle of most parasite nematodes infecting sheep is similar, although some exceptions do exist, such as *Nematodirus* spp. whose development to the infective phase occurs inside the egg (Brunsdon, 1967). Brunsdon (1982) stated that the life cycle of typical gastrointestinal (GI) parasites in all ruminant animals and the life cycle of the lungworm consist of two major stages, a stage within the host and a stage outside the host.

The development of a trichostrongyle nematode from egg to mature adult commonly follows the cycle presented in Figure 2.1. Adult parasites in the GI tract copulate, and female worms produce eggs containing the developing embryos, which are excreted onto the pasture via faeces. Under favourable conditions, the eggs hatch and release the first-stage larvae (L1) within 48 hours of deposition. These larvae actively feed on microorganisms inside the faeces and grow. After a brief period (five to seven days) the L1 moult then shed its cuticle and become L2 larvae. After a second moult, the L2 larvae develop into non-feeding infective larvae (L3). However, this process is incomplete because L3 larvae retain the cuticle of the L2 stage to protect from environmental factors until they invade the host. A majority of the L3 larvae remain on the lower 5 cm of the pasture sward and in some cases in the soil profile and are ingested by the host during grazing (Brunsdon, 1982). After the ingestion by an appropriate host, the L3 larvae develop into L4 and patent adults inside the GI tract of the host (Houdijk, Kyriazakis, Kidane, & Athanasiadou, 2012; Vlassoff et al., 2001), which takes place either in the small intestine or in the abomasum, depending on the species of parasite. For most parasite species, this development requires two to three weeks (Blackie, 2014). Additionally, McAnulty (1990) reported for the development from an egg to eggs in sheep, the minimum period required could be 28 days, with a minimum time from L3 ingested by the host until eggs detected in the faeces of 17-21 days.

Under certain conditions, parasites can arrest their growth for extending their life cycle, a phenomenon recognized as arrested larval development; hypobiosis. Urquhart, Armour, Duncan, Dunn, and Jennings (1987) defined it as a temporary cessation in the growth of a nematode at a certain point in its parasitic development. Lützelschwab et al. (2005) described that the adult parasites avert shedding eggs onto herbage when conditions around it are unfavourable, hence preventing mortality of the free-living stages. Some authors believed that many factors affect and initiate stimuli for hypobiosis on free-living infective stage larvae, such as animal management, environmental conditions and immune status of the host (Armour & Bruce, 1974; Eysker, 1993). Lützelschwab et al. (2005) concluded that the occurrence of hypobiosis was significantly influenced by environmental and seasonal factors.

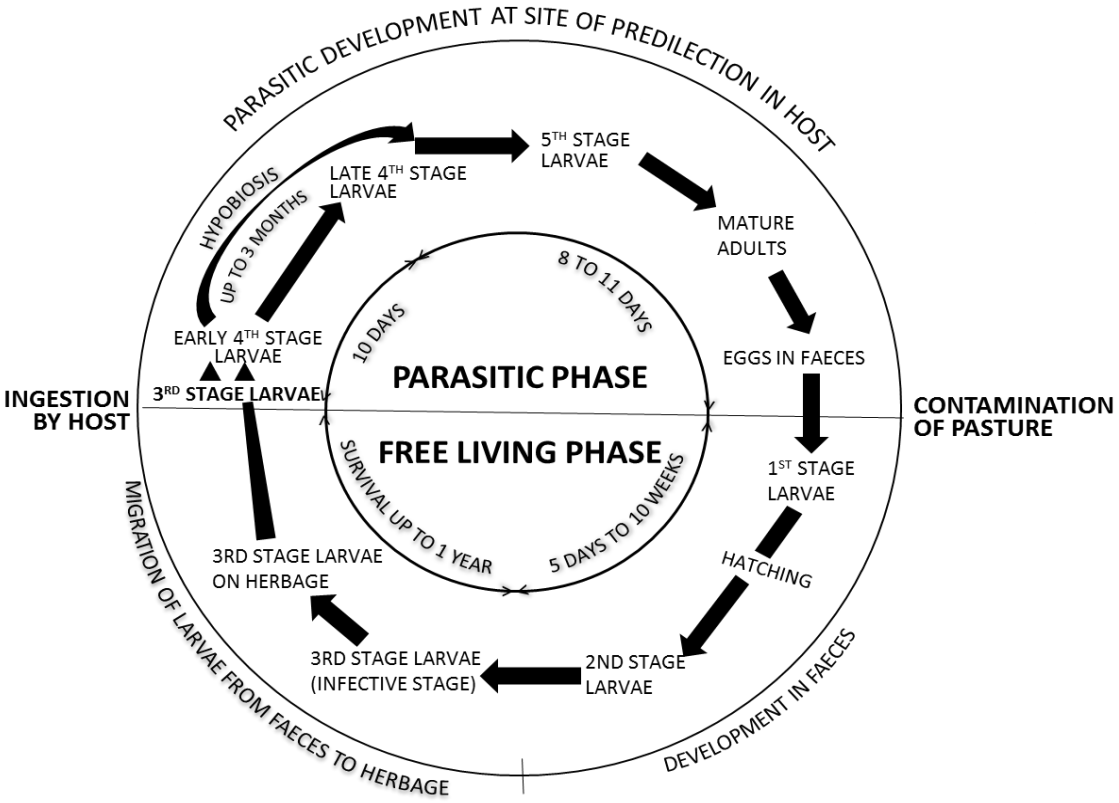


Figure 2.1 Generalised life cycle of trichostrongyle nematode (adapted from Brunsdon (1982)).

Another phenomenon that influences the normal life cycle of the parasite experienced by the ewes during late pregnancy and early lactation is the periparturient rise (PPR) in FEC (Salisbury & Arundel, 1970), which is associated with a temporary decrease in host immunity (O'Sullivan & Donald, 1973). O'Sullivan and Donald (1973) described this loss of immunity to be associated with a reduced cell-mediated immune response in the mucosa of the gastrointestinal tract. This response results in a reduction in mast cell hyperplasia, generation of globule leucocytes, and eosinophil response. Several authors believe that PPR is a major source of pasture larval contamination and infection for both lambs and ewes (Barger, 1993; O'Sullivan & Donald, 1970). The increased in pasture larval numbers results in lambs being exposed to a higher level of infection, giving rise to a greater worm burden decreased production and have an increased requirement for anthelminthic treatment (Kahn, Knox, Gray, Lea, & Walkden-Brown, 2003). The PPR has been observed in temperate climate countries (Beasley, Kahn, & Windon, 2010; Courtney, Gessner, Sholz, & Loggins, 1986; Lützelschwab et al., 2005) and in tropical and sub-tropical climate countries (Ng'ang'a, Munyua, Maingi, & Kanyari, 2004; Romjali, Dorny, Batubara, Pandey, & Gatenby, 1997; Tembely et al., 1998). Recently, studies in Canada found the occurrence of PPR in Ontario sheep herds that experienced out-of-season lambing. This finding indicating that the PPR can ensue independent to environmental conditions, likely because of immunological changes in ewes at the parturition (Falzon et al., 2013).

Factors that affect the breeding ewe to the PPR have been the focus of many studies. Urquhart et al. (1987) stated that the rise in FEC around parturition is caused by three sources: (i) maturation of inhibited larvae due to loss of host immunity, (ii) an increased establishment of infections ingested from the pastures and a diminished turnover of existing adult parasites, and (iii) an increase in fecundity of existing adult worm populations. However, there are contradictory thoughts as to the cause and occurrence of increased faecal egg shedding in periparturient ewes (Falzon et al., 2013). Dunsmore (1965) suspected that the primary reason for PPR is an increase in the concentration of plasma prolactin following parturition. Nevertheless, studies conducted by Coop et al. (1990) and Jeffcoate, Fishwick, Bairden, Armour, and Holmes (1990) show significant doubt on the effect of prolactin in the PPR. Some authors suggested that this phenomenon is associated with both immunological and nutritional changes occurring in periparturient ewes (Beasley et al., 2010; Beasley, Kahn, & Windon, 2012). Donaldson et al. (1998, 2001) revealed that the occurrence of PPR has a nutritional basis and showed that the nutritional status of periparturient ewes is a significant factor of susceptibility to the breakdown of resistance. A nutrient partitioning framework was developed to explain the effects of nutrition on parasitic infections (Coop & Kyriazakis, 1999). The authors argued that the expression of acquired immunity to gastrointestinal parasites competes with reproductive functions (pregnancy and lactation) which have a higher priority for the distribution of insufficient nutrients. The feed intake of the periparturient ewe is usually inadequate because of low-quality feeds or restricted feeding as part of the management system. During these situations, the nutrients supply, such as metabolisable protein (MP), may be limited while both reproductive functions and immunity require MP resources. The increasing MP demand for foetus development and subsequent lactation will lead to a penalty of the immune functions and therefore account for the incidence of the PPR. In support, Huntley et al. (2004) reported a decrease in the number of mucosal mast cells and globule leukocyte of ewes infected with *T. circumcincta* during the periparturient relaxation in immunity when protein supply was restricted indicating that nutrition can affect the effector arm of the immune responses. Aspects specific to the nutritional manipulation of the PPR are discussed later (section 2.8.1 Ewe requirements).

2.2.2 Seasonal pattern of larval development and survival

The development of free-living stages of GIN can be affected by many factors. The current prevalence and parasitic nematodes distribution are mostly regulated by a combination of ecological requirements for development and survival of parasites outside the host, management practices in the farm, and prevailing local climate (Vlassoff et al., 2001).

In New Zealand, the free-living phases of trichostrongylid nematodes mostly have favourable environments for developing on pasture for much of the year because of a moderate climate and sufficient rainfall. Under optimum conditions, the rate of egg development (defined as the time required for an egg to become an L3) is five to seven days, while in field circumstances the percentage varies considerably with the season and usually needs two to three weeks or more (Vlassoff & Bisset, 1991). The percentage development to the infective stage larvae of deposited eggs (termed as developmental success) is very variable at different times of the year. The developmental success is below 1% in most months; however, during more favourable periods, such as in late summer/early autumn, it can reach more than 10% (Vlassoff, 1982). After the infective stage is reached, since this stage is very resilient, it can survive for more extended periods (Vlassoff, 1982).

Typically, the seasonal pattern of variation in the numbers of L3 on herbage includes a small peak in spring/early summer which consisted of L3 that have survived over winter and L3 acquired from the rise in ewe FEC after lambing. It is followed by a broad peak in late summer/autumn which is obtained from worm eggs accumulated by lambs in summer/early autumn (Vlassoff et al., 2001) and then declines as animals develop immunity and environmental conditions become less favourable for survival.

Vlassoff (1982) described the interrelationship between seasonal patterns of parasite levels in untreated sheep and on herbage (excluding *Nematodirus* spp.) in New Zealand (Figure 2.2). Based on this figure, the primary source of pasture contamination providing to the spring peak of L3 is the rise in FEC after lambing of breeding ewes (point 1). The overwintered L3 and those from the spring peak cause the increase in the first generation of nematodes that accumulate in lambs over the summer (points 2 and 3). The infective larvae from the peak at autumn generate the second generation of worms in lambs that induce clinical diseases in winter and autumn, reaching a peak in late summer to early autumn. The lambs then become the primary source of pasture contamination in the autumn (points 3 and 4). After peaking in autumn, the larval challenge in lambs and on pasture rapidly declines from late autumn to winter (point 5). The rapid decline of that larval challenge is related to the declining temperatures resulting in a decreasing proportion of eggs surviving on pasture. These situations were assumed to occur in summer-dry conditions. The peak may also happen in summer if adequate moisture is present.

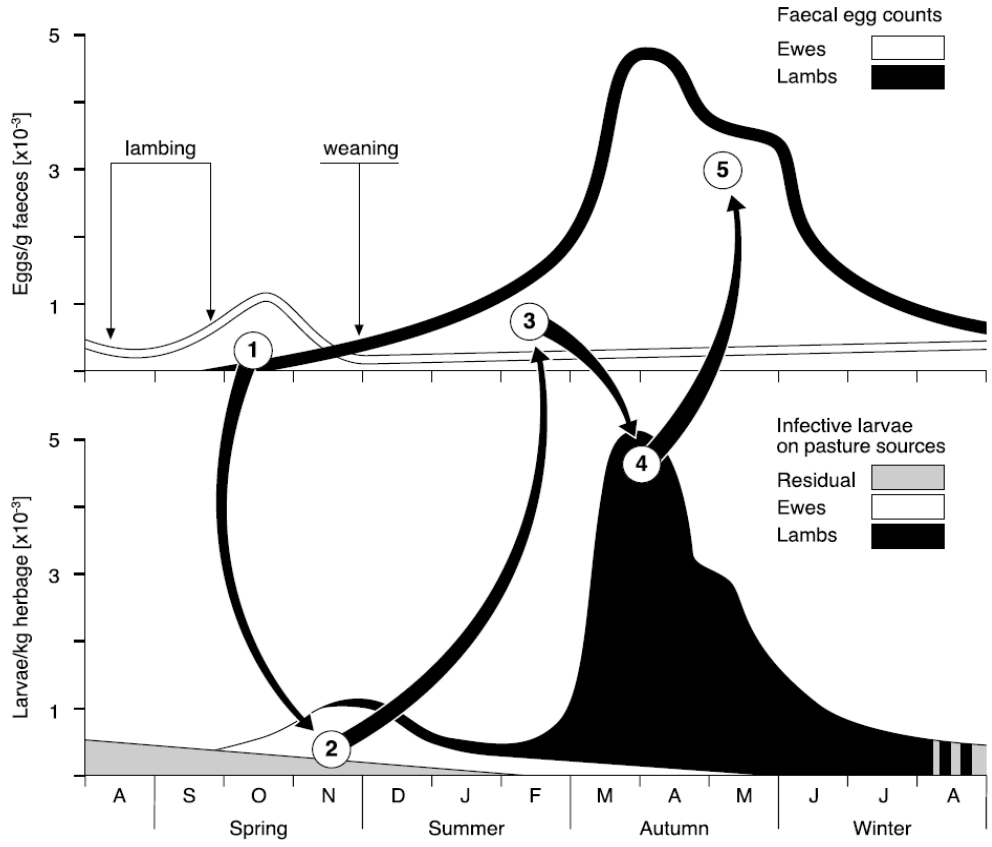


Figure 2.2 The sequential interrelationship between pasture contamination by untreated sheep and infective larvae availability on pasture (Source: Vlassoff (1982)).

Climate affects the availability of infective larval and rates of infection through direct effects on the translation of larvae onto pasture (O'Connor, Walkden-Brown, & Kahn, 2006). Leathwick (2013) stated that nematode parasites are responding to climate variables such as temperature, which govern their development. The successful development of parasites will occur regardless of the time of the year if conditions are favourable for them, as such, the profile indicated by Figure 2.2 may change if summer moisture is available. van Dijk, Sargison, Kenyon, and Skuce (2010) stated that the rates of development and death of free-living stages are mostly influenced by temperature and rainfall.

Stromberg (1997) reported that nematodes eggs hatch and develop more slowly at lower temperatures. Moreover, the development rate rises to a maximum at higher temperatures after which growth will be adversely affected, and the death of the larvae happens, with the optimum temperature range for larval development varying between species. Among the major sheep trichostrongylids, *H. contortus* has the highest (25-37 °C) and *T. circumcincta* the lowest (16-30 °C) optimum temperature, with other species in between. Ovine trichostrongylid eggs develop to the L3 larvae beyond a limit of around 4 °C. Although some species, such as *H. contortus*, have a higher minimum limit of approximately 8 °C (O'Connor et al., 2006).

The moisture effects on the free-living phases of trichostrongylid nematodes parasites have received less attention compared with temperature (O'Connor et al., 2006), but are essential. Moisture should be considered when evaluating the effects of temperature since moisture must exist to avoid desiccation and death of the developing larvae. It affects the larvae's movement and motility. In case the environment is dry, the migration of larvae onto surrounding pasture would not be possible, accordingly forcing movement into the soil below the faecal pellets (Stromberg, 1997), because it is difficult to separate relationships among moisture-related factors operating on larval microclimates, which include evaporation, rainfall, condensation, air humidity, and moisture in soil and faeces (Morgan & van Dijk, 2012). Under controlled laboratory environments, moisture is needed for trichostrongylid larvae development to the L3 stage; however, except in situations that evaporation immensely exceeds precipitation, moisture is generally sufficient within faecal pellets (O'Connor, Kahn, & Walkden-Brown, 2007, 2008). In more arid areas, shortage of rainfall restricts the development of larvae, migration out of faeces, and survival on pasture; the net effect being that larval availability decreases during the dry season (Morgan & van Dijk, 2012).

Under conditions of high evaporation, larvae in pastures generally have lower recovery rates (Heckler & Borges, 2016). The best conditions for the development and migration of the larvae are when the humidity is above 43% (Santos, Silva, & Amarante, 2012). Just as higher temperatures (above 12.8 °C) stimulate the migration of L3 into the pasture, they simultaneously prevent the movement of the larvae as they increase evaporation and thus reduce the relative humidity of the microclimate of the pasture. However, the mere presence of L3s in a pasture does not necessarily mean they are available for consumption by susceptible hosts, as most L3s are dead or migrate from faeces or forage through the soil (Aumont, Coulaud, Grude, & Gruner, 1989).

Extreme conditions, for example, during periods of very high or very low temperature and humidity, as well as infrequent or too heavy rainfall, can affect the development of L3 or inhibit the migration of L3 to pastures (van Dijk & Morgan, 2011). Aumont et al. (1989) observed that five consecutive days of rain were adequate to increase the L3 population in the pasture. Khadijah, Kahn, Walkden-Brown, Bailey, and Bowers (2013) added that rainfall immediately before or up to two days after the release of faeces into the environment promotes recovery of L3 in the pasture. Rainfall of 25-50 mm is usually required for migration (Vlassoff, 1982). Moreover, Callinan and Westcott (1986) found that the speed of vertical migration is optimal when a pasture has a humidity of around 90%, providing ideal conditions to increase the hatching, development, and survival of L3 in the pasture. This also promotes the speed of horizontal migration among the L3, which can move 15 to 90 cm away from the dung pat (Gronvold & Hogh-Schmidt, 1989). Wang, van Wyk, Morrison, and Morgan (2014)

concluded that the amount and regularity of precipitation, which favors the increase in humidity, is more important than the number of rainy days.

2.2.3 Parasite resistance and resilience

Parasite resistance is defined as the ability of the host to interact with parasites and control their life cycle (Bishop & Stear, 2003) or to limit the development or establishment of parasite (Råberg, 2014). Accordingly, hosts that are able to manage a relatively low parasite load, even when living in a parasite-infested environment, are considered resistant (Ahmad et al., 2023). Resistance is generally assessed by the ability of the host to reduce FEC, worm burden, worm length and female fecundity (Coop & Sykes, 2002), with FEC being the most practical indicator of resistance in living animals (Brown & Fogarty, 2016; Morris et al., 2010). In addition, concentrations of circulating immunoglobulins are also used to assess resistance (Aboshady, Stear, Johansson, Jonas, & Bambou, 2020; Iposu, Greer, McAnulty, Stankiewicz, & Sykes, 2010; MacKinnon, Zajac, Kooyman, & Notter, 2010). Several studies have reported increased IgA response following GIN infection, with higher rates reported in GIN-resistant breeds than susceptible breeds (Bowdridge, MacKinnon, McCann, Zajac, & Notter, 2013; McBean et al., 2016). On the other hand, resilience can be defined as the ability of the host to resist the pathogenic effects of parasites, thereby maintaining good health and productivity (Ahmad et al., 2023; Albers et al., 1987). De Barbieri et al. (2023) suggest that resilience indicators are traits related to growth, fatness, feed intake, weather tolerance, reproduction and health.

Increasing the resistance and resilience in sheep to minimize the effects of gastrointestinal nematode infection has been an area of research for many years (Ahmad et al., 2023; Baker et al., 2003; Hine et al., 2022; Hunt, McEwan, & Miller, 2008). Some researchers concluded that increased resilience and resistance to GIN infection can be achieved by manipulating the nutrition during crucial periods of the sheep's life (Kahn, 2003; Walkden-Brown & Kahn, 2002). Experimental studies have shown that increased MP intake in housed or grazed sheep during the periparturient period showed a significant improvement in resistance to GIN infection (Donaldson, van Houtert, & Sykes, 2010; Kahn, 2003; Knox, Torres-Acosta, & Aguilar-Caballero, 2006). The difference between whether nutrition promotes resistance or resilience is unclear, as nutritional supplement trials have shown increased resistance through higher immune development than non-supplemented groups. However, developing immunity and then eliminating the parasite is expected to make the animal more resilient to the constant threat of the larvae (Greer, 2005).

2.2.4 Effect of the host on the development success

Initial investigations from a laboratory experiment showed that under constant laboratory conditions, the parasite eggs proportion developing to L3 (development success) was significantly influenced by the host (Jørgensen et al., 1998). A field study by Jørgensen (2000) was conducted to support the result of the laboratory experiment. Mixed-age Perendale ewes and post-weaning lambs, which had previously selected for high and low FEC lines, were used in the field study. A significant lesser percentage of developmental success was observed in eggs derived from adult ewes than from lambs. Additionally, developmental successes were also low in eggs of ewes and lambs in the low-FEC line than those in the high-FEC line (Table 2.1) (Jørgensen et al., 1998). Presumably, this reflects nematode egg viability being influenced by the immune mechanism of the host (Jørgensen, 2000).

Table 2.1 Geometric means of faecal egg counts and developmental success of Perendale ewes and lambs selectively bred for high or low FEC.

Host	Line	Faecal egg counts (epg)*	Developmental success (%)*
Ewes	High FEC	144[a]	49[ab]
	Low FEC	80[a]	13[c]
Lamb	High FEC	847[b]	67[b]
	Low FEC	271[c]	35[a]

*= Means within colums with different letters indicate that values are significantly different (P<0.05).

Source: Jørgensen et al. (1998)

2.3 Effects of gastrointestinal parasite infections on the host

GIN parasite infections can have various effects on the host either directly or indirectly. The direct effects are associated with the pathological damage inflicted by the nematode infections on the GI tract while indirect effects happen as a consequence of response of the host to the appearance, level, and impact of parasitism in the GI tract (Sutherland & Scott, 2010). The effects are dependent on the genera of the parasite, the rate of infection, the immunological status of the host (Greer, 2005) and age and breed (Coop & Sykes, 2002). During infection with gastrointestinal parasites, many factors interacted to prevent utilisation of nutrient and regular production, such as the reduction in voluntary feed intake and increased endogenous protein losses (Coop & Kyriazakis, 2001; Knox et al., 2006), and are described below:

2.3.1 Reduced voluntary feed intake (anorexia)

The reduction in voluntary feed intake is a unique characteristic of the host's response to the parasite infection (Kyriazakis, Tolkamp, & Hutchings, 1998). The time of the first detection varies

between parasites. Anorexia in sheep infected with *T. circumcincta* occurs within two weeks of infection (Sykes & Coop, 1977). Kyriazakis, Anderson, Oldham, Coop, and Jackson (1996) found that anorexia in sheep infected with *T. colubriformis* does not ensue until 3-4 weeks after larvae ingestion. However, anorexia does not happen until after six weeks of the infection in laboratory mice infected with *Schistosoma mansoni* (Vengesa & Leese, 1979).

The magnitude of inappetance can vary, from a small decrease in feed intake (5-10%) to complete anorexia (Kyriazakis, 2010). Sykes and Greer (2003) stated that a decrease of up to 50% is commonly observed even in sub-clinical parasitism and can severely affect the nutrient economy of the host. van Houtert and Sykes (1996) estimated that between 40 and 90% of production loss could be attributed to anorexia. Sykes and Greer (2003) described that the degree of anorexia might be influenced by parasite species and infection site, and by the age, breed, and resistance status of the host. Additionally, McAnulty (1990) stated that intake of feed possibly sensitive to GIN infection, primarily when sheep are operated near to the physiological limits, such as in lactating ewes and fast-growing lamb. Kyriazakis (2010) concluded that the magnitude, duration, and recovery rate from anorexia would be affected by the content of feed given to infected hosts. Steel, Symons, and Jones (1980) demonstrated that level of infection also affects anorexia, as feed consumption of lambs was significantly depressed with larval dosing regimes (LDR) of 3,000, 9,500 and 30,000 *T. colubriformis* L3 larvae per week, by 71, 61 and 44%, respectively of intake on LDR 0 during week 8 to week 12. When anorexia during *T. colubriformis* infection was compared between lambs given access to diets of different metabolisable protein (MP) content, animals on the low MP diet suffered a higher degree of anorexia (Greer, Sedcole, et al., 2009).

A reduction in feed intake is also observed in periparturient ewes infected by *T. circumcincta* (Leyva, Henderson, & Sykes, 1982; Zaralis, Tolkamp, Houdijk, Wylie, & Kyriazakis, 2009). Leyva et al. (1982) observed a reduction of feed intake by 16% during lactation in parasitised Poll Dorset ewes but did not detect any decline of feed intake during pregnancy. Zaralis et al. (2009) reported a decrease in feed intake around 12% during late pregnancy and about 22% during lactation. They also detected significantly lower feed consumption in infected ewes than non-infected ewes during the periparturient period and suggested anorexia in periparturient ewes can ensue before any rise in FEC is detected. Kimambo, MacRae, Walker, Watt, and Coop (1988) noted that feed intake would return once animals have become immune to parasites. More importantly, it is restored rapidly in lambs following anthelmintic treatment (Kyriazakis, Anderson, Oldham, et al., 1996).

2.3.2 Endogenous protein losses

Knox et al. (2006) stated that parasite infections could cause considerable gastroenteric losses of endogenous protein in the form of whole blood, plasma, mucus, and sloughed epithelial cells. Most of these proteins are redigested before being absorbed in the site distal to infection, but not all. The increased of endogenous protein secretions into the small intestines may cause severe effects on the animal (Poppi, MacRae, Brewer, & Coop, 1986), these authors suggesting that two dominant effects will occur. Firstly, synthesizing more protein to recover losses will incur an additional cost related to that synthesis. Secondly, if the lambs are in a nitrogen-limiting condition, the incomplete resorption of endogenous secretions will reduce the availability of N for tissue growth. Additionally, parasite infection also will affect on nitrogen economy of the host because unabsorbed residues will either be digested further in the large intestine before absorbed as ammonia and excreted as urea in the urine or be excreted in the faeces (Kimambo et al., 1988; Poppi et al., 1986; Rowe, Nolan, De Chaneet, Teleni, & Holmes, 1988).

According to Coop and Kyriazakis (2001), despite some reabsorption, protein losses are abundant. Some studies estimated that the amount of unreabsorbable endogenous nitrogen leaving the terminal ileum of *Trichostrongylus*-parasitised lambs could be as high as 4-5 g N/d (Bown, Poppi, & Sykes, 1991; Poppi et al., 1986). With *H. contortus* infection, as much as 270 ml/d of the plasma volume can leak into the GI tract of sheep (Parkins & Holmes, 1989). Additionally, the study of Vaughan, Greer, McAnulty, and Sykes (2006) showed that endogenous protein losses caused by infection with *T. circumcincta* and *T. colubriformis* were independent of immuno-suppression, indicating they are a result of physical damage induced by the parasites rather than immune-pathology.

2.4 Mechanism of immune response

Animals use different mechanisms of immunity to protect themselves from the infection caused by other organisms, such as parasites, bacteria, and viruses. Nematode parasites are generally host-specific; they produce different types of antigens which unique to every developmental phase (Greer, 2005). In mammalian hosts, the immune response against infectious agents can be classified into two types, i.e., innate (non-specific) immunity and acquired (specific) immunity; both immunities have different response patterns. Immunity to parasites is developed with time. Generally, innate immunity is not improved by previous exposure to infection. The acquired immunity is associated with the memory of the immune system (Xie, 2004).

2.4.1 Innate immunity

The innate immunity is essential at the first contact to an infectious agent then initiating and driving the acquired immunity. The innate immunity is comprised of four types of defensive barriers, namely anatomic (intestinal epithelium and mucus), physiologic (intestinal microflora, biliary secretions, peristalsis), inflammatory (complement, phagocytes), and phagocytic (ingestion and destruction of antigen) (Tizard, 2000).

2.4.2 Acquired immunity

The acquired immunity is comprised of humoral (antibody or immunoglobulin-Ig based) and cell-mediated immunity (McFarlane, 1997; Tizard, 2000). Acquired immunity is considered to be the primary defence mechanism of the host against GIN parasites (Stear, Park, & Bishop, 1996), which develops over time in response to the parasite challenge, depending on the age, nutritional status, and genotype of the host (Beraldi, Craig, Bishop, Hopkins, & Pemberton, 2008; Houdijk et al., 2005; Smith, Jackson, Jackson, & Williams, 1985). Coop et al. (1995) suggested that the nutritional status of the host during GIN infection is essential. Their study showed supplementation of by-pass protein to growing lambs during GIN infections improved lamb immunity to GIN parasites. The study of Stear, Strain, and Bishop (1999a, 1999b) revealed some indicators of improved acquired immunity, which include decreased numbers of adult worms, reduced size of adult worms, and increased numbers of inhibited larvae. Moreover, Lee et al. (2011) added some indicators associated with acquired immunity, such as reduction of egg counts, increased expulsion of worms, elevated numbers of eosinophils, mast cells, plasma cells, and lymphocytes, as well as increased concentrations of parasite-specific antibodies.

2.5 The mechanism of immunity of sheep to nematode infections

The immune responses to GINs in sheep are related to a variety of effector components, including immune cells, cytokines, and antibodies. These components may function differently depending on the species and developmental stages of the parasites and the status of the host (Xie, 2004). Only antibody responses are discussed in this review.

Many researchers reported that in sheep some antibodies isotypes are correlated with GIN resistance, including IgA, IgG, and IgE (McRae, Stear, Good, & Keane, 2015; Toscan et al., 2017). Several studies identify IgA as a dominant antibody against parasite infection and fecundity (Beraldi et al., 2008; Shaw, Morris, Wheeler, Tate, & Sutherland, 2012). It represents a crucial first line of defence against pathogen invasion at the exposed mucosal surfaces (Woof & Kerr, 2004). Many

researchers stated that the mechanisms of IgA action are not entirely clear (Arsenopoulos, Symeonidou, & Papadopoulos, 2017). However, there are indications that IgA can control larval development and establishment, and egg production of *T. circumcincta* (Halliday, Routledge, Smith, Matthews, & Smith, 2007; Stear et al., 2004) by unique binding to both larvae and adult worms or to gastrointestinal nematode secretions (Venturina, Gossner, & Hopkins, 2013). On the other hand, some authors found that the role of IgG is less clear (Greer, 2005; Schallig, 2000). However, Douch, Green, Morris, and Hickey (1995) observed negative phenotypic correlations of up to 0.62 for FEC and 0.63 for worm burden with serum IgG levels. Increased levels of IgG are reported in genetically-resistant sheep (Gill, Gray, Watson, & Husband, 1993). Further a close relationship between IgE in serum and GIN infections has been shown (Shaw, McNeill, Gatehouse, & Douch, 1997), as these authors found that sheep infected with *T. axei* had significantly higher total IgE levels in serum than the controls.

The antibody responses to nematode infection may depend on the breeding line and age of the host and the developmental stage of the parasites (Xie, 2004). Studies of different lines of grazed sheep which were selected for resistance to gastrointestinal nematodes under natural infections found that the responses of the *T. colubriformis*-specific total antibody, IgG and IgM in serum to both infective larvae and adult worm antigens were all greater in low-FEC line lambs than in the high-FEC counterparts (Bisset et al., 1996). Additionally, Duncan, Smith, and Dargie (1978) showed that irradiated larvae generated protection against *H. contortus* challenge and raised abomasal mucus IgA and serum IgG levels in the adult sheep. On the other hand, neither protection nor elevated mucus IgA and serum IgG was observed in vaccinated lambs. Adams, Anderson, and Windon (1989) found the antigen from infective larvae caused a cross-reaction between *T. colubriformis* and *H. contortus* but not the antigen from the adult worms. Their study demonstrated a significant increase in titres of serum antibody against *H. contortus* infective larval antigen in those sheep vaccinated and challenged with *T. colubriformis* larvae. However, there was no similar increase of serum antibody titres against *H. contortus* adult worm antigen in the same sheep. Gill et al. (1993) revealed that IgG_1 and IgA levels in genetically resistant grazing sheep were significantly greater than the values in mixed-breed sheep after challenged between 10 and 31 days with *H. contortus*.

2.6 Control method of GIN parasites

For the majority of sheep producers in New Zealand, the use of anthelmintics is an essential part of GIN parasite control (Miller, Ganesh, Garland, & Leathwick, 2015). Even though the focus for drench treatments is mostly on the lambs, the administration of anthelmintic to adult ewes especially during the periparturient period has been a regular practice amongst sheep producers for many years

(Brunsdon, Kissling, & Hosking, 1983; Lawrence et al., 2007). The popularity of anthelmintic treatments given before lambing raised since the development and marketing of controlled release capsules (CRC) and the formulation of macrocyclic lactone actives with persistent activity (Garland & Leathwick, 2015; Leathwick et al., 2006). Leathwick et al. (2006) showed that drenching ewes around parturition, either with a CRC or after parturition using an oral dose, accelerated the development of anthelmintic resistance. If compared with untreated ewes, the development was approximately twofold when ewes were drenched annually with a CRC. Accordingly, the regular use of these drenches is likely to cause increased costs of future control. Additionally, Garland and Leathwick (2015) and Miller et al. (2015) indicated the financial returns ensuing from the administration of anthelmintic to ewes around parturition are neither consistent nor predictable, and can often be harmful, although further studies are needed.

Anthelmintic resistance has promoted research into alternative approaches that are intended to reduce reliance upon chemical to control parasite (Jackson & Miller, 2006). The various alternatives in controlling GIN parasites have recently been discussed and thoroughly reviewed elsewhere (Athanasiadou, Githiori, & Kyriazakis, 2007; Athanasiadou, Gray, et al., 2007; Chandrawathani et al., 2004; Donaldson et al., 1998; Hoste & Torres-Acosta, 2011; Knox & Smith, 2001; McClure, 2009; Thamsborg, Roepstorff, & Larsen, 1999), and only grazing management and dietary supplementation are briefly discussed in this thesis. However, dietary supplementation is discussed in a separate section (Section 2.7).

Grazing management has been extensively applied to control GIN parasites either in tropical or temperate regions. The primary purpose of grazing management is to minimise the interaction between susceptible grazing animals and the infective stages of the parasite (Hoste & Torres-Acosta, 2011), and to provide clean pastures where animals can graze safely (Barger, 1999). Grazing management can be divided into three strategies, that is preventive, evasive, and diluting strategies (Michel, 1985). Preventive strategies, including introducing unexposed animals to an uncontaminated or clean pasture (Michel, 1985) or suppressing nematode egg production by drench administration in the early part of the grazing season until the L3 level on herbage has decreased to a safe level (Barger, 1997). Evasive strategies, in contrast to the preventive approaches, do not aim at restricting the contamination on pasture by worm eggs but instead try to avoid parasite infections by relocating the animals to a clean pasture before significant numbers of larvae from those eggs contaminating the original pasture (Barger, 1997; Jackson & Miller, 2006; Michel, 1985). Rotational grazing is considered as an evasive strategy which involves the subdivision of pasture into many paddocks where each paddock is grazed by the animals for a short time and then spelled for a more extended period (Barger, 1999). Jackson and Miller (2006) stated that the control of parasitic

nematodes using this approach to be hard to employ, as well as ineffective to apply primarily in temperate regions because it greatly influenced by variations in temperature and moisture (Barger, 1997). Additionally, infective larvae on pastures in temperate areas have relatively longer survival times than that in the tropics. Waller (1997) suggested that to achieve a successful rotational grazing strategy, it is essential to possess the proper knowledge of the local ecology of the free-living phases of the parasites. For diluting strategies, Michel (1985) and Barger (1997) suggested to mixed grazing susceptible animals with helminthological inert animals, which are not a source of contamination, either with older animals from the same species or with the animals from different species. By this strategy, the worm eggs from the faeces passing onto the herbage are diluted, thus reduce the pasture infestation (Michel, 1985). Thamsborg et al. (1999) revealed that dilution could also be achieved by decreasing animal numbers in a particular pasture. Barger (1999) suggested that in order to be successful, grazing management is usually applied after a strategic anthelmintic treatment.

2.7 Economic consequences of parasitism

A number of experiments have been conducted to determine the effect of parasitism on the productivity of sheep (Brunsdon, Vlassoff, & West, 1986; Cardia, Rocha-Oliveira, Tsunemi, & Amarante, 2011; Coop, Graham, Jackson, Wright, & Angus, 1985; Greer, 2005; Leyva et al., 1982; Sykes, 1994). All studies showed that parasitism has severe effects on sheep productivity. Sykes (1994) estimated the reduction of up to 50% in liveweight gain (LWG) and feed conversion efficiency in growing lambs ingesting less than 600 nematode larvae/kg of fresh herbage/d. Greer (2005) observed a decrease of 30% in LWG in wool lambs infected with 4,000 *T. colubriformis* larvae/d. Moreover, Coop et al. (1982) revealed that LWG reduction increased with increasing larval challenge, with four-month-old crossbred lambs trickle infected with 1,000, 3,000, or 5,000 of *T. circumcincta* larvae/d having 10%, 25%, and 47%, respectively reduction in LWG, and only small amount of this loss recovered when lambs were drenched every 21 days. Reduced LWG may delay lambs reaching their finishing weight within a given period, which then may financially affect the farm. Coop et al. (1985) found lambs experiencing daily moderate levels infection of about 3,000 *T. circumcincta* larvae require four to seven weeks longer to reach a target slaughter weight of 36 to 38 kg than lambs exposed to lower larval challenge levels. Moreover, failure to sufficiently control parasite infection also reduce lamb LWG and delay lamb reaching slaughter weight (Sutherland, Shaw, & Shaw, 2010). The authors reported a 10% reduction in LWG, with lambs taking 17 days longer to reach slaughter weight when treated monthly with a benzimidazole anthelmintic compared with their counterparts treated with a fully-effective monepantel anthelmintic. Miller et al. (2012) stated that the opportunity to harvest more lambs with targeted slaughter weight and sell them sooner

would give additional benefits to the farmer, such as more herbage available for the remaining animals, save pasture, and support the introduction of new stock.

Production losses caused by GIN not only occur in lambs. Significant depression of LWG (2.4 kg) was reported in mature breeding ewes grazing high level of larval infestation of nearly 4,400 larvae/kg herbage, compared with those grazing less contaminated pasture of approximately 33 larvae/kg herbage (Brunsdon et al., 1986). Furthermore, Sutherland et al. (2010) suggested that a decreased LWG can also be associated with losses in BCS, which can adversely affect carcass composition and value, a reported loss of 14%. Reduced milk production (Leyva, Henderson, & Sykes, 1981; McAnulty, 1990; Thomas & Ali, 1983) and decreased clean wool production (Brunsdon et al., 1986) have also been detected in parasitised lactating ewes. Sutherland et al. (2010) credited a loss in wool production to the increased faecal scouring, which leads to an increased dag score, a consequential loss of wool and potential exposure to flystrike.

Familton (1991) elucidated the production losses of sheep in New Zealand caused by internal parasite infections as follows, 49.7% in lost wool production; 30.8% as in consequence of reduce ewe fertility resulting from decreased body weight at first mating; 9.2% in lost meat production; 8.7% for cost of drenches; and 1.7% as the overall estimated of cost of dagging. It is predicted that anthelmintic expenses for sheep have increased from year to year since the majority of sheep farmers depend heavily on the administration of drenches to control parasitism and maintain the performance of animal (Lawrence et al., 2007).

2.8 Host nutrition and dietary supplementation

The increasing problem of anthelmintic resistance has stimulated continued interest in nutritional approaches to improve sheep immunity and resilience to infection (Steel & Knox, 2003). Host nutrition is one of the essential factors that affect the ability of the host to maintain an effective immune response against GI nematodes (Beasley et al., 2012). Many authors have reviewed the effects of host nutrition and dietary supplementation (Caroprese, Giannenas, & Fthenakis, 2015; Coop & Holmes, 1996; Coop & Kyriazakis, 2001; Houdijk et al., 2012), particularly in the case of the periparturient ewes (Beasley et al., 2012; Donaldson et al., 1998, 2001; Houdijk, 2008; Sakkas, Houdijk, Athanasiadou, & Kyriazakis, 2012).

Coop and Kyriazakis (2001) suggested that nutrition can affect the development and consequences of nematode parasitism in three approaches. Firstly, nutrition can improve the host's ability to confront the detrimental effects of parasitism (resilience). Secondly, it can increase the host's ability to

accommodate and ultimately to defeat parasitism (resistance) by inhibiting the establishment, growth rate, fecundity and/or persistence of the population of the parasite. Thirdly, it can directly influence the population of the parasite by the consumption of antiparasitic components. Sykes (1997) stated that nutritional status has an important impact on the development and maintenance of immunity in sheep, consequently will affect the performance of the animal. Besides, the nutrition of the host can indirectly affect the resistance and resilience to GIN by enhancing the immune response (Athanasiadou et al., 2008).

Immune responses to parasites have commonly been regarded as part of body maintenance and thus may be prioritised in situations of insufficient distribution of nutrients (Houdijk, Jessop, & Kyriazakis, 2001). Nonetheless, there is increasing evidence that some aspects of immunity are sensitive to changes in the supply of nutrients (Coop & Holmes, 1996; Coop & Kyriazakis, 1999, 2001), especially during the growing and the periparturient period where the nutritional requirement increases (Coop & Kyriazakis, 1999; Walkden-Brown & Kahn, 2002). The increased nutrient demands during these periods can lead to the deficiency of nutrients, which is linked to a breakdown of acquired immunity. During periods as mentioned above, the increased nutrients, especially protein intake, lead to considerable improvements in the host's ability to deal with the parasite population (Houdijk et al., 2003; van Houtert, Barger, Steel, Windon, & Emery, 1995). Like other bodily functions, the immune system also requires energy and micronutrients like vitamins and minerals (Kyriazakis & Houdijk, 2006).

2.8.1 Ewe requirements

Sheep require metabolisable energy (ME) and metabolisable protein (MP) to meet the requirements of maintenance, wool growth, liveweight gain, pregnancy and lactation (Brookes & Nicol, 2007; Nicol & Brookes, 2007). Based on Brookes and Nicol (2007) calculations, the MP requirement for LWG in adult sheep is estimated to be 400 g MP/kg LWG. On an annual basis, breeding ewes require approximately 1 kg MP/kg lamb weaned while finishing lambs have a relatively high MP requirement relative to their ME requirement of around 7 g MP/MJ ME. Nicol and Brookes (2007) state that for adult sheep, live weight has a clear effect on the energy needed for maintenance requirement. Every extra 10 kg LW requires additional 1.0-1.5 MJ ME/d. For pregnancy, a total of approximately 50 MJ ME is required per kg lamb birth weight, for lactation and lamb weight gain needs an additional requirement of approximately 45 MJ ME/kg lamb weaning weight, while for wool growth ME requirement is estimated at around 40-50 MJ ME/kg wool. They calculated that total annual requirement for breeding ewes in flat land is 6,670 MJ ME/year, although this varies with size. These calculations may vary depending on the breed/genotype of sheep. For example, tropical genotypes are generally not selected for muscle deposition and the values tend to be larger than temperate

genotypes due to the relative energy density of adipose (Chay-Canul, Ayala-Burgos, Kú-Vera, Magaña-Monforte, & Ferrell, 2011). Consequently, tropical genotypes require more energy for ADG than temperate genotypes (Jayanegara et al., 2017). Furthermore, in high-temperature conditions in the tropics, sheep require more energy to dissipate body heat, increasing the animals' energy requirements (CSIRO, 2007). A meta-analytical study on sheep breeds in Indonesia (Priangan, fat-tailed, local breeds) by Jayanegara et al. (2017) showed that different breeds have significantly different energy and protein requirements for ADG. Those studies highlighting the importance of specific research on local conditions and breeds (Barcelos, Maeda, Santa Anna, & Pereira Jr, 2016).

The nutrient requirement for expression of immunity to *T. circumcincta* for parasitised periparturient ewes rearing twins was calculated to be about 1 g/kg metabolic body weight or 5% of the maximum MP requirements for the uninfected ewes (Houdijk, Jessop, et al., 2001). This requirement was lower than other studies (Donaldson et al., 2001; Houdijk, Jackson, & Kyriazakis, 2009; Houdijk et al., 2003) which were suggested MP requirement as high as 350 g MP/d, or 20-30% higher than conventional estimates of protein requirement of uninfected lactating ewes (Sykes & Kyriazakis, 2007). Additionally, extra MP is required for the repair and or replacement of damaged and or loss of tissues caused by parasitic infection (Kyriazakis & Houdijk, 2006).

In periparturient ewes, the PPR concurs with an increase in the nutritional requirements of the ewes due to the demands of pregnancy and lactation (Kahn, Knox, & Gray, 1999; Walkden-Brown & Kahn, 2002). While requirements for both ME and MP increase in reproductive ewes, the most substantial relative increase is for MP (Kahn, Knox, Gray, et al., 2003). The requirement for MP relative to ME in the periparturient period is high, around 7-8 g MP/MJ ME (Donaldson, 1997). Freer, Moore, and Donnelly (1997) suggested that ME requirements of a penned 50 kg single-bearing ewe maintaining maternal live weight and fed a diet providing 11.5 MJ/kg DM raised threefold by three weeks after lambing (peak lactation) while MP requirements increase 5.4 times over the same period. There is evidence from several studies that the PPR is worsened by the combination of higher demand for MP relative to ME and increased competition for essential nutrients during late pregnancy and lactation (Donaldson et al., 1998; Houdijk, Jessop, et al., 2001). Increasing the MP supply, but not ME, to penned ewes during the periparturient period has been revealed to diminish ewe FEC and worm burdens (Donaldson et al., 1998, 2001). In the study of Donaldson et al. (1998), Coopworth ewes were trickle infected with *T. colubriformis* and *T. circumcincta* and given feeds providing either 120 g CP/kg DM (Le Floc'h, Melchior, & Obled) or 200 g CP/kg DM (HP) by added fishmeal. The feeds were offered to the ewe at two levels of energy which were designed to achieve either zero or plus 50 g/d weight gain in maternal bodyweight during late pregnancy and zero or 100 g/d weight loss during the first three weeks of lactation, respectively. Increased protein supply significantly reduced FEC from

three weeks prior to lambing until three weeks after lambing, with approximately 90% reduction (from around 2,500 epg to 250 epg) at three weeks after lambing. Worm burdens were reduced by 87% (12,020 to 1,540) after three weeks of lactation in ewes that had higher MP intake. Further, Kahn, Knox, Gray, et al. (2003) revealed that when averaged over 21 weeks of the experimental period, the five-week period of pre-lambing supplementation of Merino ewes with cottonseed meal declined FEC by 43% relative to their counterparts.

2.8.2 Protein supplementation

Studies have supported the view that protein is expected to be more important in cases of parasitism than other nutrients (Coop & Kyriazakis, 1999; Kyriazakis & Houdijk, 2006), reflecting the highly proteinaceous nature of many parts of the effector immune responses (Coop & Holmes, 1996). Numerous supplementation experiments have shown that instead of energy, protein supplementation is needed to improve resistance and/or resilience during parasite infection in lamb (Coop et al., 1995; Kahn et al., 2000; van Houtert, Barger, & Steel, 1995) and the periparturient ewe. Frequently, decreased FEC and reduced worm burdens are observed both in lambs (Greer, Sedcole, et al., 2009; Louvandini et al., 2006) and ewes (Donaldson et al., 2001; Houdijk et al., 2005; Kidane, Houdijk, Tolkamp, Athanasiadou, & Kyriazakis, 2009; Zaralis et al., 2009), following supplementation with protein sources such as fish meal (Donaldson et al., 2001; Greer, Sedcole, et al., 2009), soybean meal (Louvandini et al., 2006), and xylose-treated soybean meal (Houdijk et al., 2009; Houdijk et al., 2003, 2005; Kidane et al., 2009; Zaralis et al., 2009). Supplementation of fishmeal to young sheep infected with *T. colubriformis* caused 44 and 99% reduction in worm burdens at weeks 15 and 20, respectively (van Houtert, Barger, Steel, et al., 1995).

Protein supplementation appears to assist with immunological functions, as indicated by increases in circulating and local inflammatory cell concentrations, mast cell proteases, and circulating antibodies, particularly during the phase of expression of immunity (Athanasiadou et al., 2008; Coop et al., 1995; van Houtert, Barger, & Steel, 1995). Other workers have found higher parasite-specific IgA in the plasma of infected sheep receiving higher levels of dietary protein (Houdijk et al., 2003; Strain & Stear, 2001). Protein supplementation has also been shown to enhance the proportion of thymus-derived cells, which correlated with the expression of cellular immunity in the local immune response of infected sheep (Kambara & McFarlane, 1996). Moreover, several authors reported an increased intake of MP has resulted in lower plasma pepsinogen concentrations, indicating less severe abomasal damage due to parasitism (Houdijk, Kyriazakis, Jackson, Huntley, & Coop, 2000; Houdijk et al., 2003; Zaralis et al., 2009).

2.8.3 Targeted supplementation

In New Zealand farms, pasture is the primary feed source for animals. The major disadvantage of pasture is its variability of supply between seasons of the year, between years, and between regions within New Zealand. Problems are also caused by changes in the quality of feed throughout the year (Rattray, 1978), which are often lead to insufficient nutrition that may have substantial influences on the productivity of animal (Knox et al., 2006). To overcome nutritional insufficiency, the authors proposed supplementation strategies that target the supplies of limiting nutrients which essential to help optimisation of rumen function and digestibility of available herbage. They also suggested considering the adverse effects of GIN infection on animal nutrition when designing supplementation strategies. Nutritional supplements offered to parasitised hosts must target the limiting nutrient(s) of different physiological conditions with the most vulnerable hosts at critical physiological statuses and should target periods when the requirements for nutrient are highest, such as during the growth and the periparturient period (Coop & Holmes, 1996; Coop & Kyriazakis, 1999; Knox et al., 2006; van Houtert & Sykes, 1996). Besides, the supplemental feed cost should also be considered when designing strategic supplementation regimes. Many sheep producers try to minimise the cost of supplemental feed because it is often their greatest expense. Therefore, the strategic use of nutritional supplements is the best opportunity to produce a cost-effective supplementation strategy.

Several experiments have focused on the effects of prepartum nutritional supplementation on reproductive ewes, which has been shown to increase reproductive success and enhances immunity to the GIN parasite (Donaldson et al., 1998, 2001; Houdijk et al., 2000; Kahn et al., 1999). However, there is limited information available if targeted supplementation to grazing ewes can provide epidemiological advantages to their offsprings.

2.8.4 Effect of supplementation of grazing ewes

Many workers have studied the effect of MP supply on PPR in FEC and worm burden of housed periparturient ewes (Donaldson et al., 1998, 2001; Houdijk et al., 2000). However, only a few studies evaluated the benefits of increased MP supply of periparturient ewes while grazing on pasture. These studies were using trickle infection and conducted only for a relatively short time. They have fallen short of examining the epidemiological advantage for the grazing lambs for the remainder of the grazing season. Kahn, Knox, Gray, et al. (2003), for example, worked on the effectiveness of protein supplementation and genetic selection to improve the periparturient Merino ewes resistance to infection from GIN parasites. While grazing at pasture, ewes were subjected to one of the three supplement groups that provided 0 or 250 g/day cottonseed meal (CSM) for five weeks before or six

weeks after the start of lambing. On 16, 9 and 1 days before their allocation to trial plots, each ewe was trickle infected with 3.000 L3 *Trichostrongylus colubriformis* and 1.000 L3 *H. contortus* larvae to the addition of the current nematode infection. The authors found that prepartum supplementation reduced FEC and increased ewe body weight gain. Moreover, during the prepartum period, the effects of improved MP supply on worm resistance arose when ewes were loss their maternal weight of 30 to 14 g/day. During the postpartum period, the absence of any benefits from increased MP supply to worm resistance occurred when ewes were gained maternal weight of 80–100 g/day. The authors concluded that the period of highest MP pressure was during maternal weight loss.
To extend the findings of Kahn, Knox, Gray, et al. (2003) above, Kahn, Knox, Walkden-Brown, and Lea (2003) conducted a similar study to them. The result indicated that over the postpartum period, CSM supplementation reduced 68% FEC of C ewes and 58% reduction in R ewes. These results contrast with those reported by Kahn, Knox, Gray, et al. (2003). They assumed that the smaller effect of CSM supplementation on reducing the FEC of ewes selected for worm resistance was a function of a higher priority by the gut immune system for amino acids. One possibility to explain for the difference with that reported by Kahn, Knox, Gray, et al. (2003) is that the MP pressure in the present study was considerably higher, as shown from estimated maternal weight loss. The MP pressure was sufficiently high so that an insufficient supply of MP compromised the gut immune response of R ewes. Kahn, Knox, Walkden-Brown, et al. (2003) concluded that supplementation to increase the MP supply of grazing periparturient ewes is most effective in increasing resistance to nematode parasites during periods of maternal weight loss, predominantly in ewes who may have had to mobilise their body protein during gestation.

2.9 Literature summary

Gastrointestinal parasites are an essential animal health issue for sheep in New Zealand and other countries. The unique host-parasite relationship between sheep and GIN parasites has been the focus of much research for an extended period, with the final goal to provide sheep producers with additional options for achieving sustainable and integrated parasite control (Hein, Pernthaner, Piedrafita, & Meeusen, 2010). One control method that has been investigated previously is the nutritional supplementation of the ewe during the periparturient period (Donaldson et al., 1998, 2001; Kahn, 2003). They found that dietary supplementation may provide substantial epidemiological benefits through the reduction of PPR. This PPR plays a vital function in the epidemiology of GIN because it is the primary source of infection to all sheep throughout the year.

Numerous supplementation studies have demonstrated that nutritional supplementation is needed to improve resistance/resilience during parasite infections in both the periparturient ewe and lamb

(Houdijk, 2008; Kahn, Knox, Gray, et al., 2003; Sykes & Coop, 2001). However, the information on the consequences of supplementation to grazing ewes in the periparturient period on the capacity of their lambs to resist parasitism is limited. Moreover, although indoor trials have shown that protein supply can influence the periparturient ewe immune status, but it is not clear if they consume and respond immunologically to protein supplementation while grazing pasture and if this may provide an epidemiological advantage to their grazing lambs for the rest of the grazing season. A series of experiments reported in this thesis was designed to examine these issues.

Chapter 3

General methodology

3.1 Feeding

In each year, ewes were allocated to one of two treatments, i.e., supplemented or not with a high-protein pellet (Triplet nuts, Farmlands stock feeds Ltd, New Zealand) during the first four weeks of lactation. All ewes were allowed to graze on perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture as per usual farming practice in New Zealand. In each paddock, ewes and lambs had *ad libitum* access to water and a mineral block (Summit® Multimineral Salt Block, New Zealand) containing essential trace minerals (Cu, Zn, Co, I, Se) with salt as a carrier, as per product label.

Ewes in the supplemented treatment were allowed to access commercially available high-protein sheep pellets (Triplet nuts, Farmlands Stock Feeds Ltd, New Zealand) through a feeder (Advantage Feeders NGF800, Advantage Feeders Ltd, New Zealand). The nut contained barley, wheat, soybean meal, peas, canola, wheat by-products, maize, oats, and molasses with 25% CP and 12.8 MJ/kg DM of estimated ME, as stated on the product label. All supplemented ewes were introduced to the high-protein pellets through a feeder (Advantage Feeders NGF800, Advantage Feeders Ltd, New Zealand) while at pasture. The feeder was initially restricted to supply 50 g/d/ewe three weeks before lambing and subsequently increased to 500 g/d/ewe during the first four weeks of lactation. The supplementation ceased on the last day of four week of lactation. The amount of supplement consumed for each paddock was recorded and calculated as the mean supplement intake per ewe. Amounts of the supplement were calculated to supply an additional 100 g of MP/head/d, assuming that unsupplemented ewes may consume 2 kg DM/d with a total MP intake of 160 g/d. Thus, assuming no substitution, supplementation of 0.5 kgDM/d was calculated to increase total MP supply to about 260 g/d and a total DM intake of 3.5% of body weight (Agricultural and Food Research Council, 1993). Indoor studies have indicated this MP supply may reduce worm burden in periparturient ewes by up to 50% (Donaldson et al., 2001).

Plate 3.1 Advantage Feeders (NGF800, Advantage Feeders Ltd, New Zealand) used for supplemented ewes in the current studies.

3.2 Pasture mass

Pasture mass was measured every two weeks with a manual folding plate meter (Filip's Folding Plate Pasture Meter, Jenquip, New Zealand) with measurements taken every ten steps in a transverse 'W' pattern down the field. The plate meter was calibrated using pasture cuts' from each of the paddocks taken from a 0.2 m^2 quadrant. The samples were oven-dried at 70 °C for 48 hours or until dried, then the dry matter (DM) was determined. Pasture mass from the cuts was estimated by multiplying DM by the area of the respective paddock, from which linear calibration equations were obtained, where y = pasture mass (kg DM/ha) and x = plate meter reading of height per click. Pasture mass throughout each trial was estimated using these equations and the pasture height per click of the rising plate meter for every reading and expressed as kg DM/ha.

3.3 Serum and faeces sampling and preparation

Serum. Blood samples of ewes were drawn by jugular venipuncture into 10 ml vacutainer tubes (Becton Dickinson, Rutherford, New Jersey, USA) and were immediately stored at 4 °C for 24 hours. Serum was harvested following centrifugation using an Eppendorf 5804 R centrifuge (Eppendorf AG, Hamburg, Germany) at 1,200 x *g* for 10 minutes at 4 °C, then stored at -20 °C until required.

Faeces. Faecal samples were taken from sheep rectum by grabbing with gloved fingers. Samples then placed in labelled plastic jars that were identified for each animal. Faecal samples were collected for egg count and coproculture analysis. Following collection, the samples were stored at 4 °C until processing, as described below in Section 3.6.

3.4 Animal slaughter and sampling

The slaughter of the animal was performed based on the protocols described by Donaldson et al. (2001). Animals to be slaughtered were stunned with a captive bolt gun and exsanguinated, after which the abomasum and approximately 6 m of the small intestine were retrieved. Before removal from other gastrointestinal organs, the abomasum ligated at the reticulo/abomasal and at the pyloric/duodenal junctions while the the small intestine ligated distal to the pylorus to retain the respective materials inside the abomasum and the small intestine. The contents and tissues of the abomasum and small intestine were collected for determination of worm burdens, as described below in Section 3.7.

3.5 Pasture larval counts

Pasture samples were collected from each paddock to determine the number of L3 larvae on herbage. The samples were taken by plucking herbage at every 4-5 steps while traversed the paddock in a W-shaped with four plucks of herbage were taken at each stop. Samples from each paddock were placed into a separate plastic bag at the time of collection then immediately weighed and stored at 4 °C until processing. All pasture samples were processed within a week of collection.

The pasture larval counts were carried out using the procedures described by Wright, McAnulty, Noonan, and Stankiewicz (2003). The bag containing the grass and four litres of lukewarm water was put into a modified small hand-operated washing machine (Easy pressure, Banmix Food Machine Ltd., Auckland, New Zealand). The machine was operated at 220 rpm for three minutes. The bag was removed from the machine and a small incision made and the washings allowed passing through a coarse-mesh sieve (aperture size 2 mm) into a 5 l glass beaker. The remaining herbage inside the bag then removed and washed gently with a jet of water and then as much fluid as possible was recovered from it by squeezing. The grass was then spread on a tray and dried in an oven at 70 °C for four days. When thoroughly dry, the herbage was again weighed and this weight recorded. The fresh grass weight was used in the final estimation of numbers of larvae/kg of fresh herbage with the dry weight larvae/kg DM. The resultant suspension was allowed to settle overnight at 4 °C, and then fluid was siphoned off leaving sediment and larvae which were then transferred to a measuring cylinder for second cold sedimentation. The sediment volume was recorded; the contents were shaken until mixed

thoroughly. The suspension was poured onto a 150 mm diameter filter paper (No. 2 Advantec, Tokyo, Japan) and allowed to dry. The filter paper was inverted onto a tissue paper and then put on a Baermann filter funnel filled with lukewarm water. After 36 hours, 100 ml of fluid was withdrawn from the funnel and stored in glass bottles. After storage at 4 °C for 24 hours to allow larvae to settle, the sample was reduced in volume to 20 ml by siphoning. One ml of sample was placed in a counting chamber, and a drop of iodine was added. Larvae present in the sample were counted and differentiated from free-living larvae microscopically by looking for four features typical for parasitic nematode larvae; the presence of sheath (double membrane), stain retention, completely enclosed anterior and posterior orifices and presence of tail sheath (Ministry of Agriculture Fisheries and Food, 1986; van Wyk & Mayhew, 2013). Two readings were performed for each sample and expressed as L3/kg dry matter.

Calculation of larvae per kilogram of dry herbage was as follows:

$$\text{Number of larvae/kg dry herbage} = \text{number of larvae counted} \times \frac{\text{ml sediment}}{\text{ml sediment} - \text{ml sub sample}} \times \frac{1{,}000}{\text{dry weight}} \times \frac{20}{2}$$

Plate 3.2 Modified hand operated washing machine (Banmix Food Machine Ltd., Auckland, New Zealand) used for washing larvae from the grass samples.

3.6 Faecal egg count (FEC)

FEC was determined using the modified McMaster method (Ministry of Agriculture Fisheries and Food, 1986). The sample of faeces (1.7 g) was placed into a jar containing 5 ml of water and allowed to soak overnight at 4 °C. The soaked sample was homogenised for 25 seconds using an electrical stirrer after adding 46 ml solution of saturated NaCl. Immediately, the faecal suspension was pipetted from the middle of the jar using a Pasteur pipette to fill two counting chambers of a

moistened McMaster slide. The slide was allowed to stand for a few minutes to allow the eggs to float to the surface. The number of nematode eggs presents within each grid marked in both chambers of the slide was counted using a microscope. The total number of nematode eggs was multiplied by 100 to give the number of eggs per gram (epg) of fresh faeces.

3.7 Worm burden

Abomasum worm burdens were determined in abomasal washings and abomasal digests following the guidelines proposed by Robertson and Elliott (1966) and Herlich (1956), respectively. The abomasum and the small intestine were opened separately. The ingesta and worms were removed from the mucosa by vigorously washed under a stream of water. Washings and content were combined, adjusted to a volume of 2,000 ml, and thoroughly mixed before 4 x 50 ml (10%) aliquots were taken, then preserved in a 10% formalin solution. The abomasum tissue was digested with 1% pepsin in 3% HCI at 37-40 °C for 12-16 hours to recover any immature histotrophic stages. All aliquots of the digested material were collected in a jar and stored in a 10% formalin solution. The total number of worms in the washings and the digested tissue was attained by washing 10% aliquots using a 45 µm pore size of mesh sieve that was adequate to let digested substance to pass through but hold the worms. Counts were made by pouring washed aliquots onto a petri dish with an etched grid base. Contents were examined in duplicate using a compound binocular microscope and worms differentiated and counted as L3 larvae, L4 larvae, and adult worms, and differentiated into various nematode species counts. To give a total number of worms in the abomasal digest, the number of worms counted from the abomasal digest multiplied by ten, and while to provide a total number of worms in the abomasal wash, the number of worms counted from the abomasal wash multiplied by 110. The final abomasal worm count was taken as the sum of the abomasal wash and abomasal digest count. Intestinal worm counts were determined as described in abomasal worm count and recorded as small intestinal worm counts.

3.8 Antigen preparation

T. colubriformis L3 larvae were obtained from Johnstone Memorial Laboratory (JML), Lincoln, New Zealand. The larvae were stored in water at 4 °C until the extraction was performed. Soluble somatic parasite antigen was prepared before analysis using a similar method described by Knox and Jones (1990). Briefly, *T. colubriformis* L3 larvae were transferred into 15 ml centrifuge tubes and concentrated by centrifugation using an Eppendorf 5804 R centrifuge (Eppendorf AG, Hamburg, Germany) at 1,200 x *g* for 10 minutes at 4°C with the supernatant removed while larval pellet remained. The larval pellet was then frozen and thawed (-20 °C, 37 °C) for about ten cycles before homogenisation using a homogeniser (BeadBug™ 3 Position Bead Homogeniser, Benchmark

Scientific, New Jersey, USA). Microtubes containing microbeads and the larvae were shaken several times (about 15 times) with every shaking time maximum 30 seconds with two minutes rest on ice in between to avoid protein denaturation. The resulting homogenate was clarified by centrifugation using an Eppendorf 5415D (Eppendorf AG, Hamburg, Germany) at 10,000 x *g* for two minutes at 4 ˚C and then the L3 antigen supernatant was removed. Protein concentrations of antigen supernatant were determined using Bicinchoninic Acid (BCA) Protein Assay kit (Pierce™, Thermo Scientific Ltd, lot #QL227059) following the manufacturer's instructions. Using the BSA standards, reference standard curves were plotted and protein concentrations of the somatic extracts determined. The L3 antigen then stored at -20 ˚C until required.

3.9 ELISA plates coating

Each well of 96-well microplates (Jetbiofil®, China) was coated with 50 µl of combined *T. colubriformis* L3 antigen at 2 µg/ml in coating buffer (containing distilled H_2O and 1.59 g/l Na_2CO_3, 2.93 g/l $NaHCO_3$) and left overnight at 4 °C before washed five times with phosphate-buffered saline (PBS) + 0.05% Tween 20, pH 7.2 (PBST20). Following washing, each well was blocked with 200 µl of blocking buffer (5% bovine skim milk powder in PBS) then incubated at room temperature for two hours. The microplates were washed five times using PBST20 and stored at -20 °C until required.

3.10 Indirect ELISA

Antibodies (Immunoglobulin G (IgG) and immunoglobulin A (IgA)) specific to *T. colubriformis* L3 in serum were measured using an ELISA as described by Xie et al. (2004). Serum sample was diluted to 1:2,500 for IgG and 1:10 for IgA, 50 µl of serum dilution was then added in duplicate to the microplates that had been coated with *T. colubriformis* L3 antigen then incubated at room temperature for two hours. Following washing with PBST20, each well was incubated with 100 µl of the following conjugated antibodies: for L3 IgG horseradish peroxide (HRP) conjugated polyclonal rabbit anti-sheep immunoglobulins (Pierce Immunopure Antibodies, cat #31480, lot #GI959969) at a dilution of 1:2,000, for L3 IgA HRP conjugated rabbit anti-sheep IgA (1.0 mg/ml, Bethyl Laboratories Inc, cat #A130-108P, lot #A130-108P-6) at a dilution of 1:2,000. Following incubation, the microplates were washed five times using PBST20. Colour was develop using 100 µl of 0.05 M phosphate citrate buffer (0.2 M Na_2CO_3 + 0.1 M citrate) adjusted to pH 5 with 0.02% of 30% H_2O_2 added and containing 100 µg of Tetramethyl-benzidine dihydrochloride (Sigma Aldrich, USA) for 40 minutes for both L3 IgG and L3 IgA, before stopping with the addition of 100 µl of stop solution (1.25 M H_2SO_4). The colour intensity of the serum sample was measured using a microplate reader (Multiscan Go, 1510-01462C, Thermofisher Scientific, Finland) at 450 nm. Results were expressed as the mean optical density (OD)

value of the duplicate wells and adjusted with the results of a standard positive serum which included on each ELISA plate.

3.11 Serum protein

Serum urea, serum phosphorus, serum albumin, and total serum protein concentrations were analysed using an RX Daytona Analyser (Randox, Randox Headquarters Co. Antrim, United Kingdom) following the manufacturer's instructions. Serum urea concentration was measured using urea reagent (Stear, Boag, Cattadori, & Murphy, UR3825, Randox Laboratories Ltd., UK) by the enzymatic kinetic method. Serum phosphorus concentration was analysed using Phosphorus (inorganic) assay (Stear et al., PH3820, Randox Laboratories Ltd., UK). Serum albumin concentration was measured using albumin bromocresol green reagent (Stear et al., AB3800, Randox Laboratories Ltd., UK). Total serum protein measurements obtained by the biuret method (Stear et al., TP3869, Randox Laboratories Ltd., UK). Globulin was calculated by subtracting serum albumin concentration from total serum protein concentration.

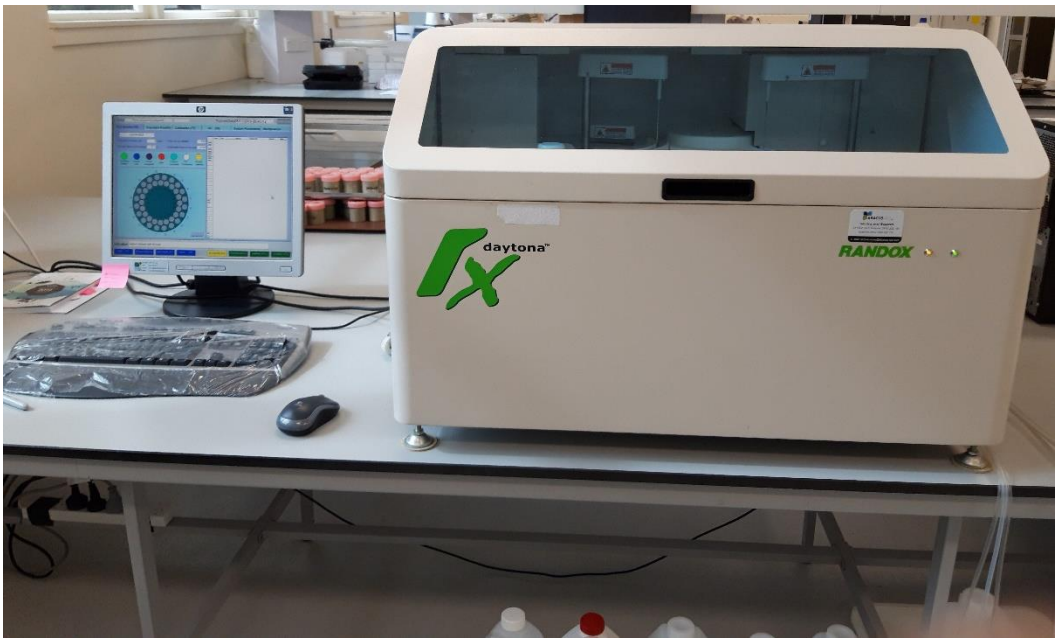


Plate 3.3 RX Daytona Analyser used for serum protein analyses.

Chapter 4

Supplementation of twin-bearing ewes on pasture to provide an epidemiological benefit for gastrointestinal parasitism

Part of this chapter has been published:
Tambunan, R. D., Logan, C. M., Bywater, A. C., & Greer, A. W. (2018). Supplementation of ewes on pasture to provide an epidemiological benefit for gastrointestinal parasitism. *New Zealand Journal of Animal Science and Production, 78*, 45-50.

4.1 Introduction

Among all sheep classes, the periparturient ewe is reported as the most significant source of pasture contamination with GIN parasite eggs (Beasley et al., 2010; Familton, 1991). During the periparturient period, ewes become more susceptible to GIN infections (Rocha et al., 2011). They experience a temporary relaxation of resistance to GIN infection, the consequence of which is an elevate in nematode eggs output in the faeces of affected ewes (Donaldson, 1998; Rocha et al., 2011). The periparturient rise (PPR) phenomenon is important because it corresponds with the availability of susceptible hosts, being the young lambs (Sebastiano et al., 2017). Since GIN is highly aggregated within the host population, susceptible hosts can lay thousands of worm eggs, which, in turn, elevates pasture contamination (McRae et al., 2015). Even though the target for anthelmintic treatments is typically the lambs, the administration of anthelmintics to adult ewes in the period immediately before or after lambing has been a common practice amongst sheep farmers in New Zealand for many years (Brunsdon et al., 1983; Lawrence et al., 2007), which may not be replicated elsewhere. Nevertheless, many researchers recognized that treating ewes at this time will accelerate the development of anthelmintic resistance (Leathwick et al., 2006; Leathwick, Vlassoff, & Barlow, 1995). Therefore, it is necessary to develop more sustainable nematode control strategies.

Several alternative methods can be used to reduce the dependence on anthelmintics for the control of GIN infection. One potential approach to control the GIN parasite and to improve ewe's immunity during the periparturient period is nutritional supplementation. Many authors reported that nutritional improvement to the periparturient ewes can provide the nutrients required to enhance an immune response against nematode parasites (Beasley et al., 2012; Houdijk, 2008; Houdijk, Kyriazakis, Jackson, et al., 2001; Houdijk et al., 2000; Kahn, Knox, Gray, et al., 2003). Donaldson et al. (1998) and Houdijk, Kyriazakis, Coop, and Jackson (2001) indicated that resistance of periparturient

ewes to GIN infection could be improved by increasing MP supply. However, the experimental design of the majority of these studies involved trickle infection with a single species of GIN larvae. Furthermore, these studies have investigated the effects of supplementation on indoor studies or while on pasture for a relatively short term. In general, there is limited data regarding the long-term effects of nutritional supplementation on grazing periparturient ewes that have a naturally acquired GIN infection where two or more parasite species are infecting ewes and lambs. Hence, this current experiment aimed to evaluate the benefits of supplementing twin-bearing ewes infected naturally by GIN on pasture to reduce the periparturient relaxation in immunity to GIN parasitism and its provision of an epidemiological benefit to grazing lambs after weaning. These studies also aimed to obtain more information on the immunological changes associated with the periparturient rise in FEC.

4.2 Materials and methods

4.2.1 Animals and treatments

This study was carried out at a summer-safe unit of LincolnSheep, Lincoln University, Canterbury, New Zealand, over two sequential years (2015 and 2016), under the authority of the Lincoln University Animal Ethics Committee (AEC) approval number 635 and 2016-25, respectively. This research unit is under irrigation but is not fully irrigated, mimicking a summer-safe environment. The 'summer safe' environment is an environment that expected to receive adequate rainfall to support pasture growth through the summer months (Smith, Mills, & Moot, 2022).

Prior to lambing in August 2015, mixed-age of twin-bearing crossbred (predominantly Coopworth) ewes (n=140) were randomly allocated to one of two farmlet treatments, *viz.*, supplemented or not, and set-stocked in each replicate paddock. The paddocks had been grazed by sheep only in previous years, ensuring the presence of a natural GIN challenge. Each treatment was replicated on two paddocks, with a total of four paddocks (29-38 ewes/paddock, based on pasture availability). Similar to the 2015 trial, in August 2016, mixed-age of twin-bearing ewes (n=128) were allocated to one of the two farmlet treatments. Each treatment was replicated on four paddocks, with a total of eight paddocks (12-20 ewes/paddock, based on pasture availability). All ewes in both years were vaccinated with 1 ml/dose of Ultravac® 5in1 with Selenium (Zoetis Inc., Auckland, New Zealand) against clostridial diseases when set stocked prior to lambing. The vaccination contained toxins for the prevention of enterotoxaemia (pulpy kidney disease), tetanus, black disease, malignant oedema (blackleg-like disease) and blackleg. Ewes remained in their farmlets across both years, allowing the cumulative of effects. The layouts of the experimental paddocks in 2015 and 2016 are shown in Figure 4.1.

Starting three weeks before lambing, twin-bearing ewes on the supplemented farmlets (n=70 for 2015 and n= 64 for 2016) were introduced to a feeder (Advantage Feeders NGF800, Advantage Feeders Ltd, New Zealand) with approximately 50 g/head/d of a commercially available high-protein sheep pellet (Triplet nuts, Farmlands stock feeds Ltd, New Zealand) and gradually increased to 500 g/head/d. All supplemented ewes were offered 500 g/head/d of pellet during the first four weeks of lactation, wherein the feeders were removed from the paddocks, and the supplementation ceased, as described in Chapter 3 (Section 3.1). The remaining ewes remained unsupplemented and were allowed to graze pasture as per usual farming practice. Ewes remained in their respective paddocks until weaning at approximately 12 weeks after the mean lambing date.

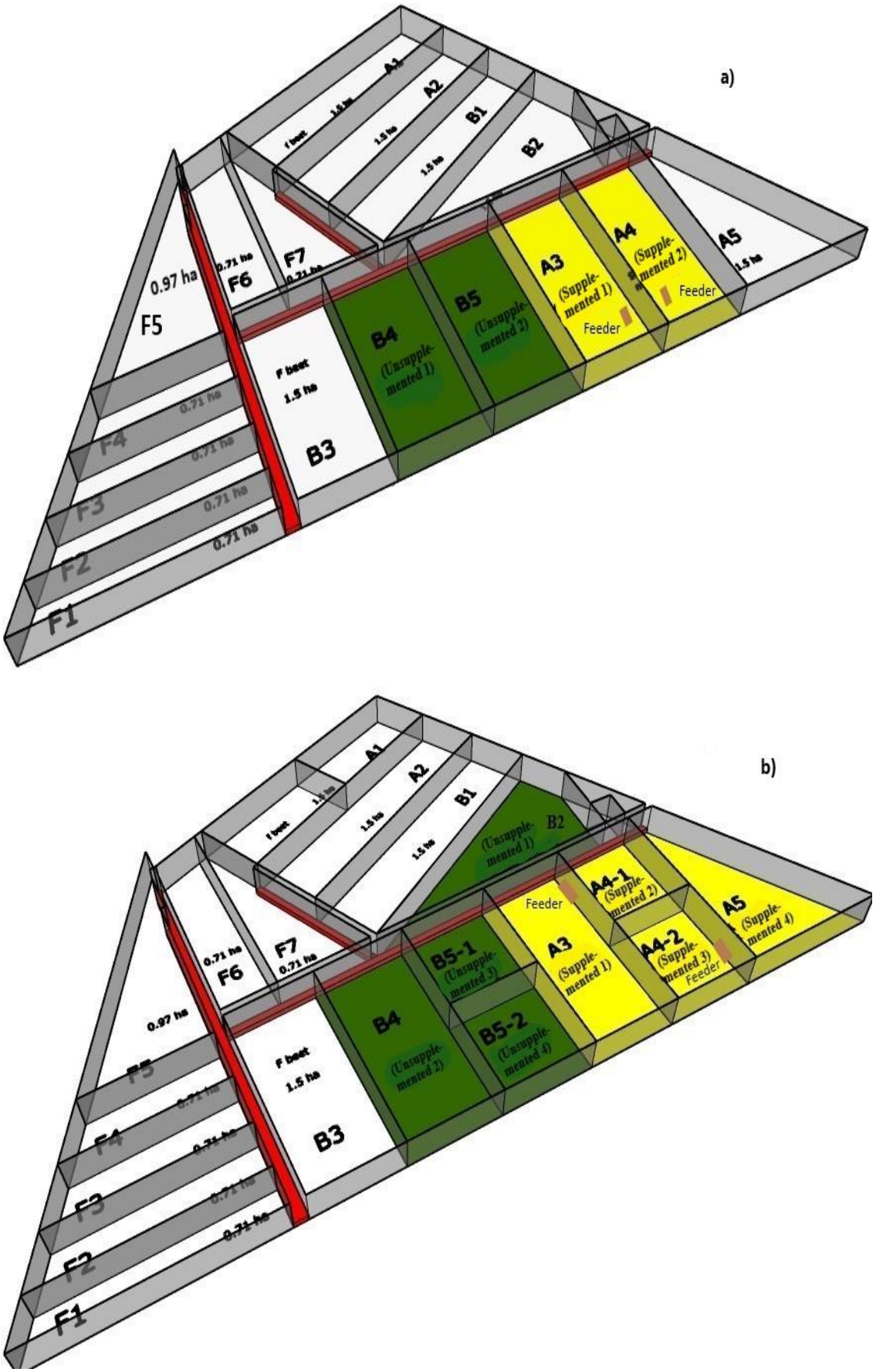


Figure 4.1 Layout of the experimental paddocks at the LincolnSheep, Lincoln University, New Zealand in a) 2015 and b) 2016.

At weaning, all lambs were drenched to remove residual parasite contamination and then exposed to a targeted selective treatment (TST) regime while grazing the areas in which ewes had been or not been supplemented to determine if any epidemiological benefit of supplementation existed. To account for any potential carry-over effect, lambs (60 lambs/replicate in 2015 and 35 lambs/replicate in 2016) originating from each treatment were stratified across the treatment area, with each replicate (n=2, for 2015 and n=3, for 2016) consisting of 50% of lambs originating from a supplemented ewes and 50% of lambs originating from unsupplemented ewes. In 2015, mean initial LW for the group of weaned lambs that grazed areas where ewes had been supplemented or remained unsupplemented, were 21.42±0.48 and 21.50±0.52 kg, respectively. In 2016, mean initial LW for the group of weaned lambs that grazed areas where ewes had been supplemented was 26.16±0.46 kg while their counterparts were 26.30±0.46 kg.

For each lamb replicate the potential for growth was assessed using sentinel lambs (n=10 in 2015 and n=6 in 2016) that were treated with 1 mg/kg LW of long-acting moxidectin injection (1 ml/20 kg LW; Cydectin, Pfizer Animal Health, Auckland, New Zealand). Selection of these was based on placement when ranked hierarchically by LW. The remaining lambs in each replicate were subjected to a TST regime where the need for anthelmintic was based on animals achieving acceptable growth rates. They were orally drenched with combination drench of 1.0 g/L abamectin, 40.0 g/L levamisole hydrochloride, 25.0 g/L albendazole (1 mL/5 kg LW Trio® Sheep, Ravensdown Ltd., Christchurch, New Zealand). In 2015, treatment thresholds were measured by the Happy Factor Model (Greer, Kenyon, et al., 2009) with the treatment threshold set to an efficiency of 0.74 (Greer, McAnulty, Logan, & Hoskin, 2010). In 2016, treatment thresholds were set at 80% of the mean growth rate of sentinel lambs as per the protocol from Sheep Improvement Limited for identifying resilient animals (Sheep Improvement Limited, 2008).

Within each treatment and replicate, lambs and ewes were rotationally grazed for the remainder of the grazing season with ewes following the lambs; therefore, they were naturally infected with GIN. The ewes were moved into a paddock on the day the lambs were moved out. To simulate on-farm conditions where lambs may be sent to slaughter, TST lambs were removed from the study once their body weight exceeded 38 kg. Sentinel animals remained on the plots throughout even if weights were above 38 kg.

After the completion of the first year, the paddocks were then grazed by the ewes for one more rotation before they were removed from the pastures and grazed on winter crops (paddocks B3 and A1, see Figure 4.1) until being set-stocked for the 2016 trial. After the completion of the 2016 study,

the same handling procedures as 2015 were given to the ewes until being set-stocked for the 2017 study (Chapter 5).

4.2.2 Animal measurements and sampling

Ewe live weight (LW) and body condition score (BCS) were monitored on all farmlets at set stocking, tailing (approximately four weeks after lambing), and every two weeks after that until weaning (lambs approximately 12 weeks of age). Animals were weighed with the use of a swing gate auto drafter (Prattely Industries Ltd, Temuka, New Zealand) fitted with an Aleis electronic tag reader and a head unit (Tru-test XR3000, Tru-test Ltd, Auckland, New Zealand) to the nearest of 0.2 kg. BCS was assessed following the palpation of the lumbar region and was undertaken by the same operator on each occasion. Scores were given using a scale of 1 to 5, with 1 being emaciated and 5 obese. BCS was then recorded into Tru-test head unit to the nearest 0.5, as a technique described by Russel, Doney, and Gunn (1969).

Lambs were weighed at each of the ewe sampling times until weaning and then weighed every two-week after weaning until they reach a slaughter weight and removed. Any individual TST lambs failing to reach their minimum target liveweight gain (LWG) was automatically drafted to one side, treated with anthelmintic and returned to graze with the remainder of the group. The frequency of anthelmintic requirement and the growth rates of lambs relative to their contemporaries receiving the long-acting anthelmintic provide measures of the epidemiological benefit provided through supplementing ewes.

4.2.3 Meteorological data

Mean rainfall and temperature during the experiments in 2015 and 2016 are presented in Figure 4.2. Total annual rainfall during the first year of the experiment (2015) was 429 mm, while during the second year (2016) was 502 mm. The total annual rainfall of both years was lower than the average long-term rainfall of the last 30 years (599 mm). This shortfall in the rain was compensated with limited irrigation (gun sprinkler). The mean air temperatures during 2015 and 2016 were 12.1 °C and 12.7 °C, respectively. The monthly air temperatures (Figure 4.2b) showed a similar trend to the long-term average air temperature.

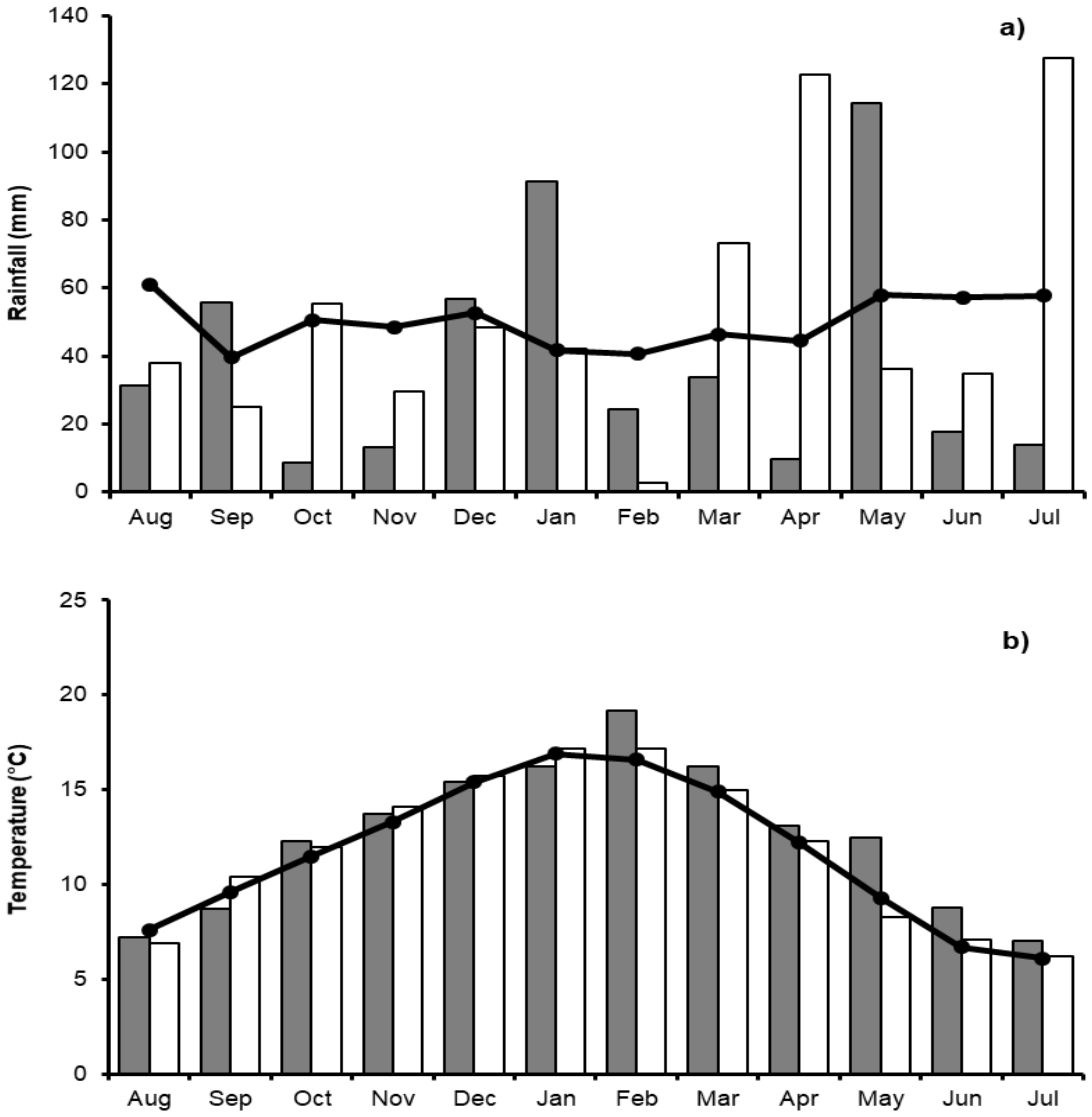


Figure 4.2 (a) Monthly mean rainfall, (b) mean air temperature for August 2015–July 2016 (closed bars) and August 2016-July 2017 (open bars), and long-term average 1981–2010 (solid line) at Lincoln, Canterbury, New Zealand. Data were taken from the National Climate Database (CliFlo) of Broadfields Meteorological Station, about 1 km from the research site.

4.2.4 Herbage chemical composition

Herbage sample was collected from each paddock at the same time as pasture larval sampling time at approximately 0900 h. The samples were immediately frozen before being freeze-dried. The dried sample was ground through a 1 mm sieve and scanned by near infra-red spectrophotometer (NIRS, NIRSystems 5000, Foss, Maryland, USA) using previous derived calibrations for perennial ryegrass-white clover to determine CP, water soluble carbohydrate (WSC), digestible organic matter (DOMD), acid detergent fibre (ADF) and neutral-detergent fibre (NDF). Metabolisable energy (ME) was calculated as MJ ME/kg DM = 0.16 × DOMD (Commonwealth Scientific and Industrial Research Organisation, 2007).

4.2.5 Parasitology

Parasite epidemiology was assessed through the use of pasture larval, which were collected every two weeks from set-stocking. Pasture grab samples were obtained using a W-shape pattern from each paddock for the measurement of pasture parasite larval concentration fortnightly from set-stocking, and L3 larvae were recovered from the pasture using a modified Baermann technique (Ministry of Agriculture Fisheries and Food, 1986). L3 larvae present were then counted and morphologically differentiated from free-living larvae under a microscope, as described in Chapter 3 (Section 3.5).

Faecal samples were collected from all ewes at set stocking, tailing, and every two-week thereafter until weaning to determine the extent of parasitic infection. Faecal samples of lambs were collected from all sentinel lambs and a sub-sample of TST lambs at weaning and every two weeks after weaning until TST lambs reach slaughter weight and were removed from the property. Ten samples (2015) and six samples (2016) per rectum were randomly collected from TST lambs within each replication that did and did not receive anthelmintic treatment and from all sentinel lambs. Faecal eggs were counted using the modified McMaster method (Ministry of Agriculture Fisheries and Food, 1986) with a sensitivity of 100 epg, as described in Chapter 3 (Section 3.6).

In the 2016 trial, after being weighed faecal samples for FEC, leftover faeces from each group were subsampled for coprocultures study to determine any effect on egg viability. Larvae recovered from the faeces were collected using the modified Baermann technique by the Ministry of Agriculture Fisheries and Food (1986). Faecal samples from each ewe in the same group replicate were weighed and pooled in a plastic beaker. These faecal samples were mixed with vermiculite to obtain the correct consistency. Before incubation, these faeces mixture was moistened with tap water then covered with a plastic bag. The coproculture samples were incubated at 25 °C for 10-14 days and moistened as necessary. After incubation, lukewarm water was added to the culture until the beaker was full. The lid of the beaker then covered with tissue paper and turned upside down into the Baermann apparatus, which was allowed to stand for a minimum of 36 hours, during which time the larvae will have migrated through the paper tissue into the bottom of the funnel. As much as 100 ml of the fluid was withdrawn from the funnel and stored in glass bottles at 4 °C until examination. Before the examination, the fluid was reduced in volume to 20 ml by siphoning. Larvae present in four 0.1 ml samples were counted and differentiated. Calculation of total larvae/g faeces = (10 x number of larvae counted)/weight of faeces.

Developmental success (%) (Jørgensen et al., 1998) = $\frac{\text{total number of L3 in sample}}{\text{mean FEC (epg) X faeces weight (g)}}$ X 100

At weaning, two lambs from each replicate (total 16 lambs) from the 2016 trial were randomly selected as tracer lambs. All tracer lambs were slaughtered at weaning for the assessment of worm burden. The slaughter and worm burden collection followed the procedures described in Chapter 3 (Section 3.7).

4.2.6 Serum analysis

Blood samples from all ewes in 2016 trial were taken at set stocking, tailing, and every two weeks thereafter until weaning by jugular venipuncture into 10 ml vacutainer tubes (Becton Dickinson, Rutherford, New Jersey, USA) and were immediately placed at 4 °C for 24 hours and then processed into serum, as described in Chapter 3 (Section 3.3).

Serum samples were analysed for IgA antibody specific to *T. colubriformis* L3 and IgG antibody specific to *T. colubriformis* L3 using an indirect ELISA, as desribed in Chapter 3 (Section 3.10), and for serum albumin, serum total protein, serum urea, and serum phosphorus using an RX Daytona Analyser, as described in Chapter 3 (Section 3.11).

4.3 Statistical analysis

The LW and BCS of ewes, FEC, FEC distribution, developmental success, pasture larval counts, herbage chemical composition, and serum analysis data were subjected to sequential comparison of ante-dependence structures for repeated measures before being analysed by the Restricted Maximum Likelihood (REML) using GenStat (16th Ed., VSN International Ltd, UK) with treatment groups (supplemented and unsupplemented) and time included as factors. Other parameters were analysed using one-way analysis of variance (ANOVA) by Minitab (16Th Ed., Minitab Inc., USA). Worm burden, developmental success, and pasture larval counts were log-transformed as log10 (count+1) while FEC was log-transformed as log10 (count+100) before analysis to obtain a normal distribution and presented as back-transformed means. Due to the weight of conceptus at the start of lambing, change in ewe LW from four weeks after lambing only was assessed. The number of drench and LWG/drench of TST lambs after weaning were analysed using ANOVA by Minitab (16Th Ed., Minitab Inc., USA). The LW and LWG of sentinel and TST lambs after weaning, and the cumulative percentage of sentinel and TST lambs that reached slaughter weight above 38 kg were analysed using the REML using GenStat (18th Ed., VSN International Ltd, UK). Where the F-test for treatment was significant ($P \leq 0.05$), treatments were compared with the Least Significant Differences test with a significance value of 5%. All values are group means and expressed as mean ± SEM unless otherwise specified.

4.4 Results

4.4.1 Pasture mass

The mean pasture mass for supplemented and unsupplemented paddocks grazed by the ewes and lambs throughout the trial in 2015 (paddocks A3 and A4 for supplemented; paddocks B4 and B5 for unsupplemented, see Figure 4.1a) and in 2016 (paddocks A3, A4 and A5 for supplemented; paddocks B2, B4 and B5 for unsupplemented, see Figure 4.1b) are presented in Figure 4.3. Pasture mass did not differ between the group at any sampling period. Climatic conditions were not favourable for pasture growth during 2015 (Figure 4.2), resulting in low pasture availability with average pasture mass declining to less than 700 kg DM/ha in all paddocks. Limited irrigation to overcome the shortage of rainfall was insufficient to support the growth of pasture.

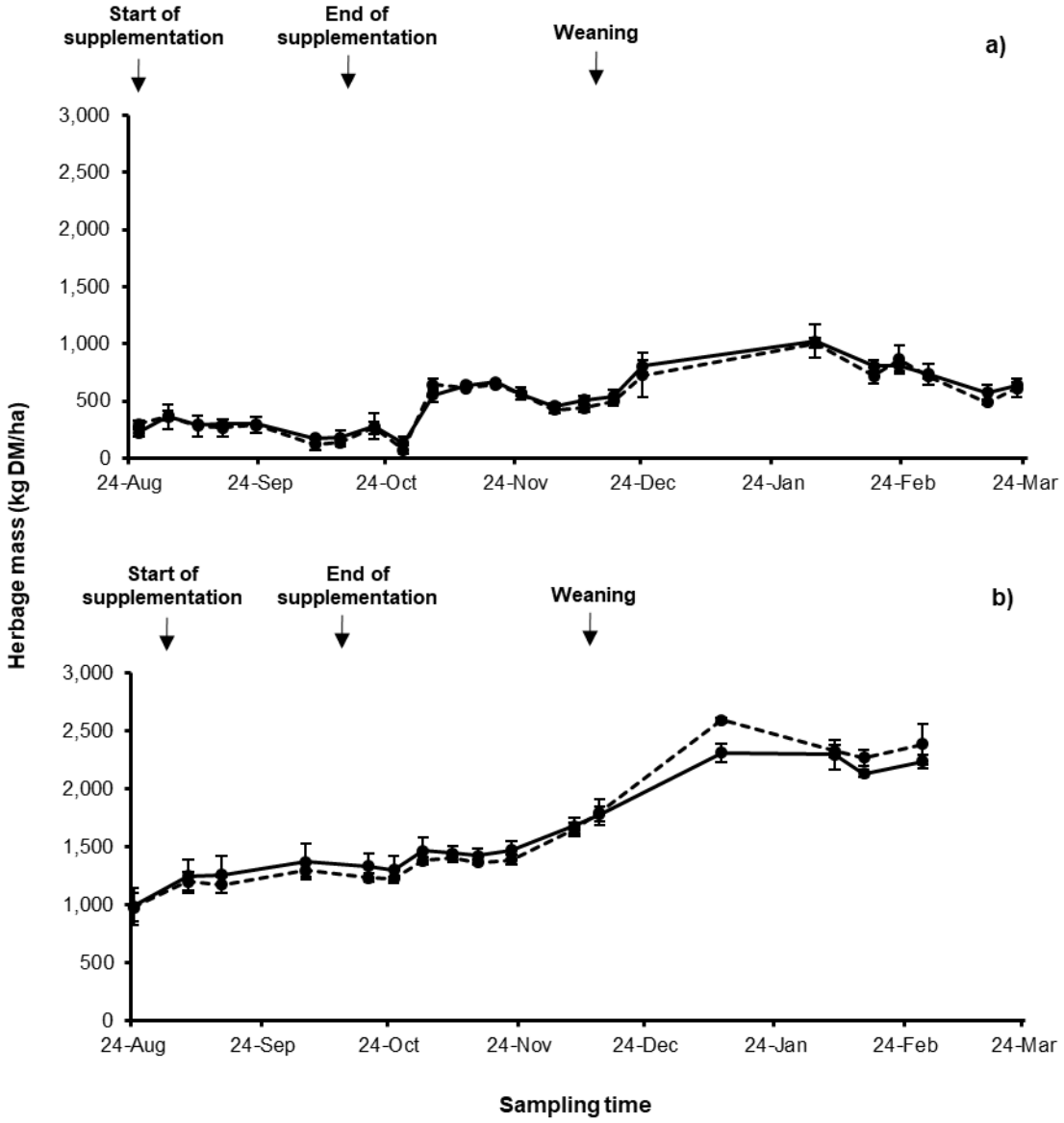


Figure 4.3 Mean pasture mass (kg DM/ha) for supplemented (solid line) and unsupplemented (dashed line) paddocks grazed by the ewes and lambs throughout the trial in a) 2015 and b) 2016.

4.4.2 Herbage chemical composition

The mean chemical composition and predicted metabolisable energy (ME) of fresh perennial ryegrass-white clover from all treatment paddocks throughout 2016 study are presented in Table 4.1. Overall, on both ewe and weaned lamb trials, chemical compositions and ME of fresh pastures from supplemented paddocks were similar with fresh pastures of unsupplemented paddocks (P>0.05 for all).

Table 4.1 The average chemical composition (% of DM) and predicted metabolisable energy (ME MJ/kg DM) determined by NIRS of fresh perennial ryegrass-white clover harvested in the morning throughout 2016 study.

Components	Ewe trial			Weaned lamb trial		
	Supplemented paddocks	Unsupplemented paddocks	P value	Supplemented paddocks	Unsupplemented paddocks	P value
ADF	22.38±0.68	22.16±0.67	0.68	27.28±0.40	28.02±0.58	0.11
WSC	20.38±0.89	19.74±0.87	0.53	22.50±1.22	22.07±1.56	0.79
DOMD	78.51±0.63	78.19±0.62	0.97	71.19±0.83	69.51±1.05	0.07
NDF	41.62±1.16	41.26±1.15	0.74	49.89±0.71	51.29±0.85	0.10
OM	91.54±0.20	91.50±0.20	0.99	93.02±0.19	93.55±0.32	0.10
CP	19.38±0.75	19.74±0.73	0.46	13.44±0.71	13.36±0.87	0.94
ME	12.56±0.12	12.51±0.06	0.97	11.39±0.13	11.12±0.17	0.07

4.4.3 Faecal egg counts (FEC)

There were no differences in parasitic load between farmlets and animals at the beginning of the study. Mean FEC of ewes and lambs in both years are given in Figure 4.4. For ewes in 2015, a significant supplementation by time interaction was detected (P=0.04), reflecting similar FEC at the start of lactation, which increased in unsupplemented, but not supplemented ewes in weeks six and eight although they were not different at weaning (week 12). In 2016 similar reductions in ewe FEC were observed; however, the decline continued until weaning. For lambs after weaning, no treatment effects were observed (P>0.05 for all) in FEC in both years.

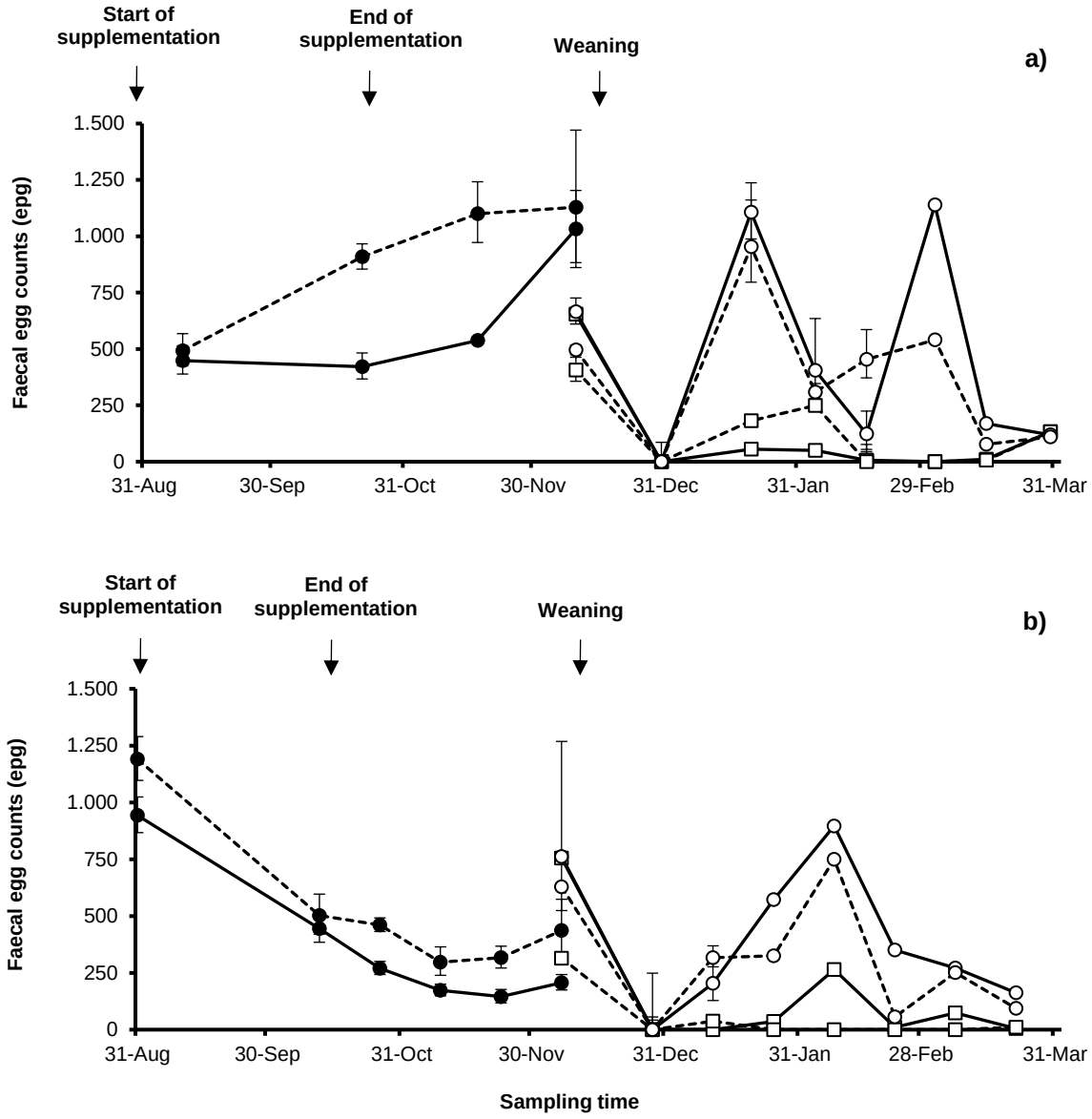


Figure 4.4 Back-transformed (log10 (count + 100)) means of faecal egg count (epg) for ewes (closed symbols) that were supplemented during the first four weeks of lactation (solid line) or not (dashed line) and for lambs (open symbols) that were suppressively drench (squares) or exposed to a targeted selective treatment anthelmintic regime (circles) that subsequently grazed areas where ewes had been supplemented (solid line) or unsupplemented (dashed line) in a) 2015 and b) 2016.

The distribution of FEC of supplemented ewes and their counterparts in 2015 and 2016 are shown in Figure 4.5 and Figure 4.6, respectively. Overall, in the 2015 trial, distributions of ewe FEC were not affected by supplementation (P>0.05 for all ranges), except for the range of >2,000 epg (P=0.03), reflecting a greater percentage of ewes that had FEC >2,000 epg in unsupplemented ewes than their supplemented counterparts. There were time effects on the range of 0-200 epg (P=0.01), 200-400 epg (P=0.01), 400-600 epg (P=0.04), and >2,000 epg (P<0.001). On 10 December 2015 (weaning), the percentage of ewes with FEC < 200 epg decreased in both groups (from around 90% at early lactation to about 40% at weaning) while the rate of ewes with FEC >2,000 epg increased to almost 9% and 14% in supplemented and unsupplemented groups, respectively. There was an interaction between treatment and time (P=0.04) at the range of 1,800-2,000 epg, reflected by a slight increase of the percentage of the ewe that had FEC 1,800-2,000 epg of the unsupplemented group over the time. Ewe FEC was affected by BCS (P<0.001). Supplementation and BCS (P<0.001) and supplementation x sampling time x BCS interactions (P=0.04) were detected on ewe FEC (Figure 4.7a).

In the 2016 trial, effects of supplementation on percentage of ewes with FEC 0-200 epg (P<0.001), 400-600 epg (P=0.01), 800-1,000 epg (P=0.01), and 1,200-1,400 epg (P=0.02) were detected, reflecting around 50% greater percentage of supplemented ewes that had FEC 0-200 epg and around 50% lower percentage of ewes that had FEC >200 epg than those of unsupplemented cohort. Time effects were detected in most of the ranges, except for the range of 400-600 epg (P=0.78), 600-800 epg (P=0.26), 800-1,000 epg (P=0.57), and 1,800-2,000 epg (P=0.84). However, no interactions between treatment and time were observed (P>0.05 for all) on FEC distribution. Additionally, ewe FEC was affected by BCS (P<0.001). No supplementation x BCS nor supplementation x sampling time x BCS interactions were observed (P>0.05 for all) on ewe FEC (Figure 4.7b).

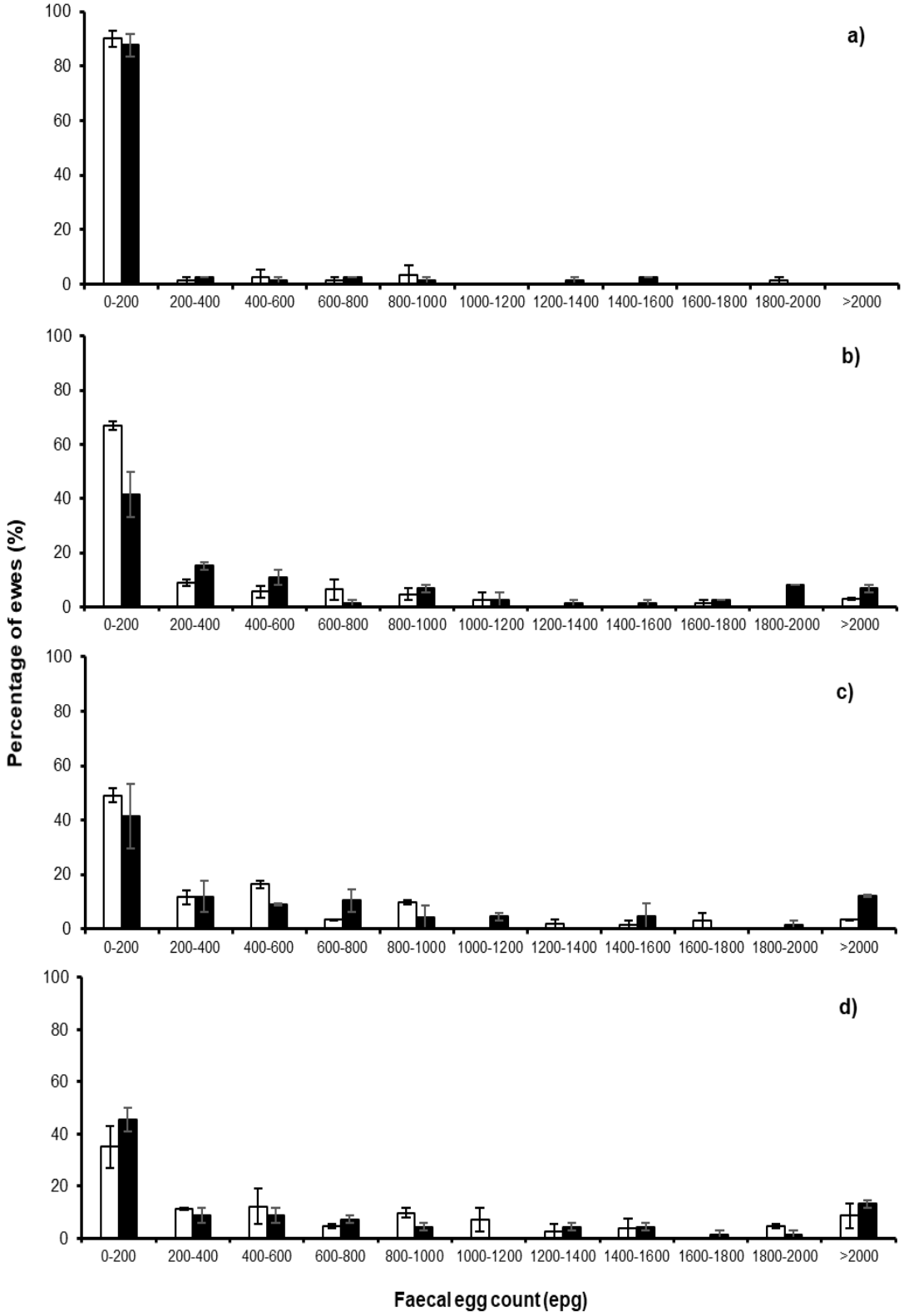


Figure 4.5 The distributions of faecal egg counts (epg) of supplemented ewes (open bars) and unsupplemented ewes (closed bars) at sampling time on (a) 9 September 2015, (b) 20 October 2015, (c) 17 November 2015, and (d) 10 December 2015.

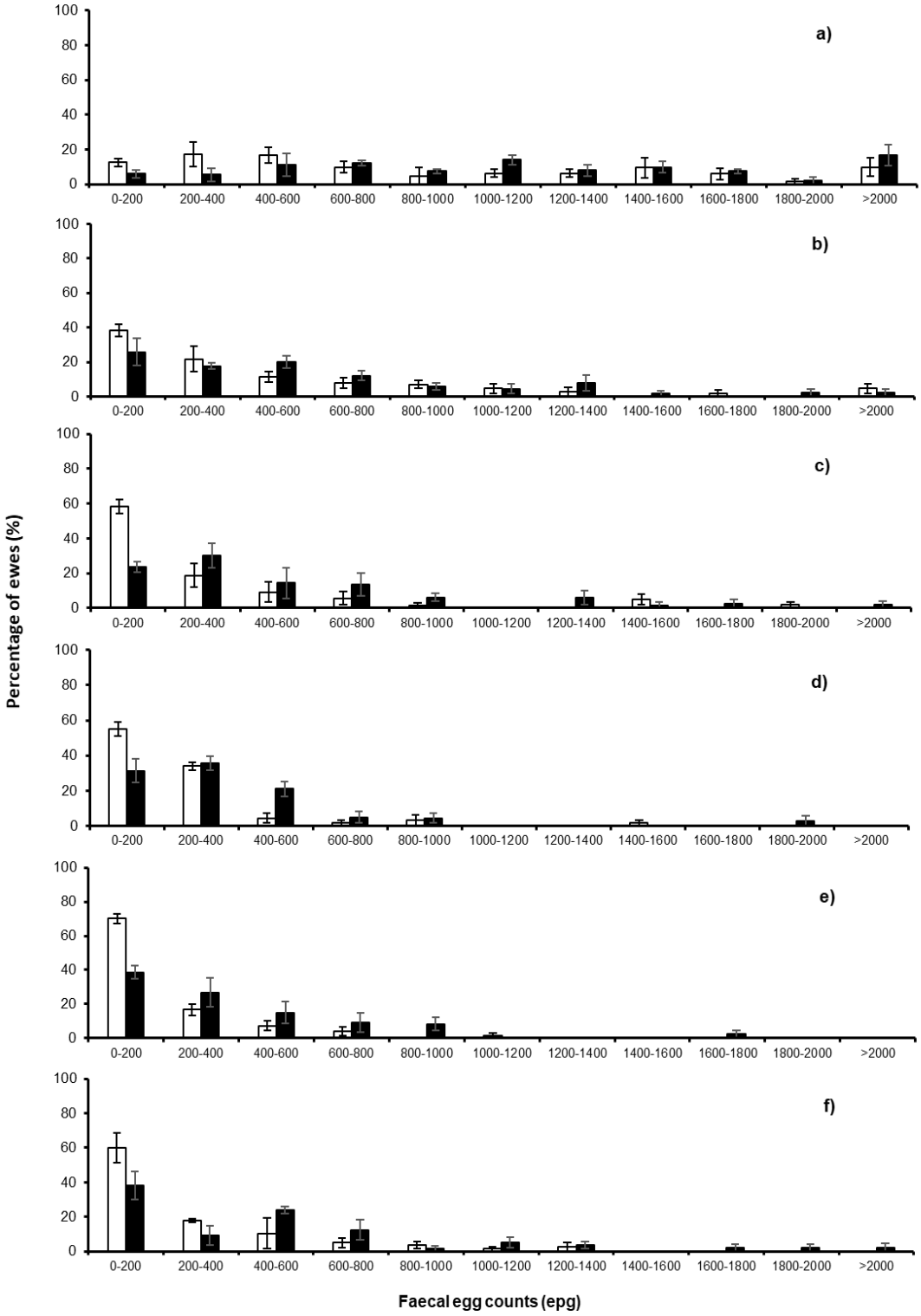


Figure 4.6 The distribution of faecal egg counts (epg) of supplemented ewes (open bars) and unsupplemented ewes (closed bars) at sampling time on (a) 31 August 2016, (b) 12 October 2016, (c) 26 October 2016, (d) 9 November 2016, (e) 23 November 2016, and (f) 7 December 2016.

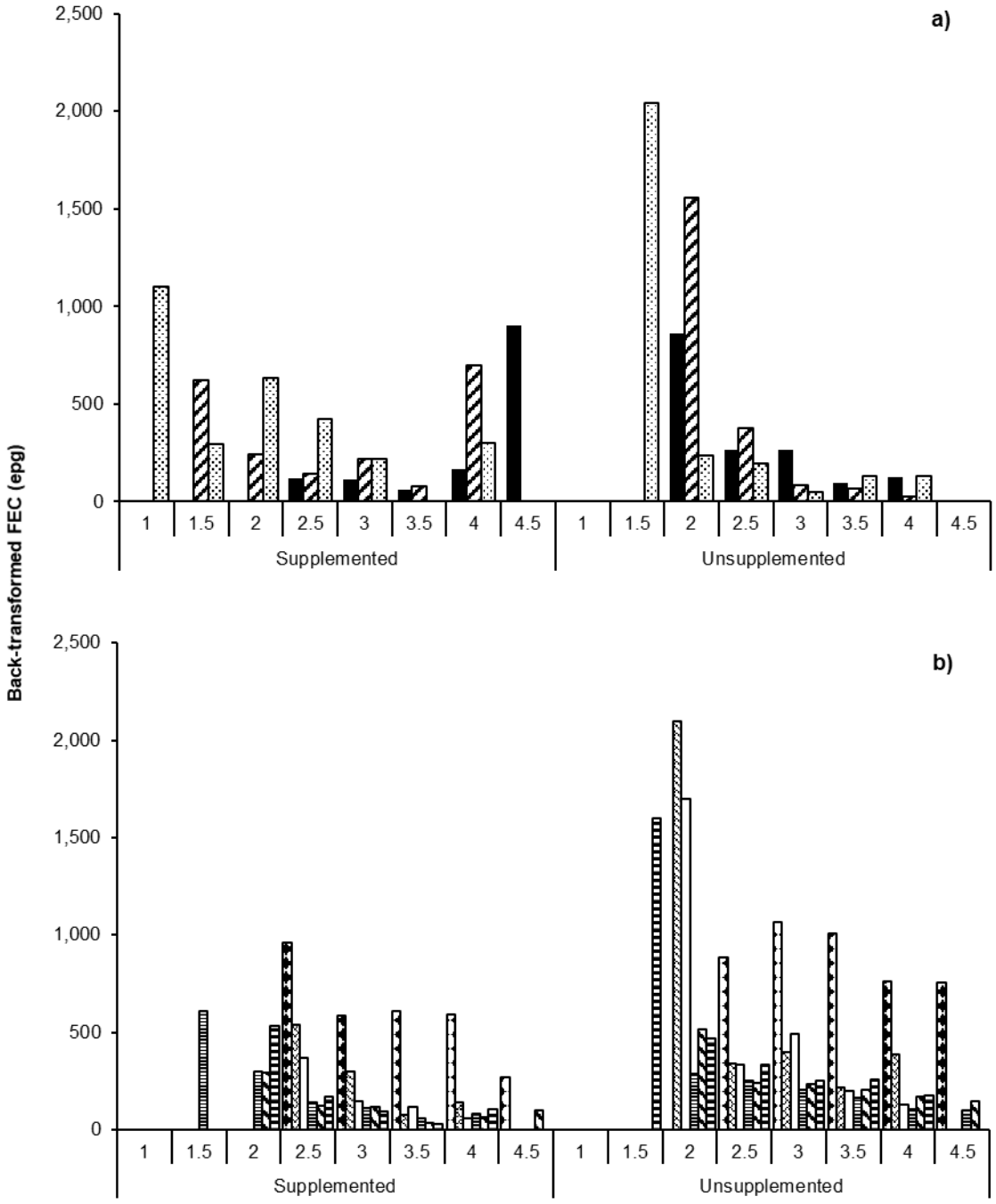


Figure 4.7 The effect of supplementation, BCS, and sampling time on ewe FEC in a) 2015 at 21 October 2015 (■), 17 November 2015 (▨), and 10 December 2015 (▩) and b) 2016 at 31 August 2016 (▩), 12 October 2016 (▩), 26 October 2016 (□), 9 November 2016 (▤), 23 November 2016 (▨), and 7 December 2016 (▤).

4.4.4 Developmental success (viability) of worm eggs to L3 larvae

Changes in the geometric mean of the percentage of eggs develop to L3 larvae (developmental success) in faecal cultures of supplemented ewes or unsupplemented ewes in 2016 are presented in Figure 4.8. Overall, there was no difference between the developmental success of worm eggs of supplemented ewes and unsupplemented ewes (P=0.53). However, interaction between treatment and time (P=0.02) and effect of time (P=0.04) were detected, reflected in a gradual increase in the developmental success of unsupplemented ewes over time. From the faecal culture of supplemented ewes were identified three species of GIN, which were *Trichostrongylus* spp. (76.50%), *Teladorsagia circumcincta* (18.65%), and *Cooperia* spp. (4.85%) and from the faecal culture of unsupplemented ewes were identified *Trichostrongylus* spp. (78.86%), *Teladorsagia circumcincta* (17.22%), and *Cooperia* spp. (3.91%).

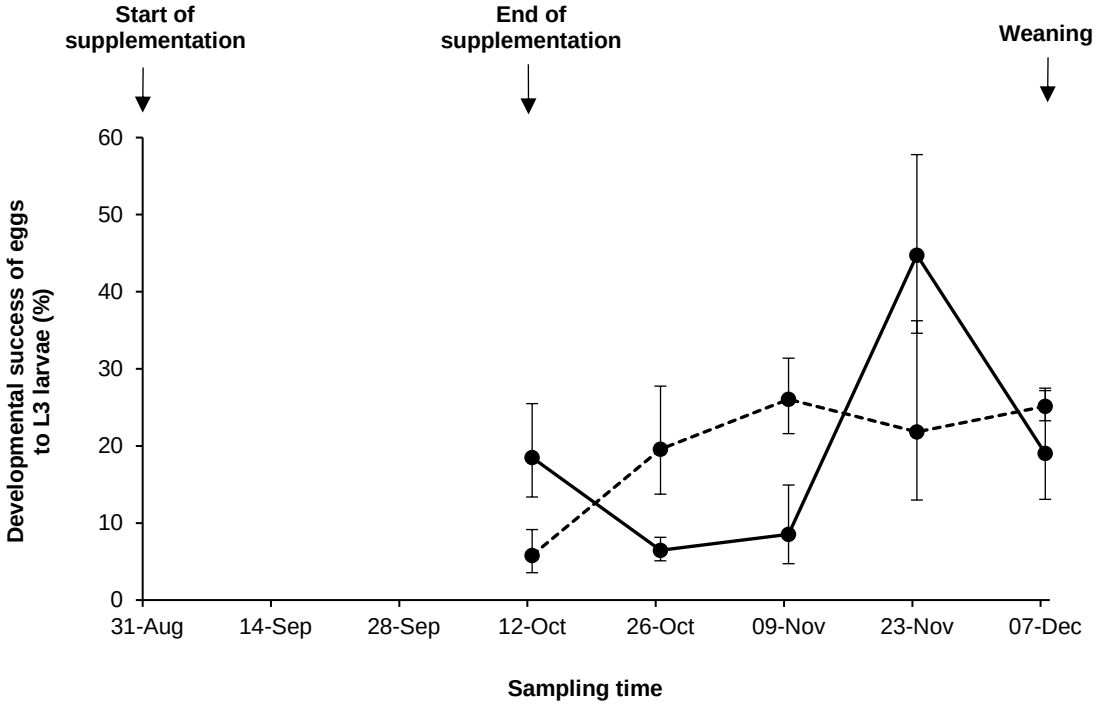


Figure 4.8 Changes in mean back-transformed (log10 (count + 1) of developmental success to L3 larvae (%) of eggs cultured from supplemented ewes (solid line) or unsupplemented ewes (dashed line) faeces in 2016.

4.4.5 L3 contamination on pasture

The mean number of L3 larvae of strongyle nematodes *(Nematodirus* spp*.* and other strongyles) present on pastures grazed by ewes and lambs before and after weaning in 2015 and 2016 are shown in Figure 4.9. Overall, no treatment differences (P>0.05 for all) nor supplementation by time interactions (P>0.05 for all) were observed in the number of L3 recovered/kg DM on herbage grazed by the ewes and the lambs before and after weaning in both years.

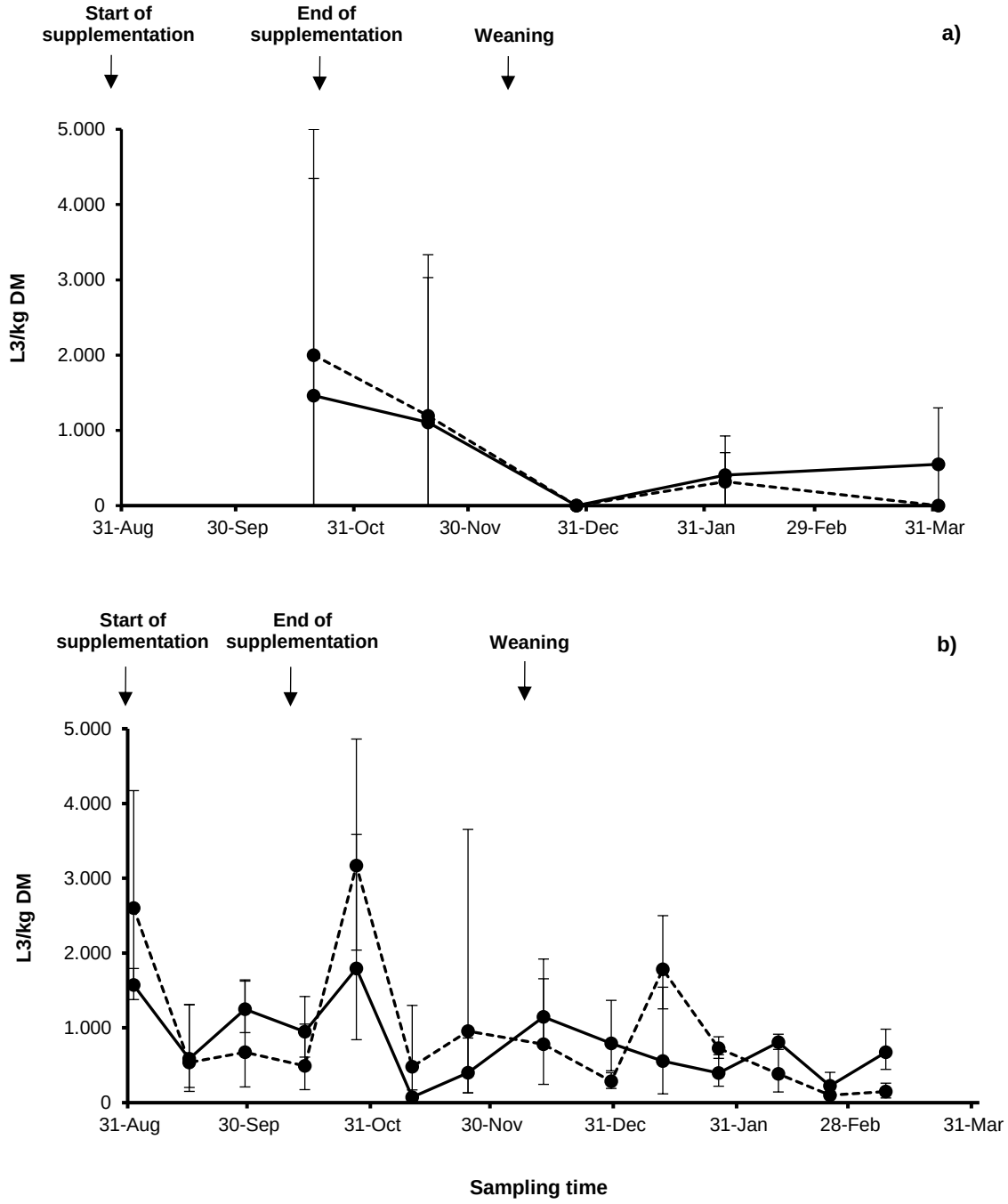


Figure 4.9 Mean back-transformed (log10 (count + 1) of the number of L3 larvae of strongyle nematodes per kg DM present in paddocks grazed by the ewes and the lambs where ewes had been supplemented (solid line) or remained unsupplemented (dashed line) in a) 2015 and b) 2016.

4.4.6 Worm burdens at slaughter

Back-transformed geometric means worm burdens at slaughter from abomasum and small intestine of weaned lambs that subsequently grazed areas where ewes had been supplemented or unsupplemented in 2016, are shown in Table 4.2 and Table 4.3. Overall, no significant effect of supplementation was observed on the number of worms recovered from the abomasum or small intestine of the weaned lambs (P>0.05 for all). Back-transformed mean of total worms in the abomasum of weaned lambs that subsequently grazed areas where ewes had been supplemented, was 8,149, consisting of 78.51% *T. circumcincta* and 21.49% *T. axei* while mean total worms for weaned lambs that subsequently grazed areas where ewes remained unsupplemented was 8,049, consisting of 83.07% *T. circumcincta* and 16.93% *T. axei*.

Table 4.2 Mean number of worms (log10 (count + 1)) recovered from abomasum of weaned lambs that subsequently grazed areas where ewes had been supplemented or unsupplemented in 2016. Back-transformed values are given in parenthesis.

Worm genera		Supplemented	Unsupplemented	P value
T. circumcincta	L3	0.82±0.51 (6)	1.02±0.59 (10)	0.81
	L4	2.76±0.29 (580)	2.88±0.04 (755)	0.71
	Adult worm	3.75±0.11 (5,581)	3.77±0.12 (5,835)	0.91
	Total	3.81±0.13 (6,398)	3.83±0.11 (6,686)	0.91
T. axei	L3	0.51±0.51 (2)	0	0.36
	L4	2.75±0.27 (558)	2.47±0.23 (295)	0.47
	Adult worm	2.92±0.32 (832)	2.94±0.07 (880)	0.94
	Total	3.17±0.27 (1,488)	3.12±0.06 (1,328)	0.86
Total worm	L3	0.89±0.57 (7)	1.02±0.59 (10)	0.86
	L4	3.06±0.28 (1,139)	3.05±0.09 (1,127)	0.99
	Adult worm	3.83±0.14 (6,723)	3.83±0.10 (6,810)	0.98
	Total	3.91±0.16 (8,149)	3.91±0.10 (8,049)	0.98

Weaned lambs that subsequently grazed areas where ewes had been supplemented had lower worm burden in the small intestine than those grazed in unsupplemented areas, even though the difference was not significant (P>0.05). Back-transformed means of total worms recovered from weaned lambs of supplemented areas was 6,847, consisting of 50.85% *Trichostrongylus* spp. and 49.15% *Nematodirus* spp. while the mean for lambs that subsequently grazed areas where ewes had been unsupplemented, was 7,020, consisting of 77.34% *Nematodirus* spp. and 22.66% *Trichostrongylus* spp.

Table 4.3 Mean number of worms (log10 (count + 1)) recovered from the small intestine of weaned lambs that subsequently grazed areas where ewes had been supplemented or unsupplemented in 2016. Back-transformed values are given in parenthesis.

Worm genera		Supplemented	Unsupplemented	P value
Nematodirus spp.	L3	0	0.995±0.58 (9)	0.14
	L4	2.19±0.17 (153)	2.60±0.13 (395)	0.11
	Adult worm	3.49±0.07 (3,089)	3.65±0.13 (4,466)	0.31
	Total	3.52±0.07 (3,288)	3.70±0.11 (5,011)	0.22
Trichostrongylus spp.	L3	1.66±0.30 (45)	1.66±0.28 (44)	0.98
	L4	2.83±0.11 (681)	2.65±0.26 (444)	0.53
	Adult worm	3.42±0.13 (2,599)	3.00±0.13 (1,008)	0.06
	Total	3.54±0.10 (3,482)	3.20±0.15 (1,591)	0.10
Total worm	L3	1.66±0.30 (45)	2.03±0.10 (107)	0.29
	L4	2.96±0.06 (911)	3.02±0.08 (1,046)	0.55
	Adult worm	3.76±0.09 (5,753)	3.76±0.10 (5,753)	0.97
	Total	3.84±0.08 (6,847)	3.85±0.09 (7,020)	0.93

4.4.7 Serum analysis

4.4.7.1 IgA

Changes in mean optical density of L3 *T. colubriformis*-specific IgA in the serum of ewes that were supplemented or not supplemented in 2016 are shown in Figure 4.10. There was a significant difference in L3 *T. colubriformis*-specific IgA absorbance in serum between the treatments (P=0.05), IgA levels of supplemented ewes was lower than their counterparts. Moreover, a significant effect of time was detected (P<0.001), reflected by a gradual increase of IgA level from the start of supplementation, peaking on week 6 of lactation (26 October 2016). The values then decreased steadily until weaning. However, there was no evidence of treatment and time interaction (P=0.21).

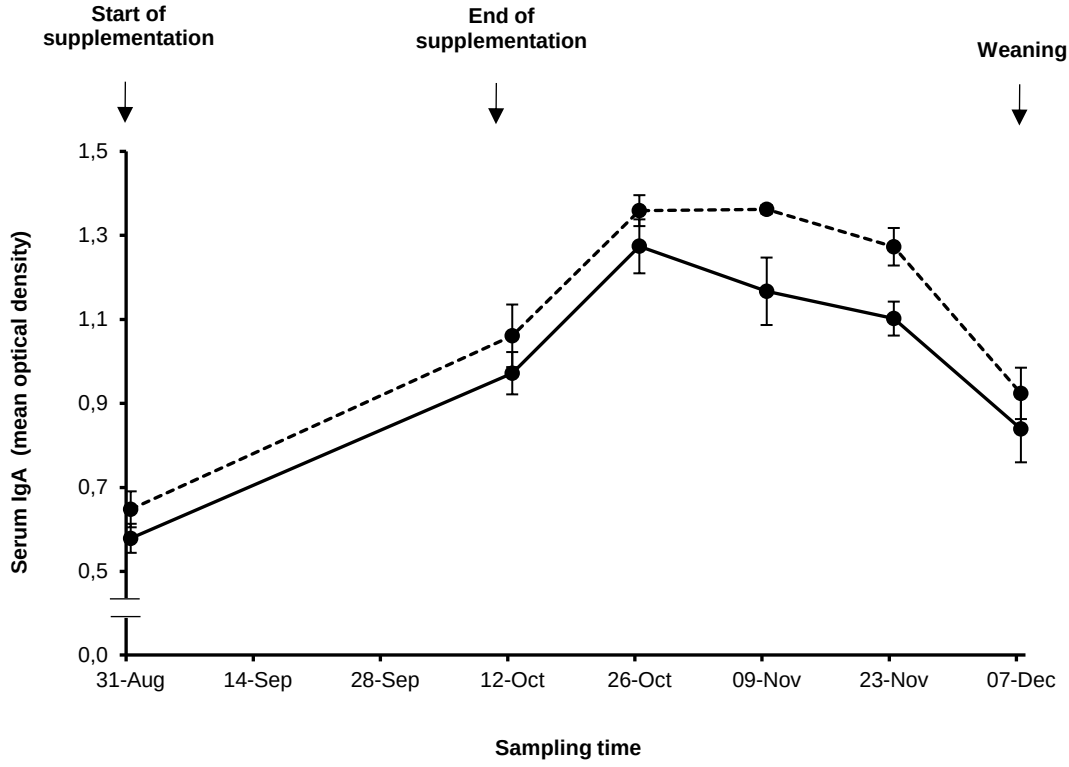


Figure 4.10 Mean optical density changes for L3 *T. colubriformis*-specific IgA antibody of serum of ewes that were supplemented during the first four weeks of lactation (solid line) or not (dashed line) in 2016.

4.4.7.2 IgG

Mean serum L3 *T. colubriformis*-specific IgG profiles for ewes that were supplemented or unsupplemented in 2016 are given in Figure 4.11. No significant supplementation effect (P=0.69) nor treatment and time interaction (P=0.71) were observed on IgG concentrations in serum of both groups. However, a significant time effect (P<0.001) was detected, reflecting an increase in mean absorbance for L3 *T. colubriformis*-specific IgG in the serum of ewes from both groups over time.

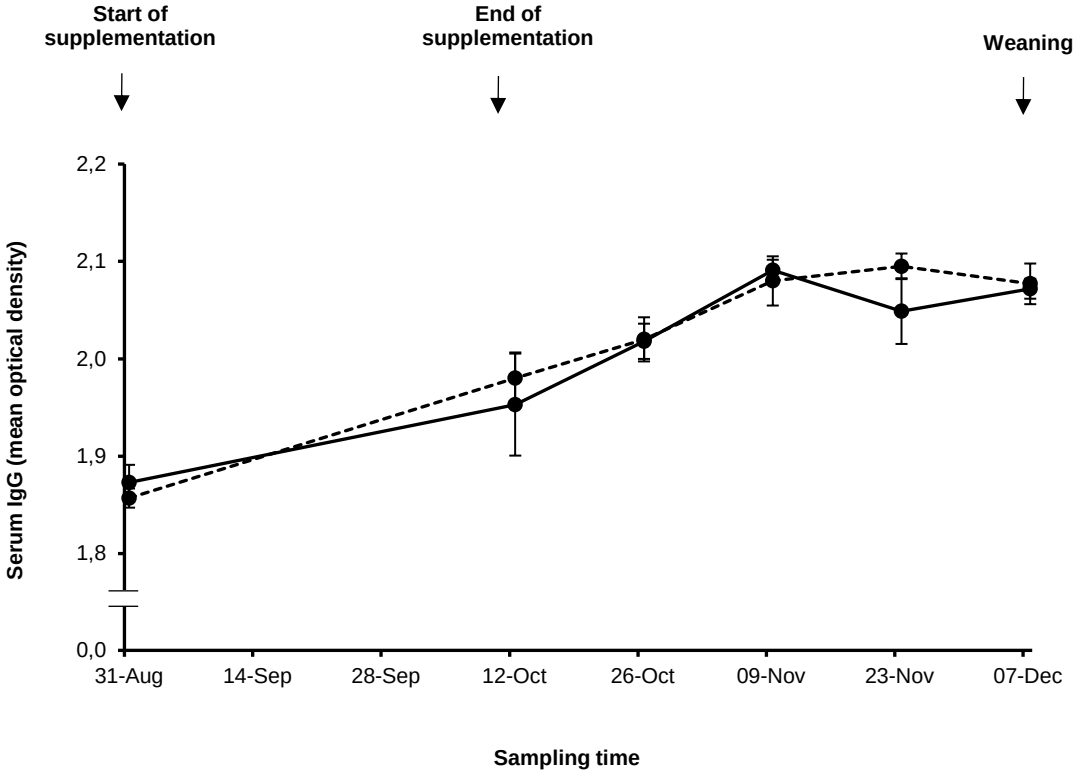


Figure 4.11 Mean optical density for L3 *T. colubriformis*-specific IgG antibody of serum of ewes that were supplemented during the first four weeks of lactation (solid line) or not (dashed line) in 2016.

4.4.7.3 Serum albumin concentration

Changes in serum albumin concentration of ewes that were supplemented during their first four weeks of lactation and those that remained unsupplemented in 2016 are shown in Figure 4.12. Overall, serum albumin concentrations were not influenced by the supplementation (P=0.40), and no significant supplementation by time interaction was observed (P=0.14). However, a significant effect of time was detected (P<0.001), reflecting an increase in serum albumin concentration over lactation.

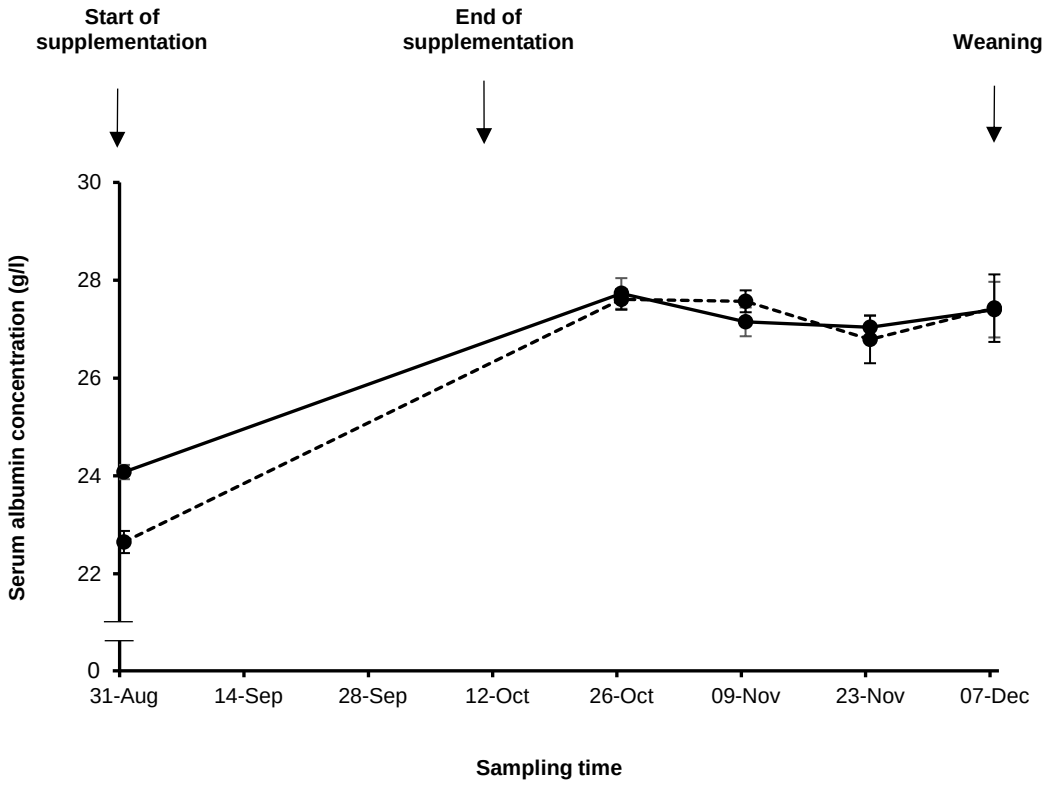


Figure 4.12 Mean serum albumin concentrations (g/l) of ewes that were supplemented during the first four weeks of lactation (solid line) or not (dashed line) in 2016.

4.4.7.4 Total serum protein concentration

Changes in total serum protein concentrations in 2016 are presented in Figure 4.13. Total serum protein concentrations were not affected by supplementation (P=0.09), mean values being 65.83±1.33 g/l for the serum of supplemented ewes, and 64.45±1.66 g/l for the serum of the unsupplemented ewes. Nevertheless, there was a significant treatment and time interaction (P=0.03), reflecting the higher rise in total serum protein concentrations from the set stock day (start of supplementation) until six weeks of lactation in both groups but being greater in supplemented ewes than their unsupplemented counterparts.

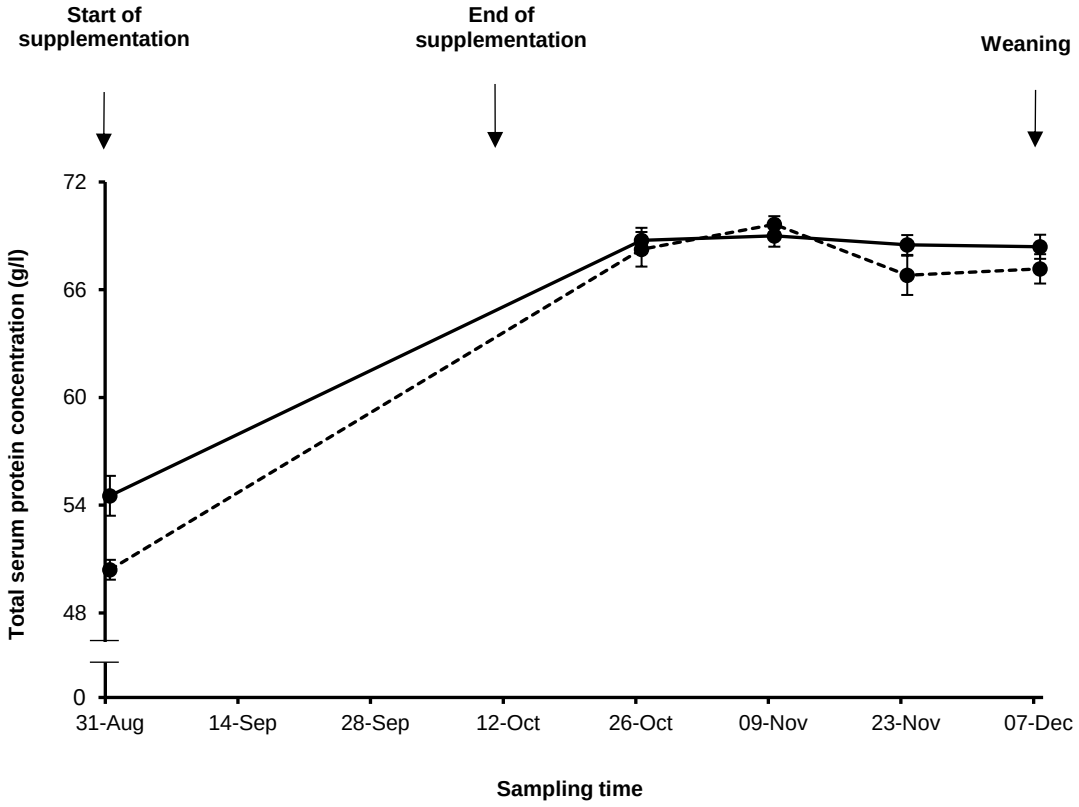


Figure 4.13 Mean total serum protein concentrations (g/l) of ewes that were supplemented during the first four weeks of lactation (solid line) or not (dashed line) in 2016.

4.4.7.5 Serum globulin concentration

Mean serum globulin concentrations of supplemented ewes and unsupplemented ewes in 2016 are shown in Figure 4.14. Serum globulin concentrations were not influenced by the supplementation (P=0.18). No evidence of treatment and time interaction (P=0.07) was observed. However, a significant time effect was detected (P<0.001), reflected by increased in serum globulin concentration over the lactation period.

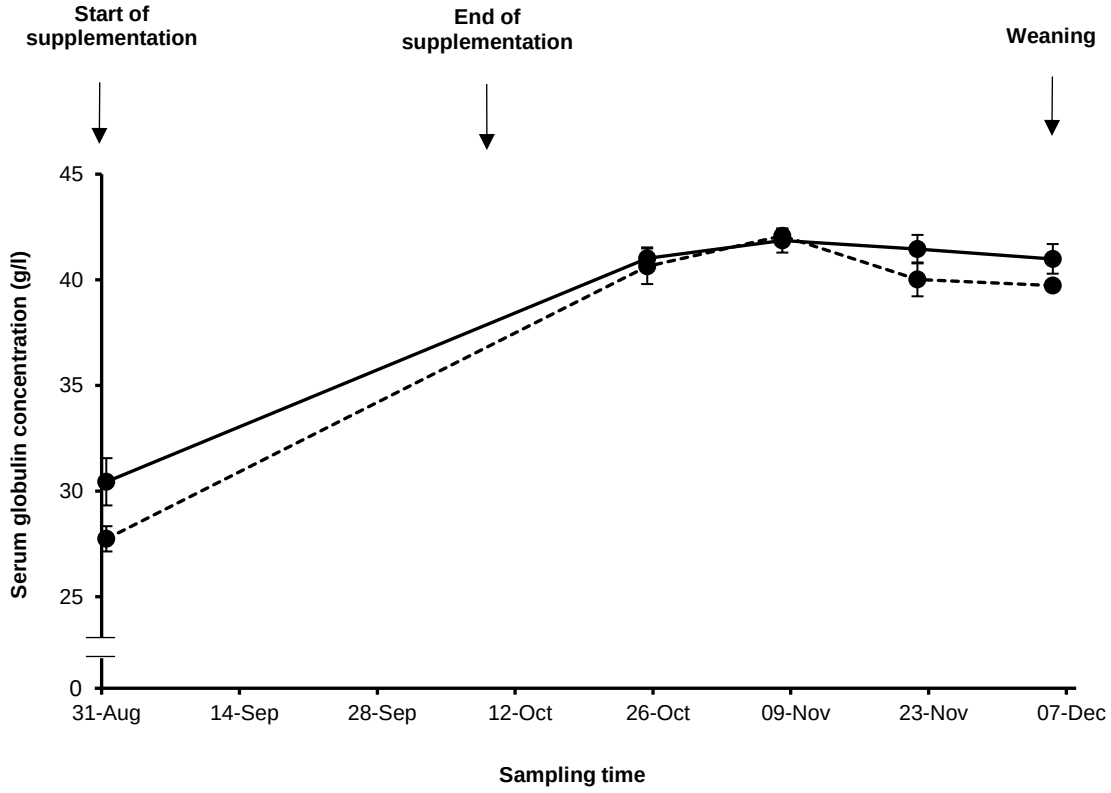


Figure 4.14 Mean serum globulin concentrations (g/l) of ewes that were supplemented during the first four weeks of lactation (solid line) or not (dashed line) in 2016.

4.4.7.6 Serum urea concentration

Figure 4.15 shows changes in serum urea concentrations of supplemented and unsupplemented ewes in 2016. The concentrations of serum urea were not influenced by ewes' supplementation during the first four weeks of lactation (P=0.19); the values range from 2.48 mmol/l to 10.29 mmol/l. Moreover, no significant interaction between treatment and time was detected (P=0.74). Nonetheless, there was a significant time effect (P<0.001), reflected by increase of serum urea concentrations over time, from set stock day (start of supplementation) until week six of lactation (26 October 2016), decreased slightly in week eight (9 November 2016) and reached its peak in week 10 then they declined again in week 12 (weaning).

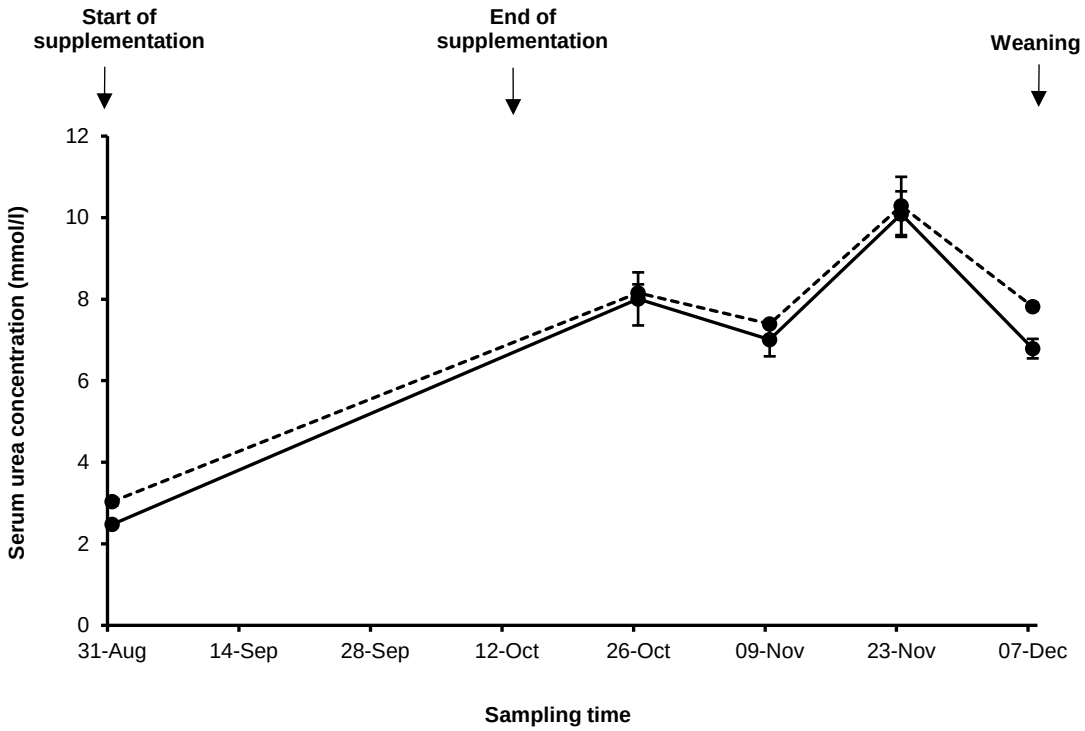


Figure 4.15 Mean serum urea concentrations (mmol/l) of ewes that were supplemented during the first four weeks of lactation (solid line) or not (dashed line) in 2016.

4.4.7.7 Serum phosphorus concentration

Changes in serum phosphorus concentrations for supplemented and unsupplemented ewes in 2016 are shown in Figure 4.16. Overall, the levels of serum phosphorus of supplemented ewes were similar to the levels of serum phosphorus of the unsupplemented cohort (P=0.39). No interaction between treatment and time was detected (P=0.99) but there was an effect of time (P<0.001), reflecting an increased level of serum phosphorus in both groups. The values were increased gradually from the start of supplementation until week six of lactation (26 October 2016), the concentrations then declined in week eight of lactation (9 November 2016) and again increased steadily until weaning.

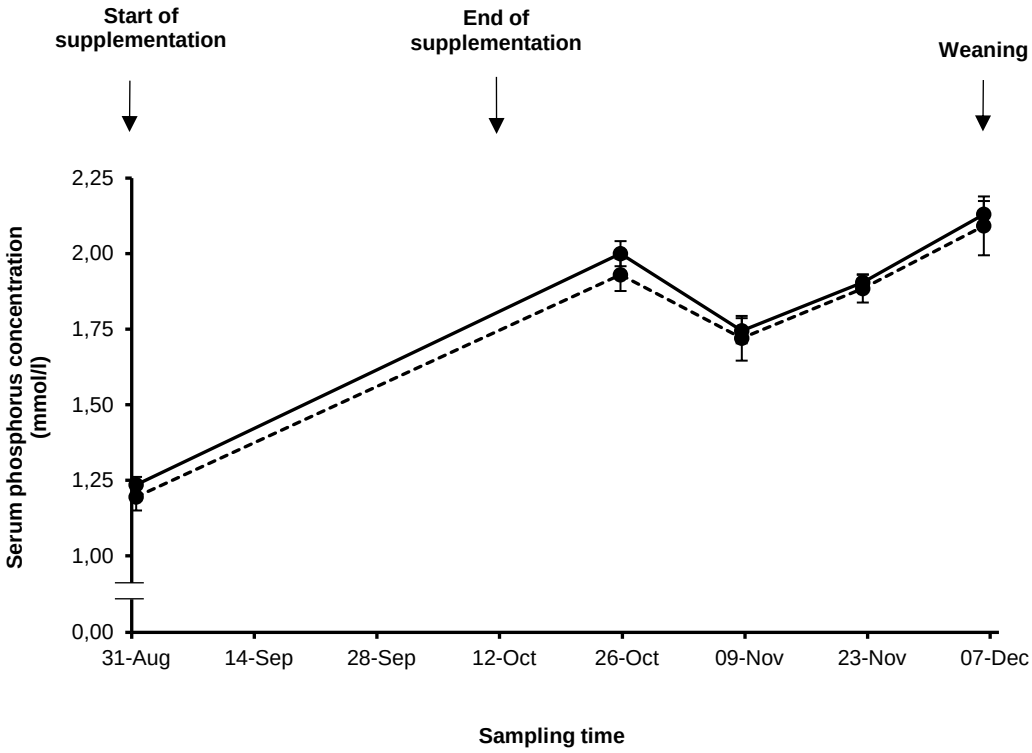


Figure 4.16 Mean serum phosphorus concentrations (mmol/l) of ewes that were supplemented during the first four weeks of lactation (solid line) or not (dashed line) in 2016.

4.4.8 Ewes and lambs performance throughout lactation

4.4.8.1 Live weight (LW) of ewes

Changes in mean ewe LW of supplemented and unsupplemented groups in both years are presented in Figure 4.17. Overall, supplementation did not affect ewe LW in both years (P>0.05 for all). However, there was a treatment and time interaction (P=0.02) on mean LW of ewes in 2015. There were effects of time on mean LW of ewes in both years (P<0.001 for all), reflected by declines in mean ewe LW in both years over the period.

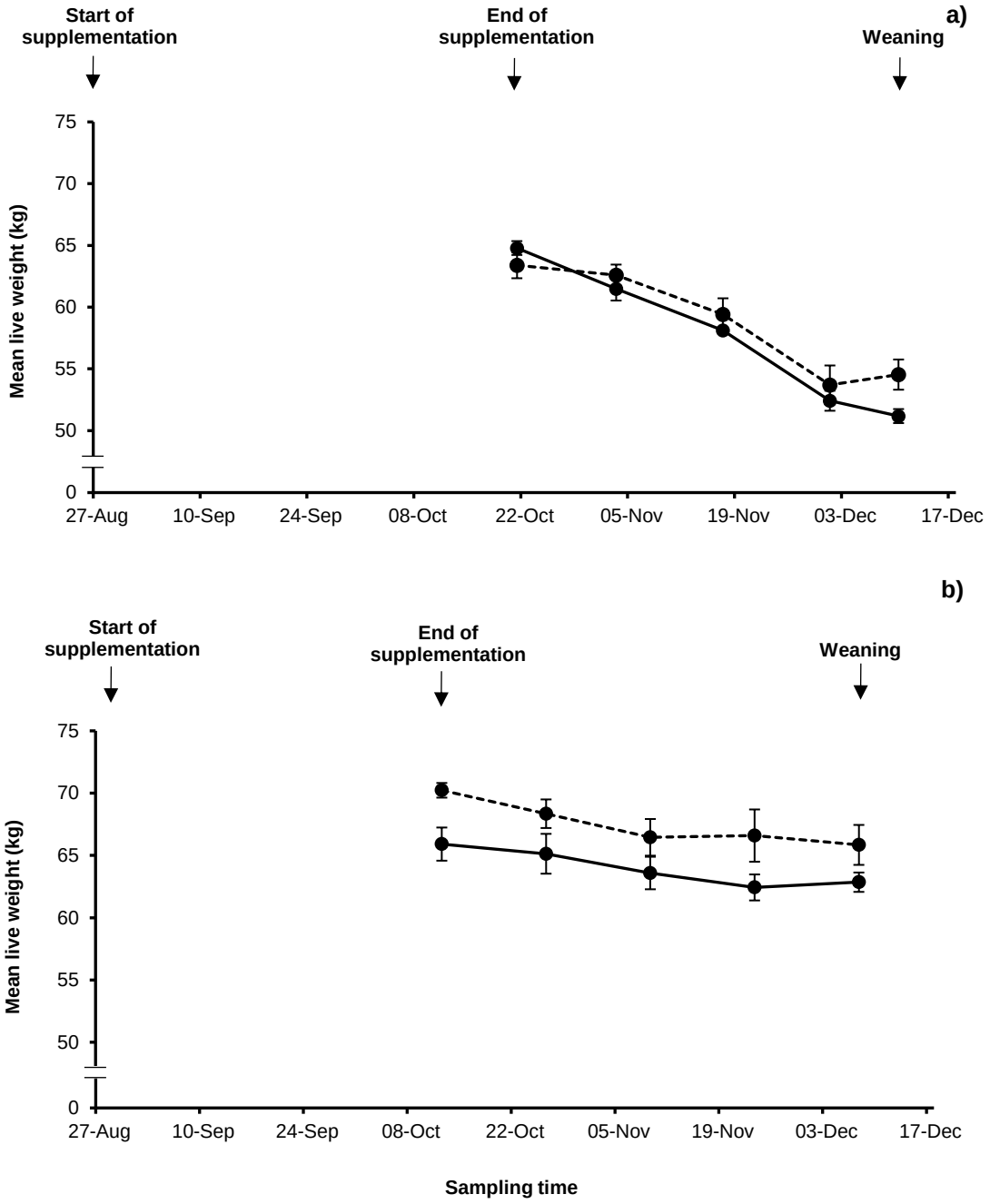


Figure 4.17 Changes in mean live weight of ewes that were supplemented (solid line) or unsupplemented (dashed line) during the lactation period in a) 2015 and b) 2016.

4.4.8.2 Body condition score (BCS) of ewes

Mean BCS of supplemented and unsupplemented ewes in both years are given in Figure 4.18. Supplementation also did not affect ewe BCS (P>0.05 for all), and no treatment and time interactions were detected (P>0.05 for all) in both years. However, there were effects of time on mean BCS of ewes in both years (P<0.05 for all), reflecting a steady decrease of mean BCS of both groups in 2015 and a slight decline in 2016 over time.

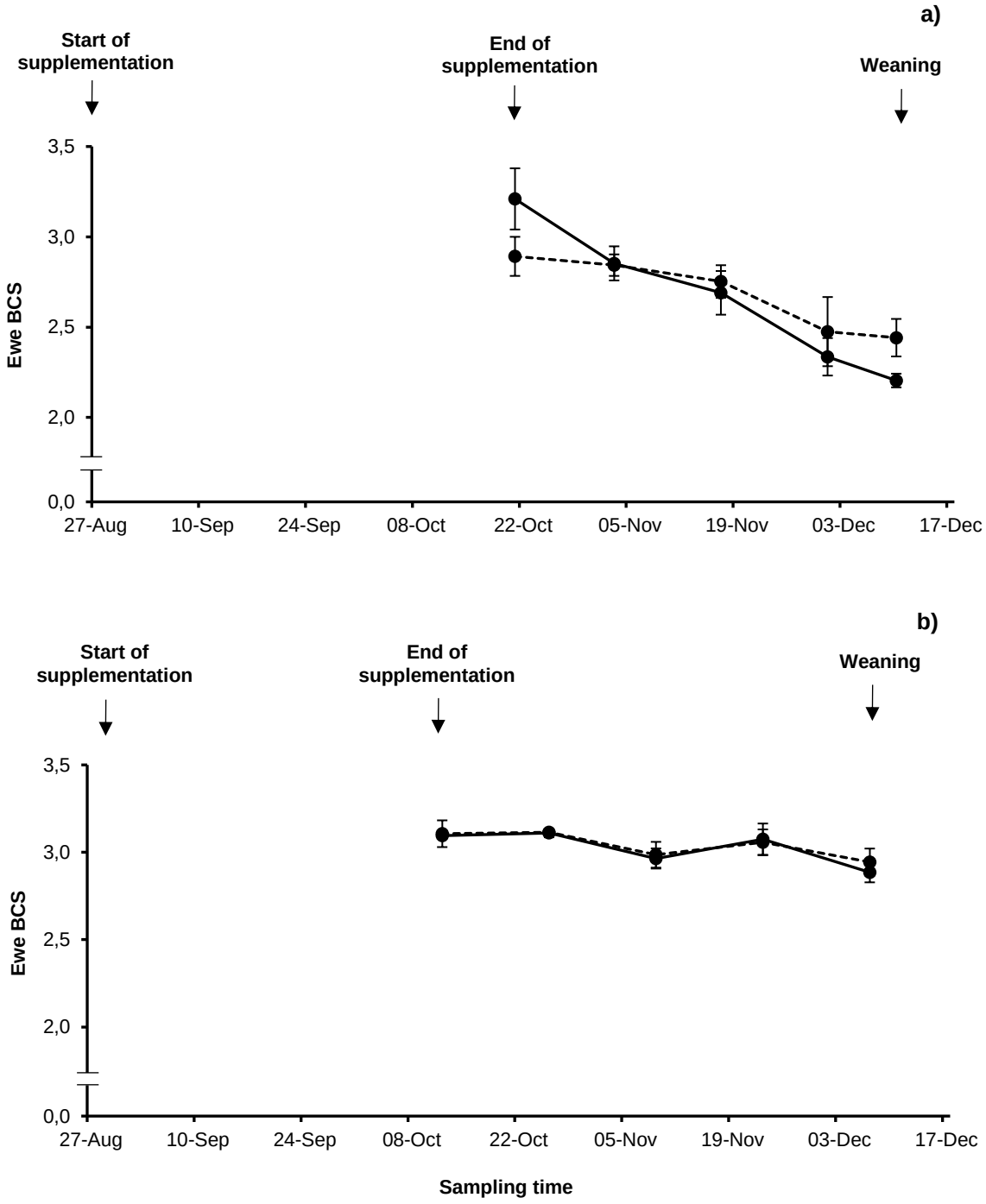


Figure 4.18 Changes in mean BCS of ewes that were supplemented (solid line) or unsupplemented (dashed line) during the lactation period in a) 2015 and b) 2016.

4.4.8.3 Live weight (LW) of lamb

The changes in mean LW of lamb throughout the lactation period in both years are presented in Figure 4.19. No treatment difference was observed between mean LW of lambs reared by supplemented ewes and unsupplemented ewes until weaning in 2015 (P=0.27). On the other hand, in 2016, lambs reared by unsupplemented ewes were significantly heavier than those raised by supplemented ewes (P=0.02). However, no treatment and time interactions were detected in both years (P>0.05 for all). Additionally, significant time effects were detected in both years (P<0.001 for all), reflected by gradual increases in mean lamb LW over time.

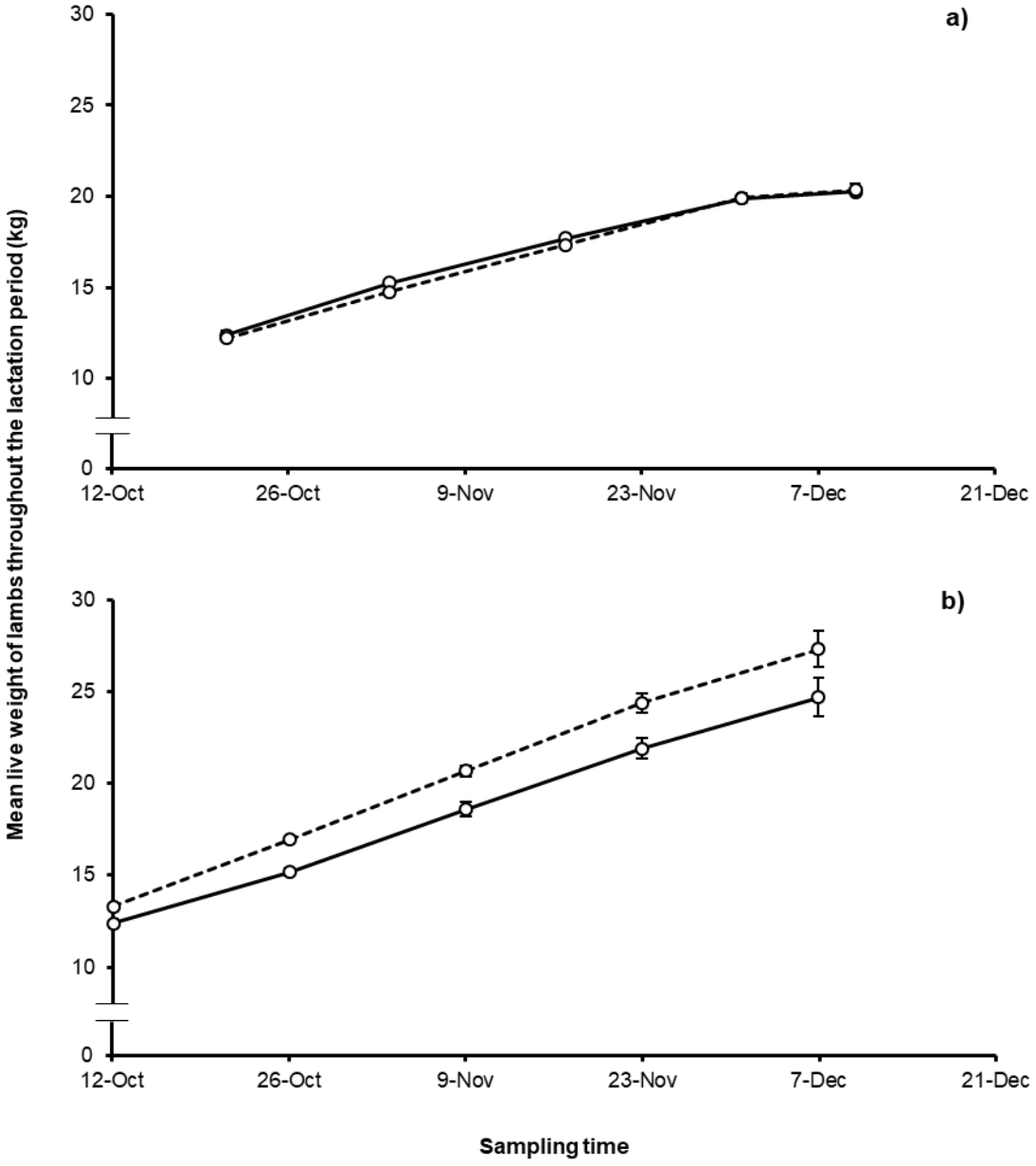


Figure 4.19 Mean LW changes of lambs reared by supplemented ewes (solid line) or unsupplemented ewes (dashed line) during the lactation period in 2015 and b) 2016.

4.4.8.4 Average daily gain (ADG) of lamb

The changes in ADG of lambs until weaning from supplemented and unsupplemented groups in both years are shown in Figure 4.20. There were no differences (P>0.05 for all) nor treatment x time interactions (P>0.05 for all) in mean ADG of lambs raised by supplemented and unsupplemented ewes throughout the lactation period in both years. However, time effects (P<0.001, in 2015 and P=0.004, in 2016) were reflected by a steady decline in mean ADG of both groups throughout the lactation period in 2015 and a slight decrease in mean ADG of both groups over time in 2016. The lowest mean ADG of both groups in both years occurred around weaning.

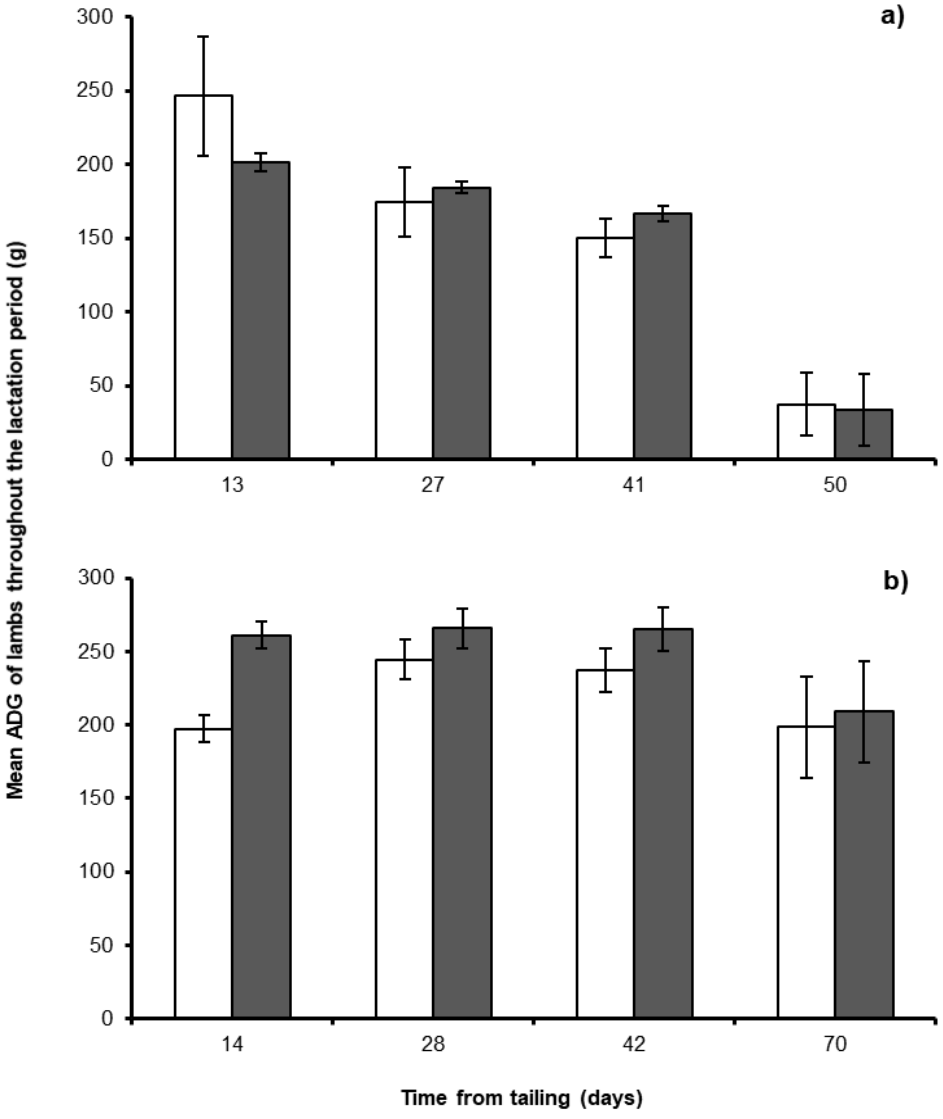


Figure 4.20 Mean average daily gain (g) of lambs reared by supplemented ewes (open bars) or unsupplemented ewes (closed bars) during the lactation period in 2015 and b) 2016.

4.4.8.5 Weight of lamb weaned per ewe

The weight of lamb weaned per ewe (WLWE) of the supplemented group and unsupplemented groups are shown in Figure 4.21. Supplementation to twin-bearing ewes did not affect the WLWE of both years (P>0.05 for all). The average value in 2015 was 32.47±1.56 and 29.98±0.89 kg for lamb reared by supplemented and unsupplemented ewes, respectively. In 2016 trial, the average value was 48.01±1.97 kg for lamb raised by supplemented ewes and 50.02±1.14 kg for lamb reared by the unsupplemented cohort.

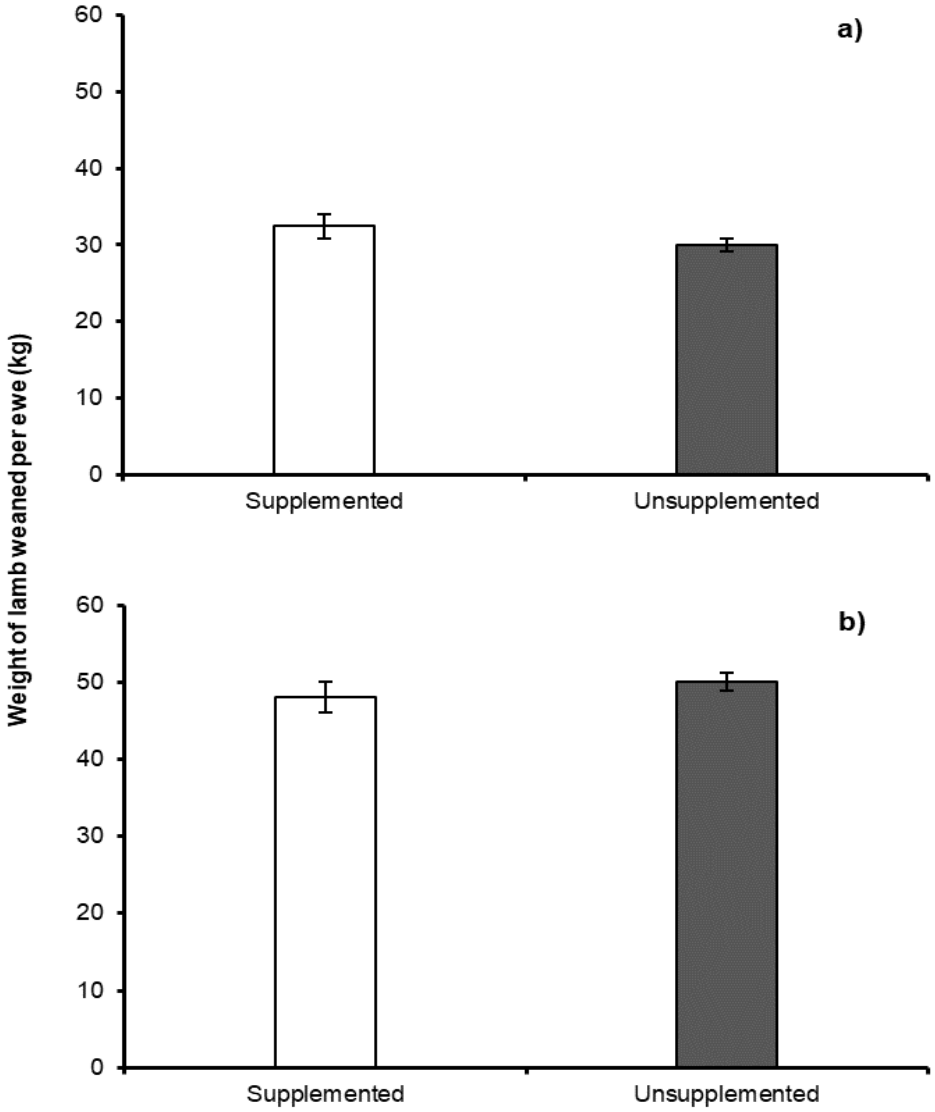


Figure 4.21 Effect of ewe supplementation on the weight of lamb weaned per ewe throughout the lactation period in a) 2015 and b) 2016.

4.4.9 Lamb performance after weaning

4.4.9.1 The numbers of drench and LWG/drench

The numbers of drench and LWG/drench of targeted selective treatment (TST) lambs that grazed areas where ewes had been supplemented or remained unsupplemented in 2015 and 2016 are shown in Table 4.4. Overall, there were no consistent differences (P>0.05 for all) on the number of drench and LWG/drench of TST lambs between the two treatment groups in all years, except for a higher number of drenches administered per lamb of TST lambs in areas where ewes had not been supplemented in 2015 (P=0.04).

Table 4.4 The effect of supplementing ewes on pasture on the number of drench and LWG/drench of TST lambs.

Parameter	Supplemented	Unsupplemented	P value
Number of drench			
2015	3.03±0.01	3.20±0.04	0.04
2016	2.22±0.06	2.35±0.12	0.40
LWG/drench (g/d)			
2015	48.44±0.83	51.61±0.88	0.12
2016	81.97±6.08	77.78±4.00	0.59

4.4.9.2 Live weight of lamb after weaning

Changes in mean LW of lambs after weaning in 2015 and 2016 are shown in Figure 4.22. There were no effects of treatment (P>0.05 for all) on LW of sentinel and TST lambs in both years but there were treatment x time interactions (P=0.01) in sentinel lambs in 2015 and TST lambs (P=0.04) in 2016. In both years, there were significant effects of time (P<0.001 for all) on LW in both sentinel and TST lambs, reflected by increase LW of all groups over time.

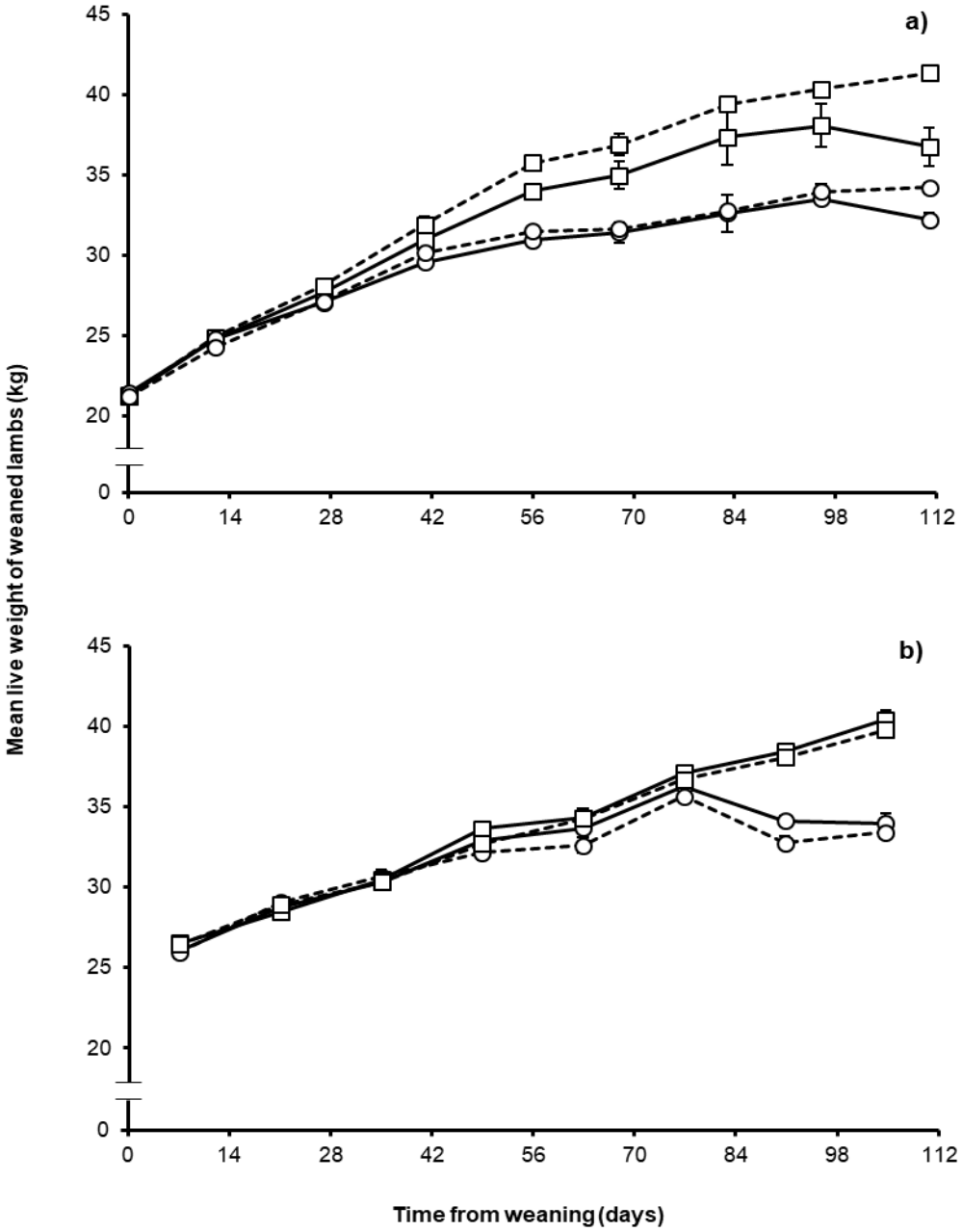


Figure 4.22 Mean live weight changes of weaned lambs that were suppressively drenched (squares) or exposed to a targeted selective treatment anthelmintic regime (circles) that subsequently grazed areas where ewes had been supplemented (solid line) or unsupplemented (dashed line) in a) 2015 and b) 2016. Sentinel lambs with LW above 38 kg remained in the plots throughout the phase of lamb finishing.

4.4.9.3 Average daily gain (ADG) of lamb after weaning

Mean ADG of sentinel and TST lambs that grazed areas where ewes had been supplemented or remained unsupplemented in 2015 and 2016 are presented in Figure 4.23. No treatment differences (P>0.05 for all) nor treatment and time interactions (P>0.05 for all) were detected in mean ADG of TST and sentinel lambs grazed areas where ewes had been supplemented and unsupplemented in both years. However, effects of time were observed (P<0.001 for both TST and sentinel lambs in 2015, P<0.001 for TST lambs and P=0.01 for sentinel lambs in 2016), reflected by a decline in mean ADG throughout the phase of lamb finishing in both years.

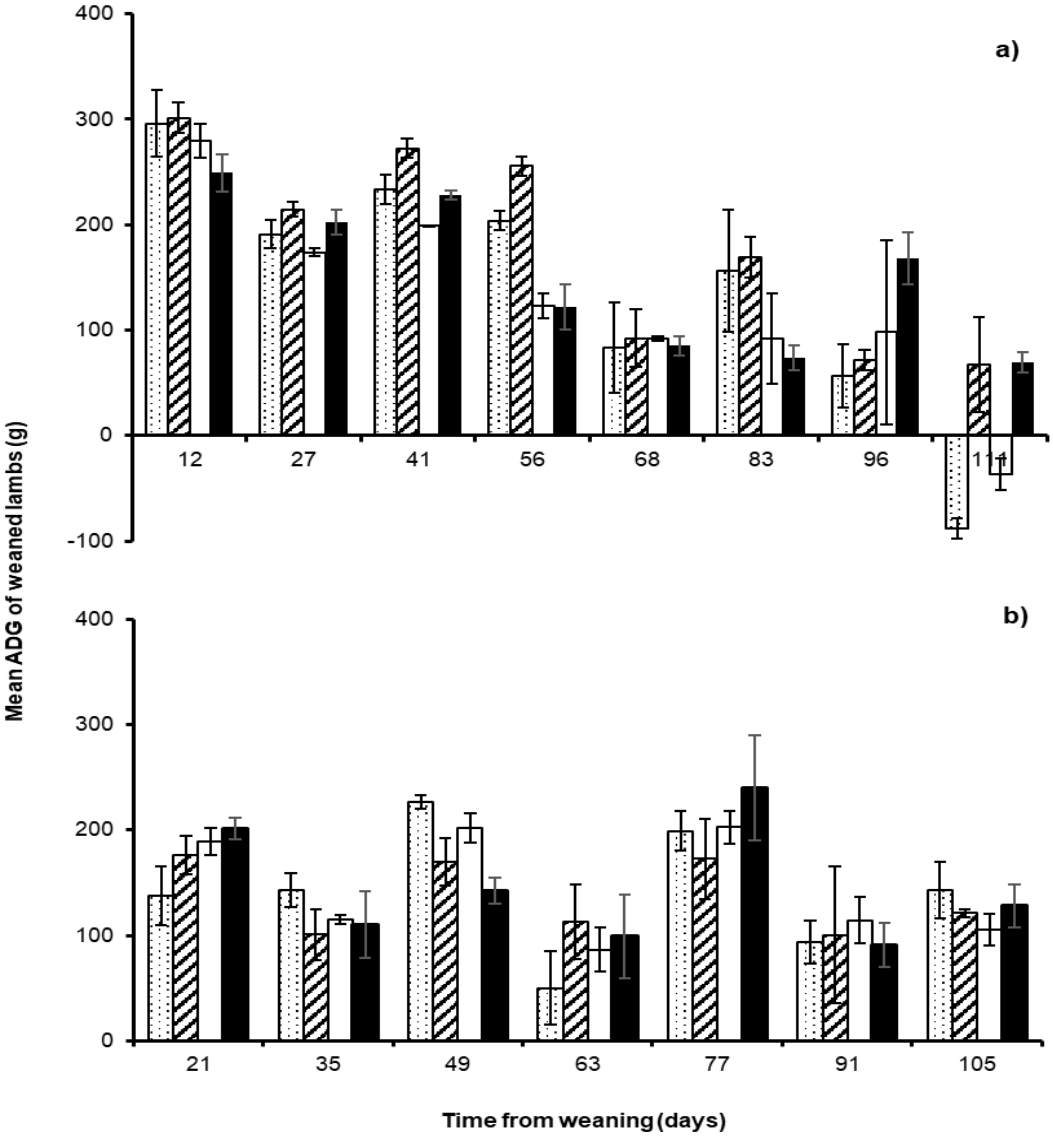


Figure 4.23 Mean average daily gain (g) of sentinel lambs that subsequently grazed areas where ewes had been supplemented (▭) or unsupplemented (▨) and TST lambs that subsequently grazed areas where ewes had been supplemented (▭) or unsupplemented (■) in a) 2015 and b) 2016. Sentinel lambs with LW above 38 kg remained in the plots throughout the phase of lamb finishing.

4.4.9.4 Cumulative percentage of lambs reached slaughter weight

The cumulative percentage of sentinel and TST lambs that reached slaughter weight above 38 kg in 2015 and 2016 are presented in Figure 4.24. Overall, in both years, no treatment effects (P>0.05 for all) nor treatment and time interactions (P>0.05 for all) were detected on the cumulative percentage of sentinel and TST lambs reaching slaughter weight above 38 kg. However, the effects of time were detected (P<0.001 for all), reflected by the increasing percentage of lambs reached slaughter weight over time. In the 2015 trial, 55% and 75%, respectively, sentinel lambs that fed areas where ewes had been supplemented or had been unsupplemented, reached the slaughter weight at 111 days after weaning. While at the same date, 36% of TST lambs that grazed the areas where ewes had been supplemented and 45% of lambs that grazed areas where ewes had been unsupplemented reached the slaughter weight. In the 2016 trial, the cumulative percentage of sentinel lambs that grazed the areas where ewes had been supplemented and those fed in the areas where ewes had been unsupplemented reached the slaughter weight was 55.56% and 44.44%, respectively while 56.32% of TST lambs from both groups reached slaughter weight at 105 days after weaning.

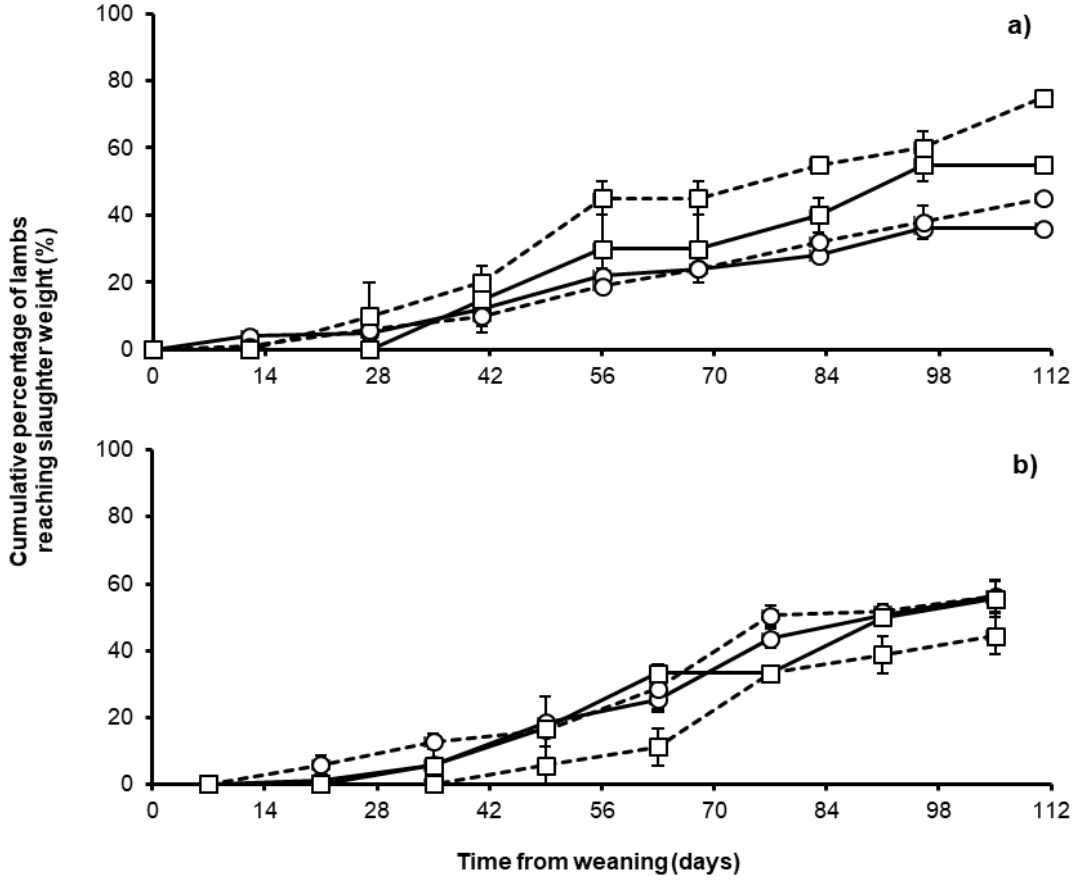


Figure 4.24 Cumulative percentage (%) of weaned lambs that were suppressively drenched (squares) or exposed to a targeted selective treatment anthelmintic regime (circles) that subsequently grazed areas where ewes had been supplemented (solid line) or unsupplemented (dashed line) reaching slaughter weight above 38 kg in a) 2015 and b) 2016.

4.5 Discussion

The main objective of the current study was to examine the benefits of supplementing lactating ewes on pasture and its provision of an epidemiological parasite benefit to grazing lambs after weaning. The short period of targeted supplementation was chosen to provide a cost-effective supplementation regime that provided additional nutrient supply to the ewes at the time of greatest need, such as until peak lactation. The results of this experiment confirm the earlier findings of some benefits of protein supplementation for reducing FEC. However, this reduction in FEC was insufficient to provide a measurable and consistent epidemiological benefit to the grazing lambs.

Overall, supplementation of lactating ewes in both years was successful in reducing 50% of ewe's FEC during lactation periods. Although MP supply was not measured in this study, the 50% reduction in FEC is comparable with the 50% reduction in worm burden observed in parasitised lactating ewes relative to the expected MP supply shown by Donaldson et al. (2001). However, in 2015, this effect was transient, as FEC of supplemented ewes increased and was not different at week 12 of lactation. In part, this increase in FEC by weaning was unexpected as the demand for nutrients for lactation may be supposed to be relatively low. However, climatic conditions were not favourable for pasture growth during this year (Figure 4.3), resulting in low pasture availability, which may have resulted in unintentional nutritional stress, with mean pasture mass declining to less than 700 kg DM/ha in all paddocks, below-recommended levels (Corner-Thomas et al., 2015) and may have resulted in increased concentration of eggs in the faeces. In 2016, the reduction in ewe FEC was more consistent throughout lactation, indicating a longer-term benefit to the ewe as this extended beyond the pre-patent period of any larvae that would have been ingested after supplementation. Additionally, the declining number of eggs observed in 2016 at late pregnancy until week eight of lactation agrees with the increase of IgA at the same time (Figure 4.10), which may be due to an improvement in immunity. Nevertheless, while supplementation appeared to offer some benefit in reducing the periparturient relaxation in immunity to nematode parasites, the FEC in neither treatment in either year declined to low levels by weaning, which coincided with a decrease of IgA, possibly indicating that the nutritional stress caused by lactation extends well beyond peak lactation.

Given the only measured difference between the ewes' groups and their performance was the reduction in FEC, it seems reasonable to suggest that rather than substitution, this reflects the diversion of nutrients into immune functions rather than increases in ewe body weight gain or lactation performance. This interpretation would be in-line with the nutrient partitioning framework proposed by Coop and Kyriazakis (1999), as the additional nutrients supplied by supplementation appeared to be utilised by the ewes for maintenance of immunity, which possibly reflects the

nutritional cost of the immune response in lactating ewes. It is supported by a lack of supplementation effect on change in ewe live weight, ewe BCS, or lamb production. The lack of an impact on ewe LW or BCS or lamb weaning weight per ewe observed here is comparable to what has been reported in farm studies with interventions aimed at breaking the PPR with long-acting drenches (Garland & Leathwick, 2015; Miller et al., 2015). However, the design of the aforementioned studies was such that it did not allow an evaluation of the epidemiological benefit, which may have accrued through interrupting the PPR.

After weaning, supplementation of lactating ewes did not provide a clear benefit to lambs grazing the areas where ewes had been supplemented. Interpretation of the lamb LWG may be influenced by the potential differences between the pastures of each paddock, as such, comparisons between the TST and sentinel lambs are preferred as these animals grazed the same areas. This aside, those lambs grazing areas on which ewes were supplemented did appear to have a lesser need for drench, although this only occurred in 2015. This indicates a small epidemiological advantage may have been conferred through the supplementation of ewes, although such effects were transient and were not great enough to result in a consistent difference in pasture larval contamination. Further, this apparent benefit is relatively low given the extent of the difference in FEC in the ewes for much of the lactation period. In part, this may reflect the design of the study, whereby the ewes followed the lambs to mimic grazing practices on-farm. This design may have contributed to the lack of effect due to the net removal of parasites by grazing non-lactating ewes (Leathwick et al., 2008). This may have been further exacerbated due to the low pasture availability in 2015, resulting in ewes grazing further down in the sward where a majority of the parasite population is believed to exist (Gazda, Piazzetta, Dittrich, Monteiro, & Thomaz-Soccol, 2009; Holasová, Pavlasek, & Kotrlá, 1989; Vlassoff, 1982). Alternatively, the lack of benefit may reflect the relatively low transmission of disease from contamination supplied by lactating ewes. In the current study, pasture larval concentrations generally reduced during lactation despite the ewe FEC indicating a reasonable number of nematode eggs were being deposited. Nematode egg viability has been shown to be influenced by the immune mechanisms of the host, with eggs from periparturient animals having lower viability (Jørgensen, 2000), an effect which may be compounded by the relatively low egg development that has been reported during colder periods of winter and early spring (Leathwick, Miller, & Waghorn, 2011; Waghorn et al., 2011). In the current study, even though developmental success was not significantly affected by supplementation, over time, eggs passed by supplemented ewes tended to produce fewer infective stage larvae than those shed by unsupplemented ewes (Figure 4.8).

Parasitological and immunological parameters are typically used as resistance indicators against GIN (Toscan et al., 2017). In sheep, the production of some antibody isotypes, including IgA, IgG, and IgE,

are correlated with immunity against GIN (McRae et al., 2015; Toscan et al., 2017). In 2016, the *T. colubriformis*-specific L3 IgA absorbances in supplemented ewes were significantly lower than their unsupplemented counterparts throughout the experiment (Figure 4.10). Lower *T. colubriformis*-specific L3 IgA absorbance in supplemented ewes than those in unsupplemented was unexpected, and the reasons for this are unclear. However, it may reflect differences in the larval challenge. It was expected that supplemented ewes would maintain greater parasite-specific IgA concentrations than unsupplemented ewes, indicative of better immune capacity. This hypothesis was supported by Strain and Stear (2001), who reported an increase in the IgA response in lambs depending on adequate dietary protein. Watson, Colditz, Andrew, Gill, and Altmann (1994) and Watson and Gill (1991) stated that the magnitude of antibody responses and resistance to parasite infection in sheep are affected by many factors, such as the age of animals and prior exposure to the parasites. Moreover, the capacity of older animals to improve immune responses to parasites was contributed to by their previous antigenic stimulation and resultant immunological memory due to prior exposure to internal parasites, even though in the current study, there was limited support for a preceding difference in the larval challenge during 2016. Douch, Green, and Risdon (1994) suggested that the reasons for the difference in IgA concentrations among studies could be a result of the different breeds of sheep, species of infective larvae used for the challenge, the protocols of dosing, and the sensitivity or specificity of the ELISA procedure applied in the study, albeit the reason for the lack of benefit of supplementation is not clear.

Higher concentrations of IgA antibodies against somatic *H. contortus* L3 and adult antigens in the serum of ewes that received high MP diets have been reported (Rocha et al., 2011). In their study, the feed was calculated to supply either 0.8 times (low MP diet) or 1.3 times (high MP diet) of the estimated requirement of MP from three weeks before lambing until eight weeks of lactation. One possibility to account for the difference in IgA concentrations with that reported by Rocha et al. (2011) is the number of lambs reared during the periparturient period as they used single-rearing Ile de France and Santa Ines ewes compared with twins in the current study. Several workers observed lower effects of GIN infection (lower FEC and smaller worm burdens) in single-rearing than in twin-rearing ewes due to the reduction of MP demand (Donaldson et al., 1998; Houdijk, Kyriazakis, Jackson, et al., 2001). Houdijk (2008) suggested that a decrease in demand for nutrition because of rearing fewer lambs would be expected to improve the availability of nutrients to the periparturient ewes for expression of immunity to GIN. Moreover, Houdijk, Jackson, Coop, and Kyriazakis (2006) found it can enhance the resistance of lactating ewes to *T. circumcincta*. O'Sullivan and Donald (1970) reported that ewes showed a significant and rapid reduction in FEC related to the part of their existing worm burden rejection and a marked rise in the resistance to new infection when they were separated from their lamb(s) at birth or after a period of lactation.

The reduction in FEC following supplementation was accompanied by an increase in *T. colubriformis*-specific L3 IgA absorbance in 2016. This result was in accord with the previous studies of Jeffcoate et al. (1992) and Xie et al. (2004), who observed the same pattern of elevated anti-parasite IgA antibody levels as that of FEC. Jeffcoate et al. (1992) stated that PPR in FEC was closely related to a significant rise in the concentration of anti-parasite IgA antibody in ewe plasma, which occurred at a time when IgA is transported from the gastrointestinal tract to milk during early lactation. Additionally, Xie et al. (2004) suggested that the same pattern of anti-parasite IgA antibody levels as that of FEC may adequately reflect elevated antigenic stimulation during the re-establishment of immunity after periparturient relaxation.

Overall, serum biochemical parameters (serum albumin, total serum protein, serum globulin, serum urea, and serum phosphorus) of the supplemented ewes were not significantly different from unsupplemented counterparts. However, supplemented ewes tended to have higher biochemical concentrations than their counterparts. In contrast with the current study, Abdelatif, El-Nageeb, Makawi, and Fadlalla (2009) showed that dietary supplementation to ewes significantly increased total serum protein, serum albumin, and serum urea. There are some possible reasons for these differences. One reason for the difference in the reported results may be the length of supplementation. In the present study, ewes were supplemented with high-protein sheep pellets only from approximately three weeks before lambing until four weeks of lactation, while in the study of Abdelatif et al. (2009), supplemented ewes received 500 g/head/d of a concentrate mixture (crushed sorghum grain and cottonseed cake) started from pre-mating until the third month of lactation while they were grazing residues of harvested fields of sorghum and grass (*Cynodon dactylon*). Alternatively, it remains possible in the current study that some substitution occurred, although this cannot be confirmed. Even if substitution did occur, the difference in FEC indicated some benefits of supplementation to improve the nutrient status. It is possible that the lack of effects on biochemical parameters, although surprising, may reflect nutrient partitioning to immune function.

One consideration for the present research where the animals were infected naturally by GIN is that it can not be guaranteed the supplemented ewes had the same larval exposure as their unsupplemented counterparts throughout the experiment. Further, although it is acknowledged the timing of larval accumulation may have differed, the lack of difference in worm burden of the tracer lambs at weaning suggests cumulative larval challenge was not different. Muñoz-Guzmán, Cuéllar-Ordaz, Valdivia-Anda, Buendía-Jiménez, and Alba-Hurtado (2006) concluded that the number of ingested larvae could not be controlled when using a natural infection in the experiment, apparently

because the behaviour of individual animals grazed in the pasture. So, it remains a variable that was unable to be controlled for.

In summary, supplementation of grazing ewes during the first four weeks of lactation did not affect ewe performance but was successful in temporarily reducing faecal egg by 50%, presumably reflecting better maintenance of immune function through greater nutrient supply, even though this was not detected in parasite specific-immunoglobulin. However, the reduction in parasite contamination was insufficient to provide a measurable and consistent epidemiological benefit to the grazing lambs that may assist with parasite control. These results demonstrated that twin-bearing ewes supplemented on pasture during the periparturient period significantly had lower *T. colubriformis*-specific L3 IgA concentrations than their counterparts. No significant effects of supplementation were observed on serum albumin, serum urea, total serum protein, serum globulin, and serum phosphorus. It is suggested that for strategies to help break the parasite lifecycle through targeting the relaxation in immunity in the periparturient ewe, a reduction in ewe FEC by more than 50% is required.

Chapter 5

Epidemiological impact on gastrointestinal parasitism through targeted supplementation of twin-bearing ewes treated with an anthelmintic pre-lambing

5.1 Introduction

The primary sources of initial GIN infection for young lambs at pasture are larvae that have survived from the previous season and those which develop from eggs secreted by the periparturient ewe (Coop, Sykes, & Angus, 1977; Jackson, Jackson, & Williams, 1988; Nunns, Rawes, & Shearer, 1965). The increase in faecal egg output around parturition derives from the development of pre-existing populations and newly acquired infections (Reid & Armour, 1975). To remove pre-existing GIN parasites populations obtained from the previous grazing time and also to diminish the possible contamination of pasture that will be eaten by susceptible lambs, a usual practice operated by sheep producers in New Zealand is to drench ewes around lambing (Brunsdon et al., 1983; Lawrence et al., 2007). Under those conditions, the PPR in FEC acquires only from the new establishment of GIN parasites.

In the previous trials (2015 and 2016 trials in Chapter 4), the ewes were not drenched; accordingly, this allowed the continuation of pre-existing parasite infections. The results from those studies showed that supplementation of twin-bearing ewes could reduce FEC at an average of 50% for an extended period. However, a 50% reduction in the FEC of lactating ewes is not sufficient for an epidemiological advantage to the grazing lamb. After weaning, supplementation of lactating ewes did not provide a clear benefit to lambs grazing the areas where ewes had been supplemented.

Moreover, the immune response is multi-factorial, one component of which is aspects that may influence the establishment of larvae, and which has been shown to be sensitive to protein supply (Jackson et al., 2004). Therefore, the objective of this study was to evaluate an epidemiological advantage of greater than 50% reduction in FEC, which can be obtained through supplementation with ewes when the existing population is removed through treatment with anthelmintic.

5.2 Materials and methods

5.2.1 Animals and treatments

This study was carried out at a summer-safe unit of LincolnSheep, Lincoln University, Canterbury, New Zealand, under the authority of the Lincoln University Animal Ethics Committee (AEC) approval number 2017-16.

At set stocking, 160 mixed age twin-bearing crossbred (predominantly Coopworth) ewes were allocated to one of two treatments, namely supplemented and unsupplemented, as in the previous trials (Chapter 4). Starting three weeks before lambing in August 2017, 80 twin-bearing ewes on the supplemented farmlets were introduced to an advantage feeder (NGF800, Advantage Feeders Ltd, New Zealand) with approximately 50 g/head/d of a commercially available sheep pellet (Triplet nuts, Farmlands stock feeds Ltd, New Zealand). The supplementation then increased to 500 g/head/d during the first four weeks of lactation wherein the feeders were removed from the paddocks, and supplementation ceased, as described in Chapter 3 (Section 3.1). The remaining cohort of 80 twin-bearing ewes was remained unsupplemented and allowed to graze pasture as per usual farming practice. All ewes were orally dosed with a combination drench of 1.0 g/L abamectin, 40.0 g/L levamisole hydrochloride, 25.0 g/L albendazole (1 mL/5 kg LW; Trio® Sheep, Ravensdown Ltd., Christchurch, New Zealand) and vaccinated with 1 ml/dose of Ultravac® 5in1 with Selenium (Zoetis Inc., Auckland, New Zealand) against clostridial diseases when set stocked prior to lambing. The ewes lambed in three weeks and were stratified by lambing dates into representative paddocks. Each week during lactation, ewes which had lambed in that week and their lambs were moved into a separate paddock, providing four groups of ewes per treatment, each with a similar lambing date. Within 24 hours of parturition, lamb weight was recorded, and lambs were tagged and matched to their dam. Figure 5.1 depicts the layout of the experimental paddocks in 2017.

As a control, after lambing, a total of sixteen lactating ewes with twins at foot were randomly selected from two farmlets (eight ewes from the supplemented group and eight ewes from the unsupplemented group. The ewes were allocated into one of four groups. The first group was supplemented and undrenched (n=4, mean initial LW was 64.65±1.30 kg). The second group was unsupplemented and undrenched (n=4, mean initial LW was 68.90±2.12 kg), the third group was supplemented and drenched (n=4, mean initial LW was 67.15±2.11 kg). The fourth group was unsupplemented and drenched (n=4, mean initial LW was 67.20±1.37 kg), creating a 2x2 factorial design. Drenched ewes were treated with 1 mg/kg LW of long-acting moxidectin injection (1 ml/20 kg LW; Cydectin, Pfizer Animal Health, Auckland, New Zealand).

At weaning, all lambs received a combination drench of 1.0 g/L abamectin, 40.0 g/L levamisole hydrochloride, 25.0 g/L albendazole (1 mL/5 kg LW; Trio® Sheep, Ravensdown Ltd., Christchurch, New Zealand) to remove residual parasite loading. Nine days after weaning, 180 lambs were set-stocked in paddocks providing the replicate. Half of the lambs from each farmlet were transported to the other farmlet (balanced for LW), creating a crossover design with a combination of lamb groups, similar to the design described in Chapter 4. Each treatment was replicated three times. Mean initial LW for weaned lambs that grazed areas where ewes had been supplemented was 27.75±0.29 kg while those that grazed areas where ewes had been unsupplemented was 28.17±0.32 kg. Six lambs from each replicate (three of each sex) that were representative of sex and weight based on placement when ranked hierarchically by LW within the sex of the remainder of the population were treated with 1 mg/kg LW of long-acting moxidectin injection (1 ml/20 kg LW; Cydectin, Pfizer Animal Health, Auckland, New Zealand) and used as sentinel lambs. All remaining lambs were subjected to a TST regime in which individuals growing at less than 80% of the mean of the suppressively drenched controls were treated with a combination drench of 1.0 g/L abamectin, 40.0 g/L levamisole hydrochloride, 25.0 g/L albendazole (1 mL/5 kg LW; Trio® Sheep, Ravensdown Ltd., Christchurch, New Zealand) and returned to graze with the remainder of the group. TST lambs were removed from the study once their body weight exceeded 38 kg. Sentinel animals remained even when reaching an arbitrary slaughter weight above 38 kg. Within each farmlet, lambs were rotationally grazed for the remainder of the grazing season with lambs grazing freshly grown forages, followed by the ewes.

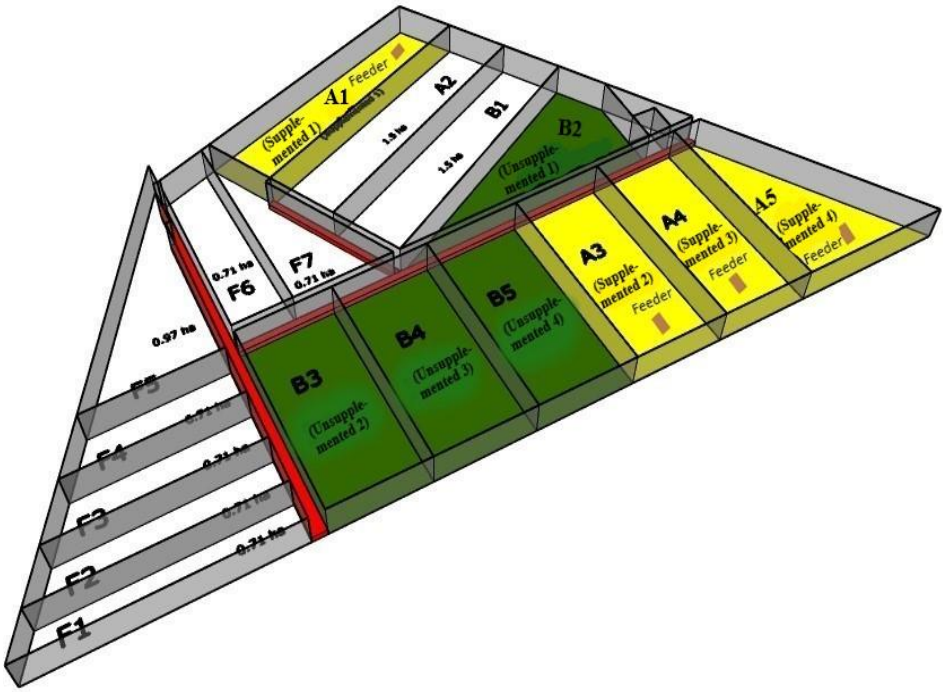


Figure 5.1 Layout of the experimental paddocks at the LincolnSheep, Lincoln University, New Zealand in 2017.

5.2.2 Animal measurements and sampling

LW, BCS, and FEC of all ewes were monitored on all farmlets at approximately four weeks, eight weeks, and 12 weeks after parturition. Ewes were milked as described in section 5.2.6 at weeks four, eight and 12, with milk samples were also collected from each ewe at the same day of those samplings.

Lamb weight was recorded within 24 hours of birth, at their fourth week, eighth week and twelfth week of age, and every two weeks after weaning (as part of a TST regime) until they reach slaughter weight. Animals were weighed with the use of a swing gate auto drafter. Ewe BCS was assessed following the palpation of the lumbar region as described in Chapter 4 (Section 4.2.2).

5.2.3 Parasitology

Pasture larval concentrations were monitored every two weeks from set stocking until the end of the study and were measured as described in Chapter 3 (Section 3.5). Ewe faecal samples were collected from all animals at week four, week eight, and week 12 of lactation. During the TST phase of lamb finishing, all six sentinel and six non-sentinel lambs were faecal sampled every two weeks from weaning. FEC was counted using the modified McMaster method described in Chapter 3 (Section 3.6).

At weaning, two lambs from each replicate (total 16 lambs) were randomly selected as tracer lambs. They were slaughtered at weaning for the assessment of worm burden. The slaughter and worm burden collection followed the procedures described in Chapter 3 (Section 3.7).

5.2.4 Meteorological data

Monthly mean rainfall and air temperatures during the experimental period of 2017/2018 are shown in Figure 5.2. Annual rainfall during 2017 was 609 mm while the mean air temperature was 12.3 °C. The total annual rainfall was slightly higher than the average long term rainfall of the last 30 years (599 mm). The monthly air temperatures (Figure 5.2b) showed a similar trend to the long-term average air temperature.

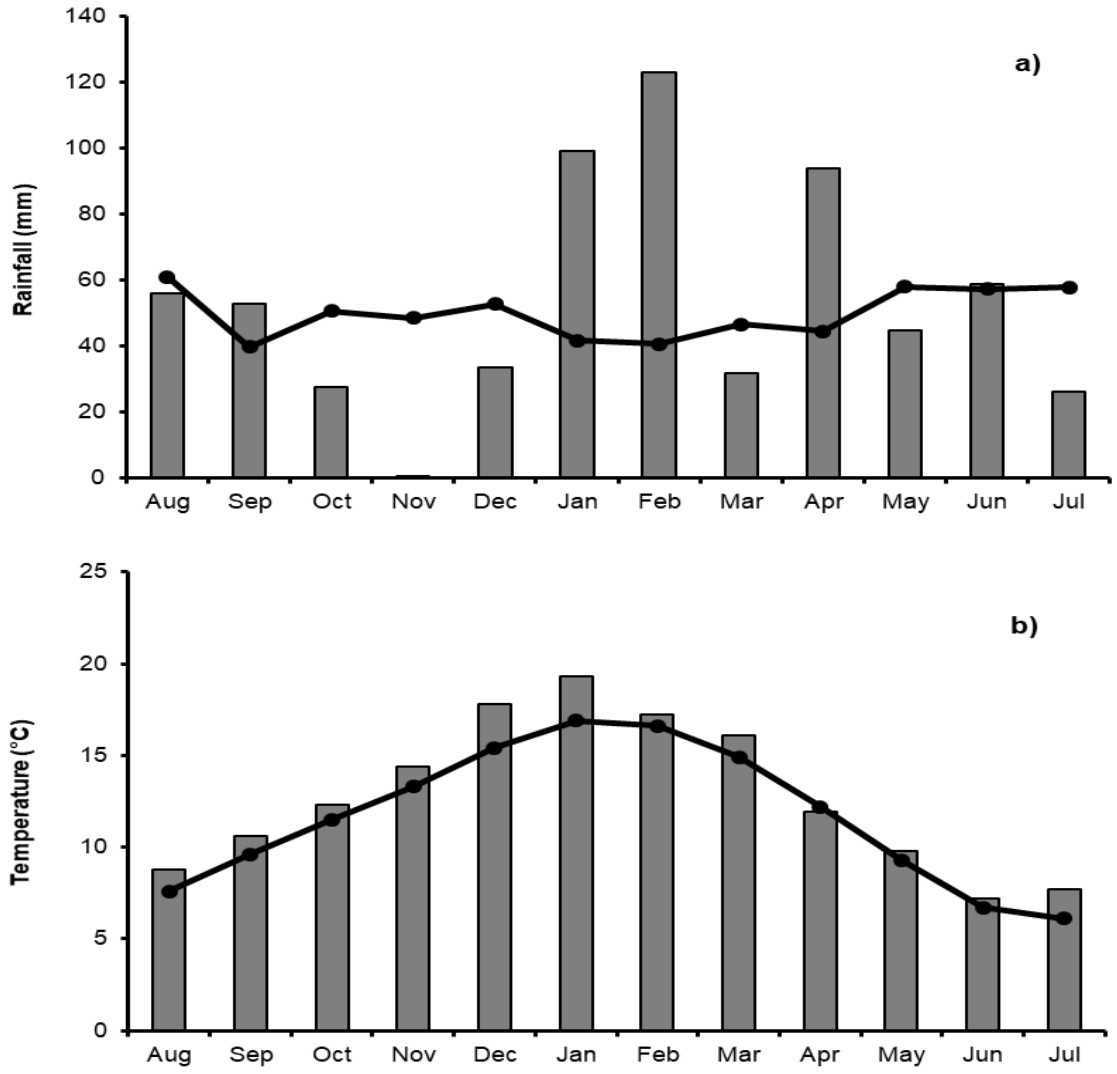


Figure 5.2 (a) Monthly mean rainfall, (b) mean air temperatures for August 2017–July 2018 and long-term average 1981–2010 (solid line) at Lincoln, Canterbury, New Zealand. Data were taken from the National Climate Database (CliFlo) of Broadfields Meteorological Station, about 1 km from the research site.

5.2.5 Serum analysis

Blood samples from all ewes were taken at weeks four, eight and 12 of lactation. Samples were drawn by jugular venipuncture into 10 ml vacutainer tubes (Becton Dickinson, Rutherford, New Jersey, USA) and were immediately stored at 4 °C for 24 hours and then processed and serum collected, as described in Chapter 3 (Section 3.3).

Serum samples were analysed for IgA antibody specific to *T. colubriformis* L3 and IgG antibody specific to *T. colubriformis* L3 using an indirect ELISA, as described in Chapter 3 (Section 3.10), and for serum albumin, total serum protein, serum urea, and serum phosphorus using an RX Daytona Analyser, as described in Chapter 3 (Section 3.11).

5.2.6 Milk analysis

Milk sampling was conducted on the same day as LW, BCS, faecal and blood sampling, at four, eight, and 12 weeks-of-lactation. Milk yield was measured by a four-hour milk test to estimate milk yield and to determine milk composition, which was similar to the experiments conducted by Afolayan et al. (2009) and Hunter et al. (2015). At each time of milk sampling, ewes from each treatment group were allocated into batches of 10 to ensure accurate timing of sampling between milkings. The lambs were separated from their dams and kept in their respective paddock with access to grass and water. After that, the ewes were milked out using a milking machine (DeLaval Type DVP170/340/EF-601516001-TJ Tumba, Sweden) to empty the udder and the time of first milking was recorded. After four hours from the time of first milking, the ewes were administered with 1.0 ml of oxytocin (0.0167mg/ml at 10IU/ml, Kela N.Y. Hoogstraten, Belgium, lot# 26824A10) intramuscularly and after one minute they were milked again and milk volume and sub-samples collected using a sheep-calibrated herd testing sampler (#C0180 and C0001, Livestock Improvement Corporation (LIC) Ltd., Christchurch, New Zealand). The milk sub-samples were preserved with bronopol (0.1%) and analysed at LIC laboratory to determine somatic cell counts (SCC), fat, protein, and lactose contents using MilkoScan (Foss Electric, Hillerød, Denmark) and for total solids content. Following the second milking, the ewes were returned to their lambs, and animals were returned to graze pasture in their respective paddock.

Plate 5.4 The ewes milked out using a milking machine.

5.3 Statistical analysis

The LW and BCS of ewes, FEC, milk performance, pasture larval counts, and serum analysis data were subjected to sequential comparison for ante-dependence structures prior to analysis as repeated measures using an REML by GenStat (18th Ed., VSN International Ltd, UK) with treatment groups (supplemented and unsupplemented) and time included as factors. The effects of supplementation difference, long-acting drench administration, and their interaction were examined by two-way ANOVA procedures using GenStat (18th Ed., VSN International Limited, UK). Other parameters were analysed using ANOVA by Minitab (17Th Ed., Minitab Inc., USA). Worm burden, pasture larval counts, and SCC were log-transformed as log10 (count+1) while FEC was log-transformed as log10 (count+100) before analysis to obtain a normal distribution and presented as back-transformed means. At week four, milk production data of one replication from each treatment consisted of 20 samples per replication, were not assessed due to laboratory error and were subsequently not included in the statistical analysis. At week 12, the sub-samples from 65 animals were insufficient for milk composition, and subsequent comparisons from these ewes were excluded from the results. The relationships between lamb ADG and milk production of ewe were analysed using General Linear Model by GenStat (18Th Ed., VSN International Ltd, UK). The number of drench and LWG/drench of TST lambs after weaning were analysed using ANOVA by Minitab (17Th Ed., Minitab Inc., USA). The LW and LWG of sentinel and TST lambs after weaning and the cumulative percentage of sentinel and TST lambs that reached slaughter weight above 38 kg were analysed using the REML using GenStat (18th Ed., VSN International Ltd, UK). Where the F-test for treatment was significant ($P \leq 0.05$), treatments were compared with the Least Significant Differences test with a significance value of 5%. All values are group means and expressed as mean ± SEM unless otherwise specified.

5.4 Results

5.4.1 Treated pre-lambing groups

5.4.1.1 Faecal egg counts (FEC)

Mean back-transformed FEC of ewes and weaned lambs in 2017 are shown in Figure 5.3. For ewe FEC (Figure 5.3a), no effect of treatment ($P=0.19$) nor treatment by time interaction ($P=0.25$) was observed, but a significant effect of time ($P<0.001$) was detected due to the FEC of both groups at the start of supplementation was zero due to anthelmintic administration. The highest egg outputs in the faeces of ewes for both groups were at four weeks of lactation, with mean values of 273 epg and 384 epg for supplemented and unsupplemented ewes, respectively. For lambs (Figure 5.3b), there were no effects of treatment ($P>0.05$ for all) or treatment and time interaction ($P>0.05$ for all) in FEC of sentinel and TST lambs after weaning. However, there were significant effects of time ($P=0.01$ for sentinel lambs and $P<0.001$ for TST lambs), reflecting a gradual increase of FEC of TST lamb from unsupplemented groups to a peak of 617 epg at 51 days after weaning (31 January 2018) and 802 epg at 65 days (14 February 2018) of supplemented groups.

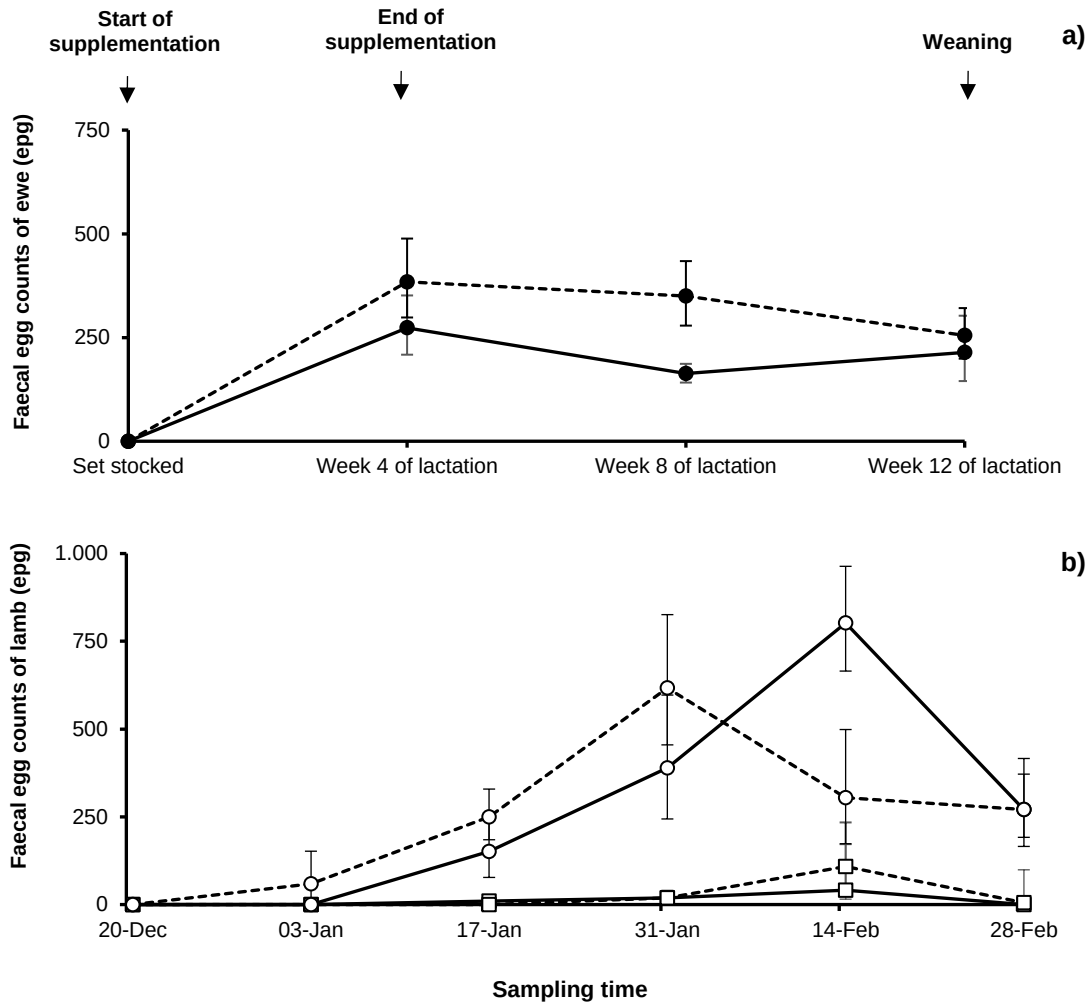


Figure 5.3 Back-transformed (log10 (count + 100)) means of faecal egg count a) for ewes (closed symbols) that were supplemented during the first four weeks of lactation (solid line) or not (dashed line), and b) for lambs (open symbols) that were suppressively drench (squares) or exposed to a targeted selective treatment anthelmintic regime (circles) that subsequently grazed areas where ewes had been supplemented (solid line) or unsupplemented (dashed line) in 2017.

The distributions of FEC of supplemented ewes and their counterparts in 2017 are presented in Figure 5.4. Overall, no effects of treatment nor time (P>0.05 for all) were observed on the distributions of ewe FEC. No treatment x time interactions (P>0.05 for all) were detected, except for the range of 200-400 epg (P=0.01), reflecting a gradual increase of the percentage of ewe of the unsupplemented cohort had FEC between 200 and 400 epg over the lactation period. Ewe FEC was affected by BCS (P=0.01). No evidence of supplementation x BCS nor supplementation x BCS x sampling time interactions (P>0.05 for all) were detected on ewe FEC (Figure 5.5).

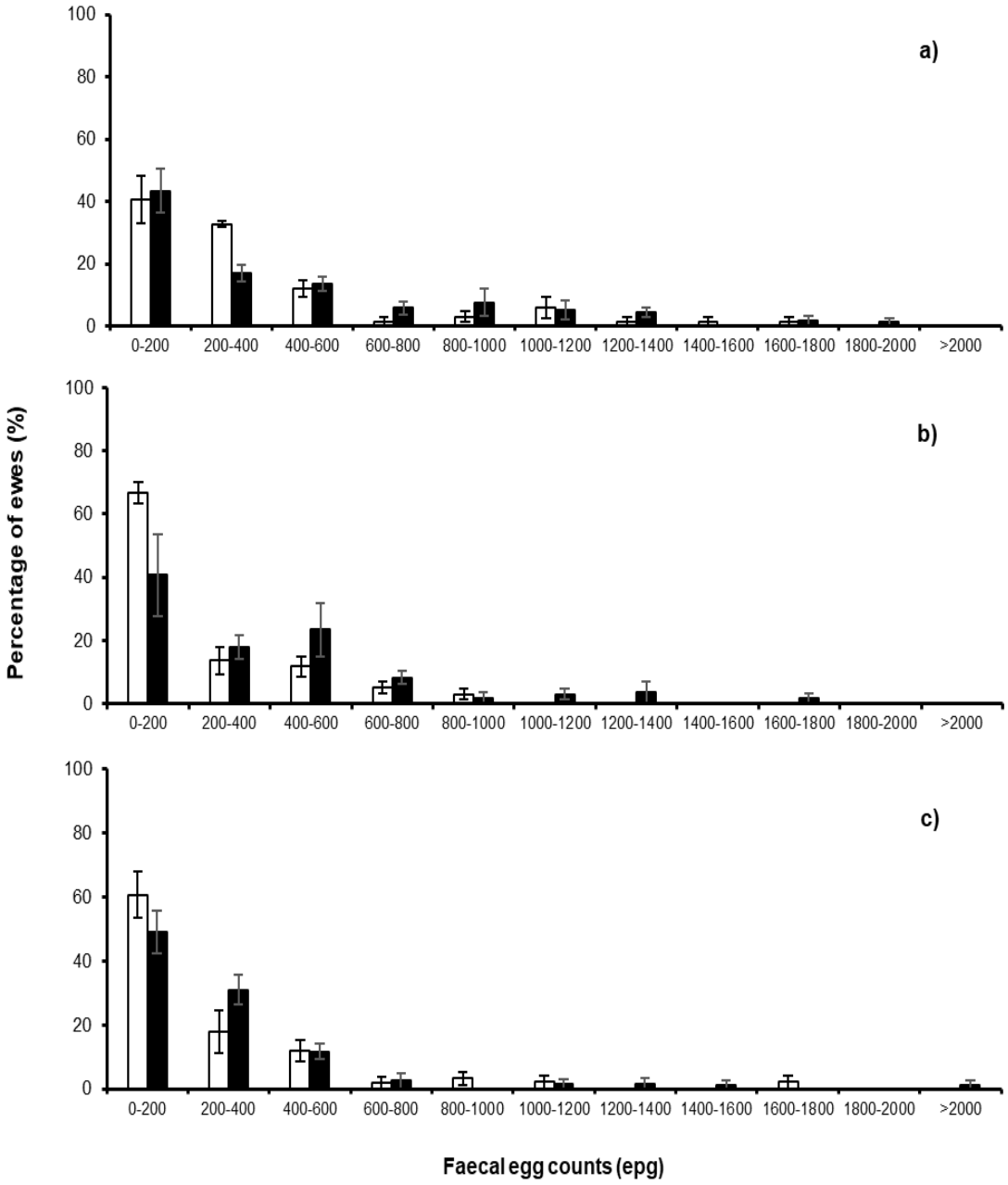


Figure 5.4 The distribution of faecal egg counts (epg) of supplemented ewes (open bars) and unsupplemented ewes (closed bars) at (a) week four of lactation, (b) week eight of lactation, and (c) week 12 of lactation in 2017.

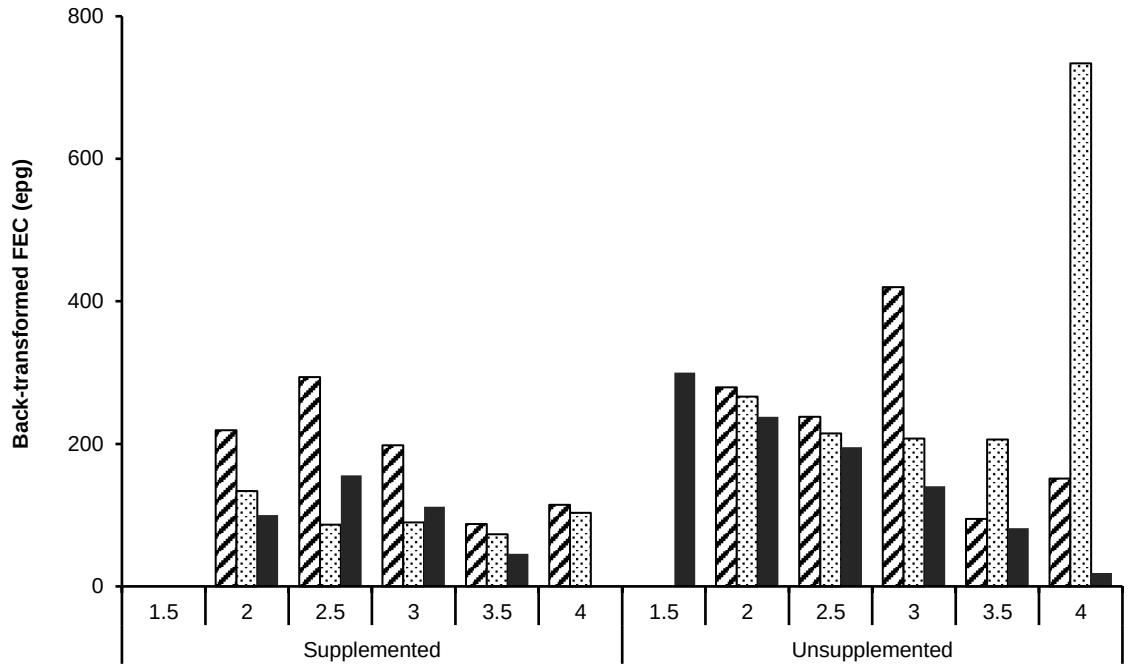


Figure 5.5 The effect of supplementation, BCS, and sampling time on ewe FEC at week four of lactation (▨), week eight of lactation (▩), and week 12 of lactation (■) in 2017.

5.4.1.2 L3 contamination on pasture

The number of L3 of strongyles and L3 of *Nematodirus* spp. recovered/kg DM of pastures grazed by ewes and lambs before and after weaning at supplemented and unsupplemented paddocks in 2017 is given in Figure 5.6. Before weaning, no effects of supplementation (P=0.26) or time were detected (P=0.38); nevertheless, there was a supplementation by time interaction (P=0.03) in numbers of L3 larvae of strongyles recovered/kg DM of pastures (Figure 5.6a), reflecting a gradual increase of numbers of L3 larvae for the unsupplemented group over time. For larvae of *Nematodirus* spp. (Figure 5.6b), there were no treatment differences (P=0.18), nor an interaction between treatment and time (P=0.97) in numbers of L3 larvae recovered/kg DM on pastures grazed by the ewes and the lambs.

After weaning, for both strongyles and *Nematodirus* spp. larvae, there were no significant effects of supplementation (P=0.25 for strongyles and P=0.40 for *Nematodirus* spp.), nor were there treatment and time interactions (P=0.38 for strongyles and P=0.48 for *Nematodirus* spp.) in numbers of L3 larvae recovered/kg DM on pastures grazed by the ewes and the lambs. However, there were significant effects of time (P=0.01 for strongyles and P<0.001 for *Nematodirus* spp.), reflected in increasing values of both groups over time.

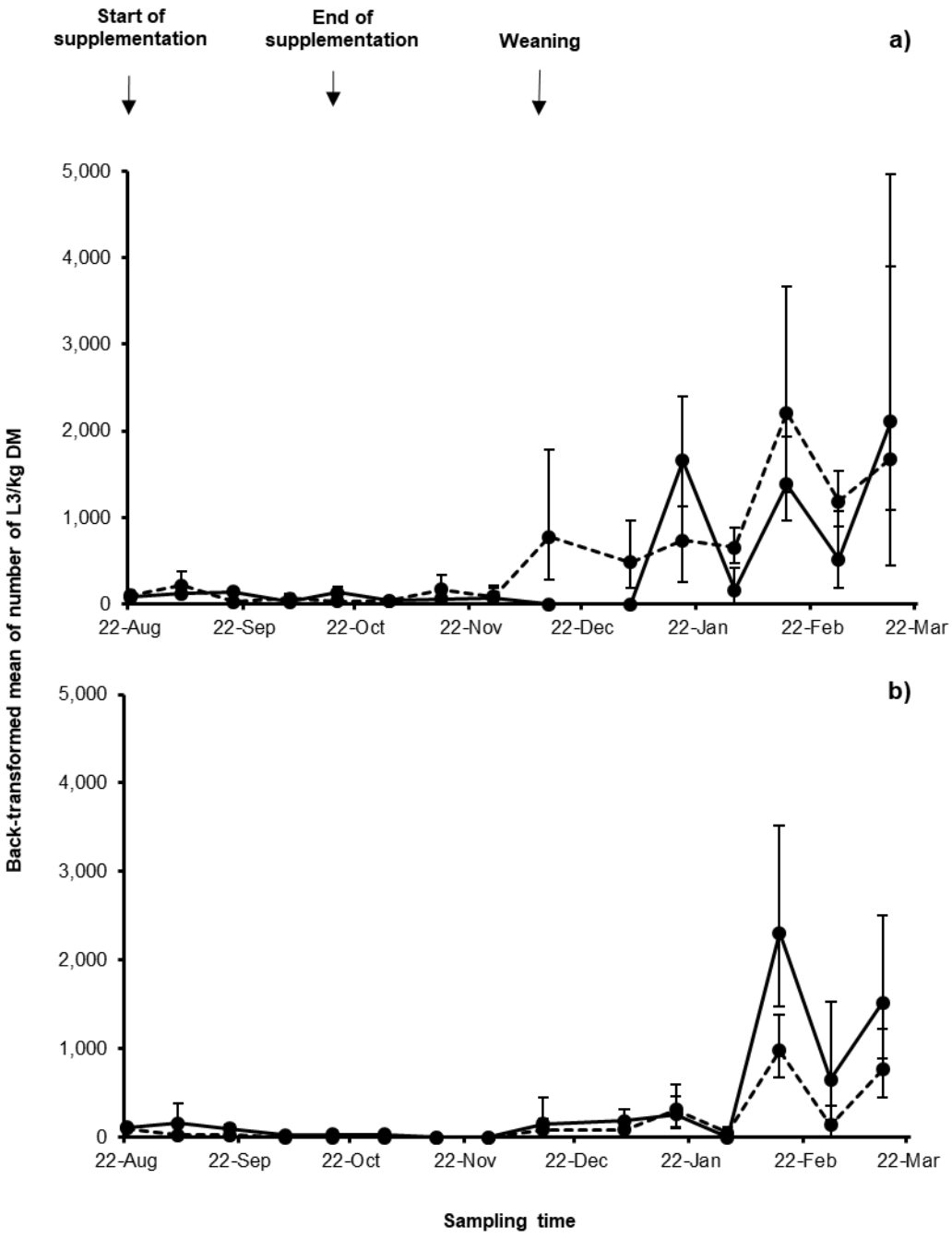


Figure 5.6 Back-transformed (log10 (count + 1)) means of the number of (a) L3 of strongyles and (b) L3 of *Nematodirus* spp. per kg DM present in herbages grazed by the ewes and the lambs where ewes had been supplemented (solid line) or remained unsupplemented (dashed line) in 2017.

5.4.1.3 Worm burdens at slaughter

Table 5.1 and Table 5.2 show the back-transformed geometric mean worm burdens at slaughter from abomasum and small intestine of weaned lambs that subsequently grazed areas where ewes had been supplemented or unsupplemented in 2017. Overall, there were no effects of supplementation (P>0.05 for all) on the number of worms recovered from both the abomasum and small intestine of the weaned lambs. In the abomasum of weaned lambs that subsequently grazed areas where ewes had been supplemented, back-transformed mean of total worms was 846, consisting of 63.71% *T. axei* and 36.29% *T. circumcincta* while for their counterparts back-transformed mean for total worms was 1,260, consisting of 62.46% *T. circumcincta* and 37.54% *T. axei*.

Table 5.1 Mean number of worms (log10 (count + 1)) recovered from abomasum of weaned lambs that subsequently grazed areas where ewes had been supplemented or unsupplemented in 2017. Back-transformed values are given in parenthesis.

Worm genera		Supplemented	Unsupplemented	P value
T. circumcincta	L3	0	0	-
	L4	0.44±0.29 (2)	1.52±0.25 (32)	0.08
	Adult worm	2.47±0.11 (291)	2.85±0.12 (711)	0.24
	Total	2.47±0.58 (293)	2.88±0.18 (750)	0.23
T. axei	L3	0	0	-
	L4	0	0	-
	Adult worm	2.73±0.21 (539)	2.68±0.11 (473)	0.82
	Total	2.73±0.21 (539)	2.68±0.11 (473)	0.82
Total worm	L3	0	0	-
	L4	0.44±0.44 (2)	1.52±0.25 (32)	0.08
	Adult worm	2.93±0.24 (842)	3.09±0.08 (1,222)	0.54
	Total	2.93±0.24 (846)	3.10±0.08 (1,260)	0.52

The back-transformed mean of total worms recovered in the small intestine of weaned lambs that subsequently grazed areas where ewes had been supplemented was 34.66% lower than their counterparts, although this difference was not significant. For weaned lambs grazed supplemented areas the mean was 1,627, consisting of 89.24% *Nematodirus* spp., 5.65% *Trichostrongylus* spp. and 5.10% *Cooperia* spp. while for lambs that subsequently grazed areas where ewes had been unsupplemented the mean was 2,490, consisting of 87.47% *Nematodirus* spp., 10.80% *Trichostrongylus* spp., and 1.73% *Cooperia* spp.

Table 5.2 Mean number of worms (log10 (count + 1)) recovered from the small intestine of weaned lambs that subsequently grazed areas where ewes had been supplemented or unsupplemented in 2017. Back-transformed values are given in parenthesis.

Worm genera		Supplemented	Unsupplemented	P value
Cooperia spp.	L3	0	0	-
	L4	0	0	-
	Adult worm	0.44±0.44 (2)	0.44±0.44 (2)	1.00
	Total	0.44±0.44 (2)	0.44±0.44 (2)	1.00
Nematodirus spp.	L3	0	0.51±0.51 (2)	0.36
	L4	1.50±0.51 (31)	2.29±0.09 (196)	0.18
	Adult worm	3.13±0.08 (1,358)	3.29±0.08 (1,961)	0.21
	Total	3.16±0.07 (1,452)	3.34±0.09 (2,178)	0.16
Trichostrongylus spp.	L3	0	0	-
	L4	0.63±0.42 (3)	0.195±0.195 (1)	0.38
	Adult worm	1.91±0.35 (81)	2.43±0.16 (268)	0.23
	Total	1.98±0.35 (95)	2.43±0.16 (269)	0.29
Total worm	L3	0	0.51±0.51 (2)	0.36
	L4	1.73± (53)	2.30±0.09 (197)	0.16
	Adult worm	3.18± (1,512)	3.36±0.09 (2,269)	0.22
	Total	3.21± (1,627)	3.40±0.09 (2,490)	0.18

5.4.1.4 Serum analysis

IgA

The effects of supplementation of ewes on pasture during the first four weeks of lactation on serum L3 *T. colubriformis*-specific IgA concentrations in 2017 are shown in Figure 5.7. Overall, no effects of supplementation (P=0.80) and time (P=0.32), nor evidence for supplementation and time interaction (P=0.82) were observed on serum L3 *T. colubriformis*-specific IgA levels.

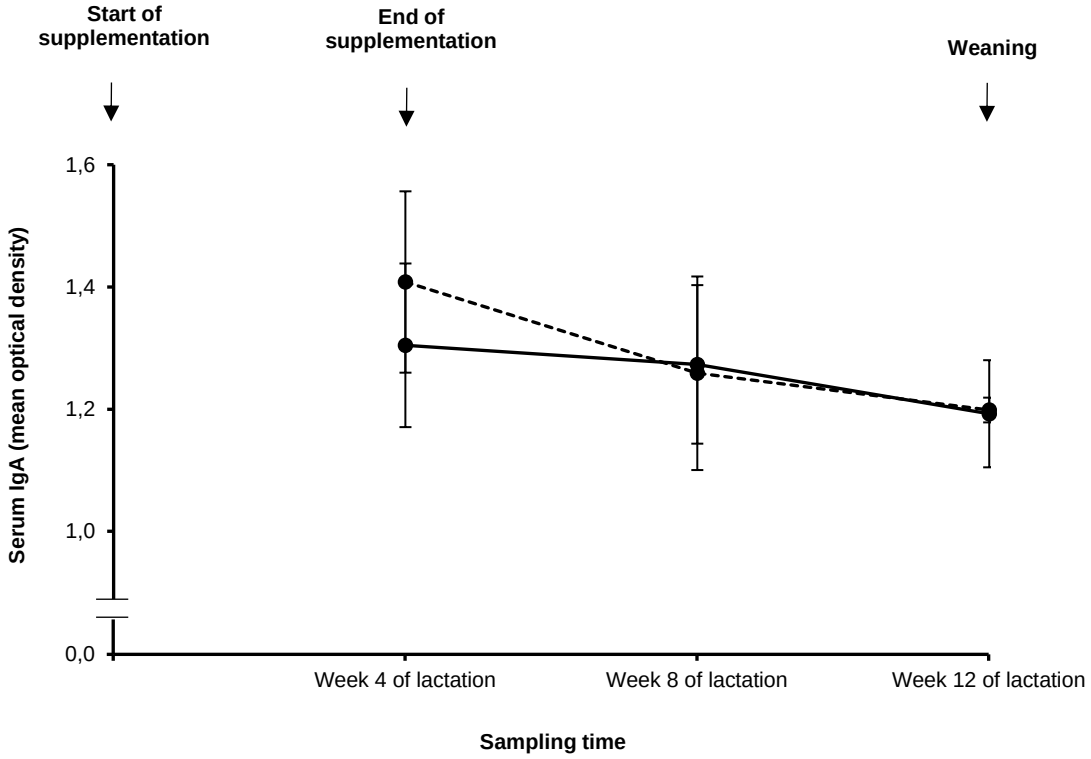


Figure 5.7 Changes in mean optical density for serum specific IgA antibody against L3 *T. colubriformis* of ewes that were supplemented during the first four weeks of lactation (solid line) or not (dashed line) in 2017.

IgG

The effects of ewe supplementation on serum specific IgG antibody levels to L3 *T. circumcincta* in 2017 are presented in Figure 5.8. A significant effect of time (P=0.03) was detected on serum IgG concentrations. This reflecting high IgG responses at week four of lactation, which then decreased with time in all groups. The decline was greater in unsupplemented ewes than supplemented counterparts at week eight of lactation. However, the level of serum IgG of supplemented ewes was similar (P=0.97) with the concentration of serum IgG of unsupplemented counterparts and no interaction between treatment and time were observed (P=0.41).

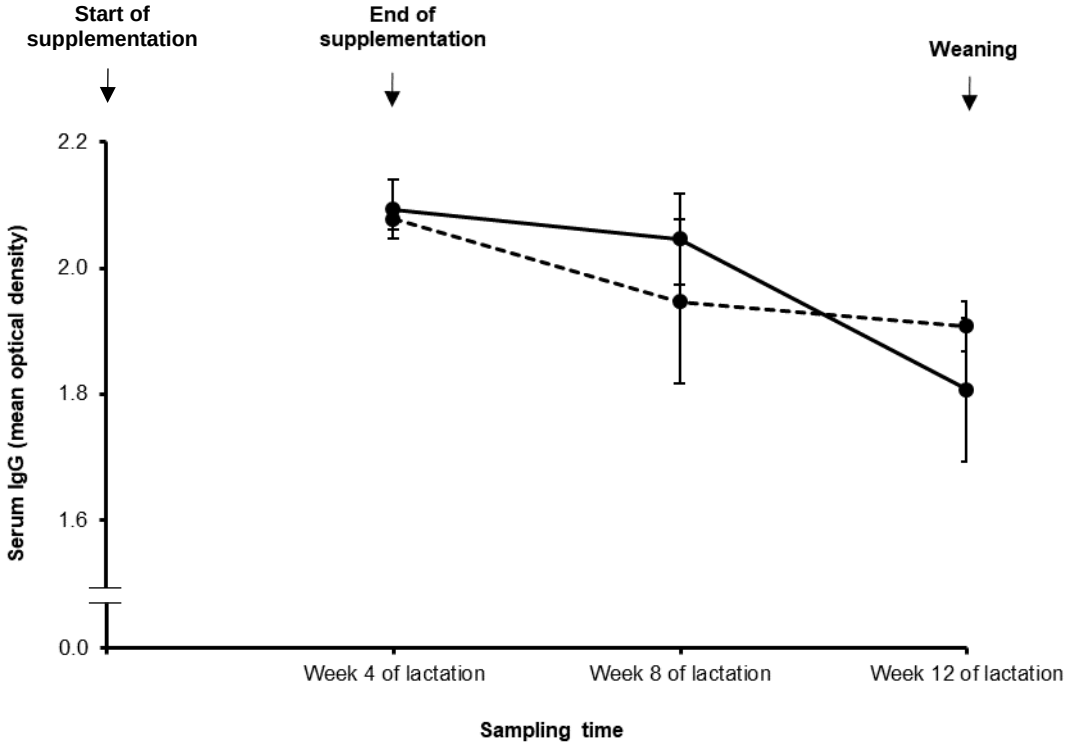


Figure 5.8 Changes in mean absorbance for serum specific IgG antibody to L3 *T. colubriformis* of ewes that were supplemented during the first four weeks of lactation (solid line) or not (dashed line) in 2017.

Serum albumin concentration

Mean serum albumin concentrations of supplemented ewes and unsupplemented ewes in 2017 are given in Figure 5.9. Serum albumin levels of ewes that were supplemented during their first four weeks of lactation were similar (P=0.24) with serum albumin concentrations of unsupplemented ewes. The average of serum albumin concentration of supplemented ewes was 29.72±0.46 g/l while for unsupplemented ewes was 28.88±0.34 g/l. There was no effect of time (P=0.39) nor the interaction between treatment and time (P=0.81).

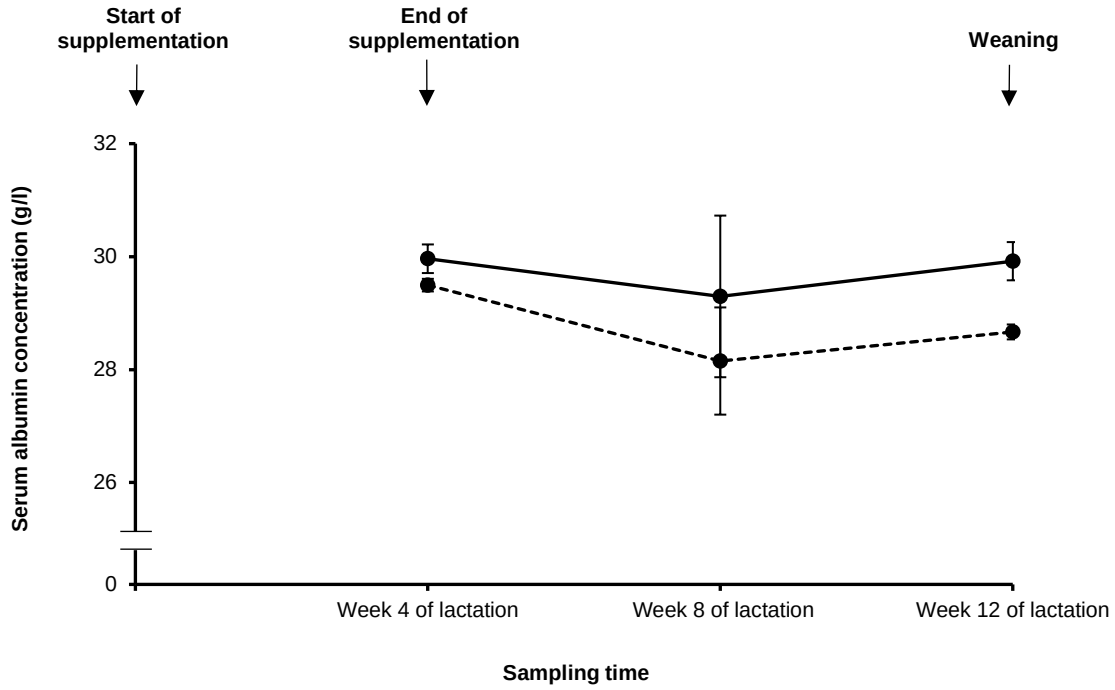


Figure 5.9 Mean serum albumin concentrations (g/l) of ewes that were supplemented during the first four weeks of lactation (solid line) or not (dashed line) in 2017.

Total serum protein concentration

Mean total serum protein concentrations of ewes in 2017 are presented in Figure 5.10. Total serum protein levels were not affected (P=0.79) by supplementation of ewes during the first four weeks of lactation; mean values were 65.18±0.99 g/l for the serum of supplemented animals and 64.70±1.07 g/l for the serum of unsupplemented counterpart. There was no effect of time (P=0.14) nor the interaction between treatment and time (P=0.93).

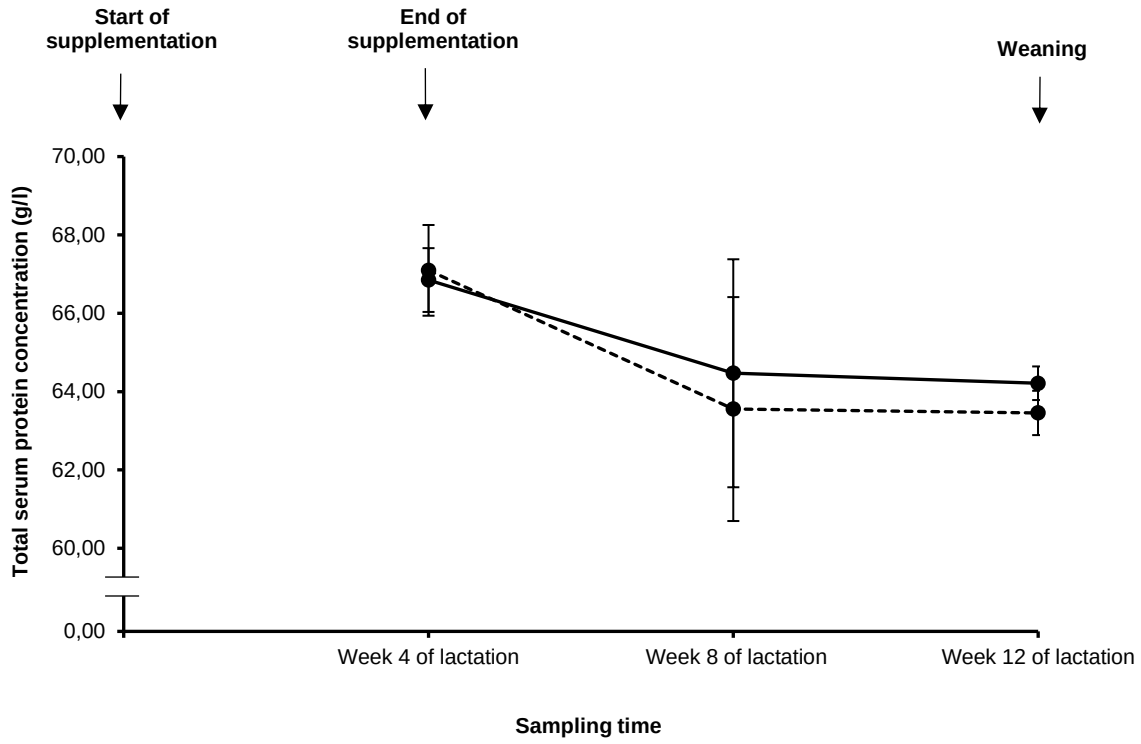


Figure 5.10 Mean total serum protein concentrations (g/l) of ewes that were supplemented during the first four weeks of lactation (solid line) or not (dashed line) in 2017.

Serum globulin concentration

Changes in mean serum globulin concentrations of ewes that were supplemented or remained unsupplemented in 2017 are presented in Figure 5.11. The mean serum globulin concentrations for supplemented ewes was 35.45±0.85 g/l while the average value for unsupplemented ewes was 35.93±1.18 g/l. No significant effect of supplementation (P=0.68), time (P=0.06) nor an interaction between treatment and time were detected (P=0.97) on serum globulin concentration.

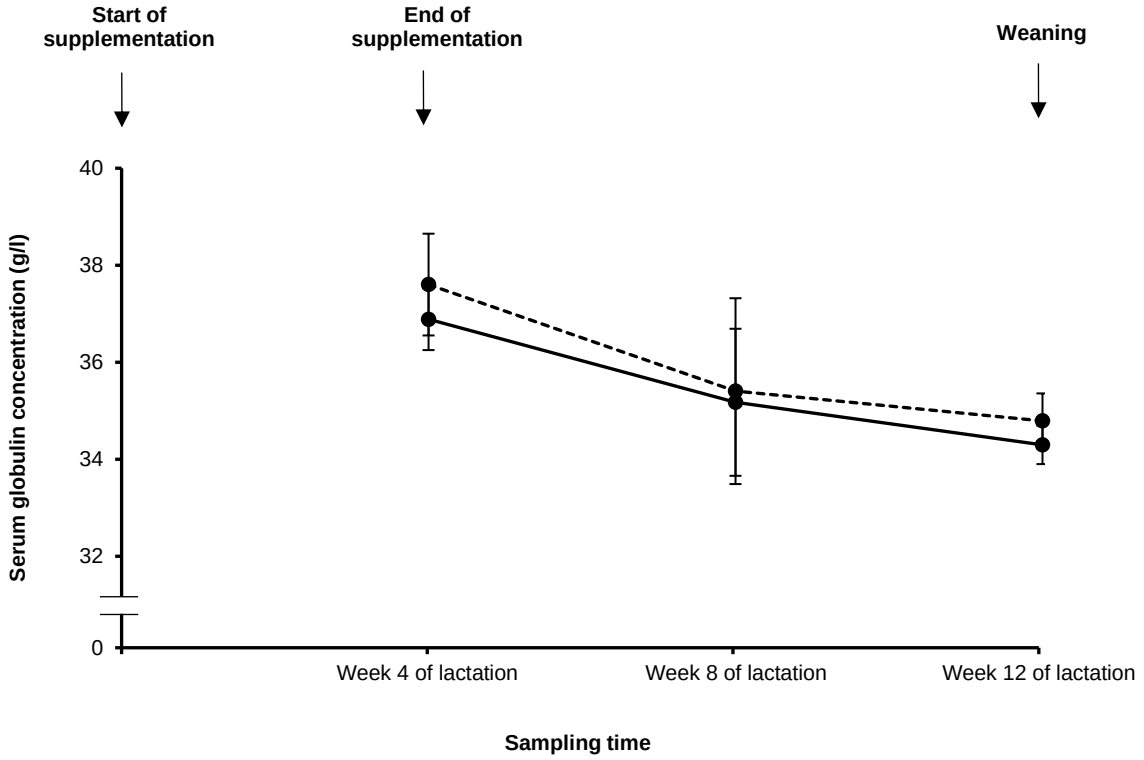


Figure 5.11 Mean serum globulin concentrations (g/l) of ewes that were supplemented during the first four weeks of lactation (solid line) or not (dashed line) in 2017.

Serum urea concentration

Mean serum urea concentrations of ewes that were supplemented or remained unsupplemented during the lactation period in 2017 are presented in Figure 5.12. The average of serum urea concentration for supplemented ewes was 6.47±0.58 mmol/ while the average value for unsupplemented ewes was 6.25±0.41 mmol/l. Overall, no supplementation effect (P=0.68) nor an interaction between time and treatment (P=0.15) were observed on serum urea levels. However, a significant time effect (P<0.001) was detected, reflected in the decrease in urea levels over the lactation period in both groups.

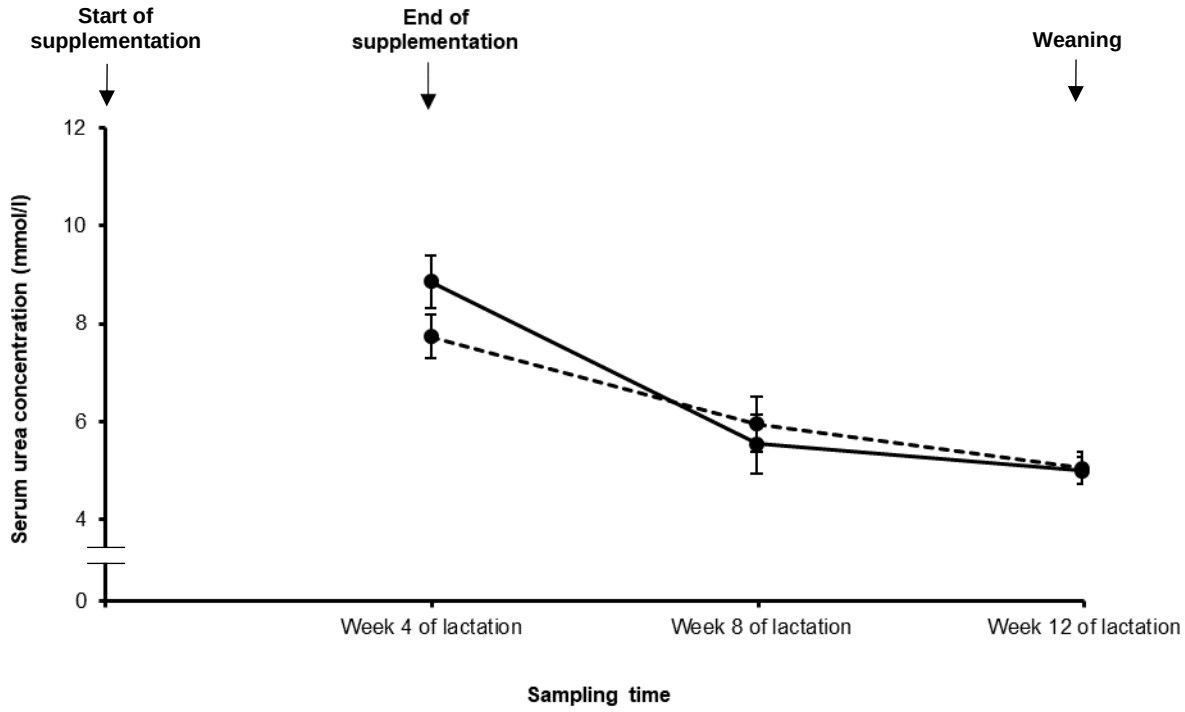


Figure 5.12 Mean serum urea concentrations (mmol/l) of ewes that were supplemented during the first four weeks of lactation (solid line) or not (dashed line) in 2017.

Serum phosphorus concentration

Mean serum phosphorus concentrations in both groups throughout the lactation period in 2017 are shown in Figure 5.13. No effect of treatment (P=0.54), time (P=0.19) nor an interaction between treatment and time (P=0.81) were detected in serum phosphorus concentrations. The average of serum phosphorus concentration of supplemented ewes was 1.67±0.03 mmol/l while the mean value for unsupplemented ewes was 1.64±0.06 mmol/l.

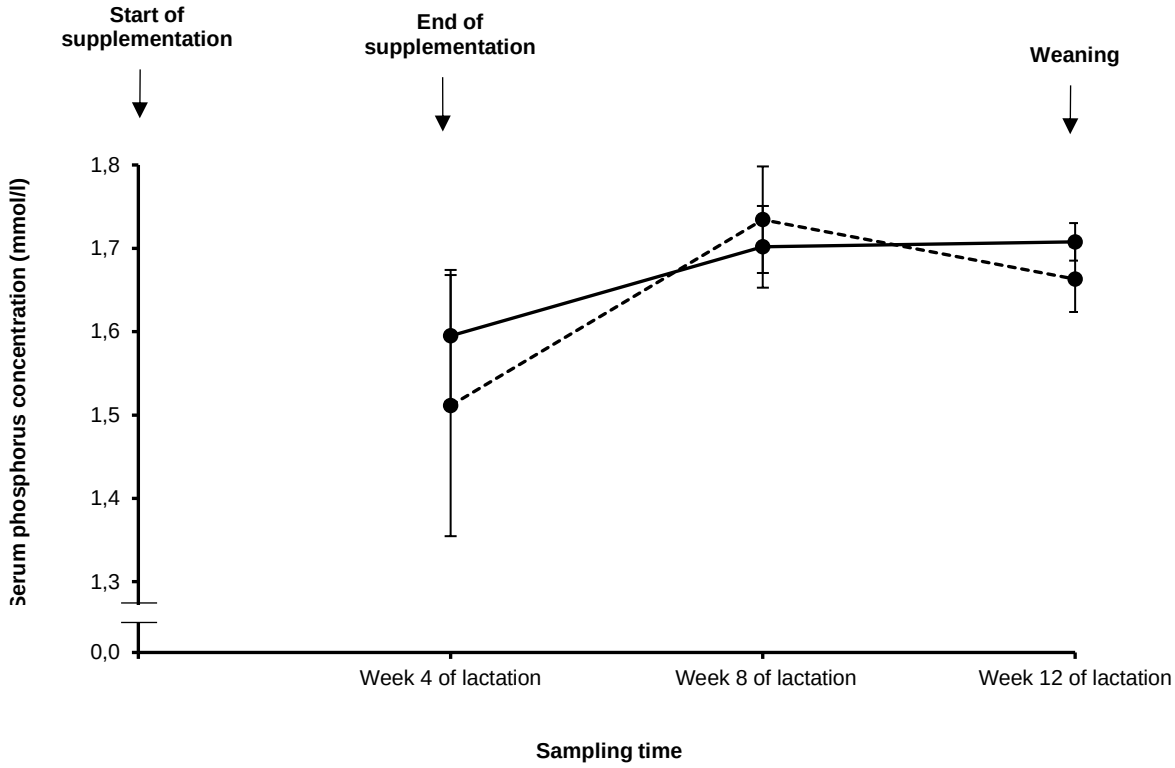


Figure 5.13 Mean serum phosphorus concentrations (mmol/l) of ewes that were supplemented during the first four weeks of lactation (solid line) or not (dashed line) in 2017.

5.4.1.5 Ewes and lambs performance throughout lactation

Live weight (LW) of ewe

The mean LW changes of supplemented and unsupplemented ewes in 2017 are shown in Figure 5.14. Overall, ewe LW during the lactation period was unaffected by supplementation (P=0.82) and time (P=0.45). No treatment x time interaction was observed (P=0.96) on LW of ewes.

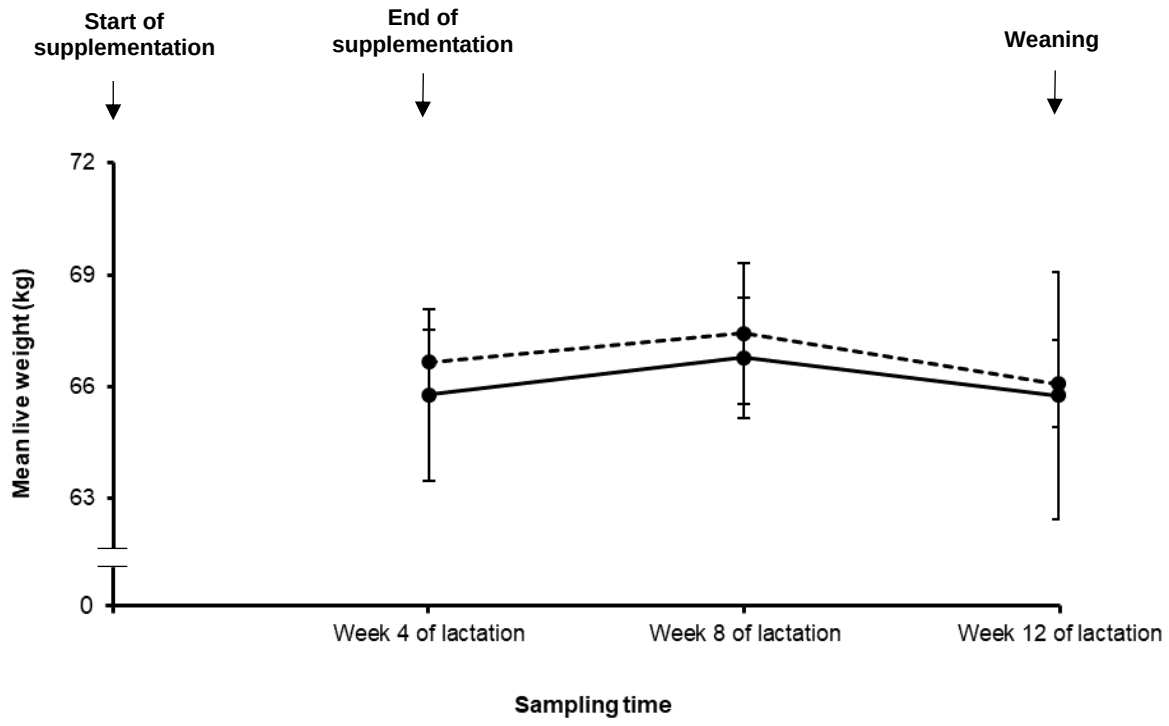


Figure 5.14 Mean live weight changes of ewes that were supplemented (solid line) or unsupplemented (dashed line) during the lactation period in 2017.

Body condition score (BCS) of ewes

Mean BCS changes of supplemented and unsupplemented ewes in 2017 are presented in Figure 5.15. Overall, there was an effect of time (P=0.05), reflecting a decline in ewe BCS throughout lactation. Between week four and week eight of lactation, mean BCS decreased 0.13 units for supplemented and 0.15 units for unsupplemented ewes. At week 12 of lactation, mean BCS of supplemented ewes remains the same as the value at week eight of lactation while in unsupplemented ewes mean BCS declined 0.21 units. However, no effect of supplementation (P=0.56) nor an interaction between treatment x time (P=0.41) was detected.

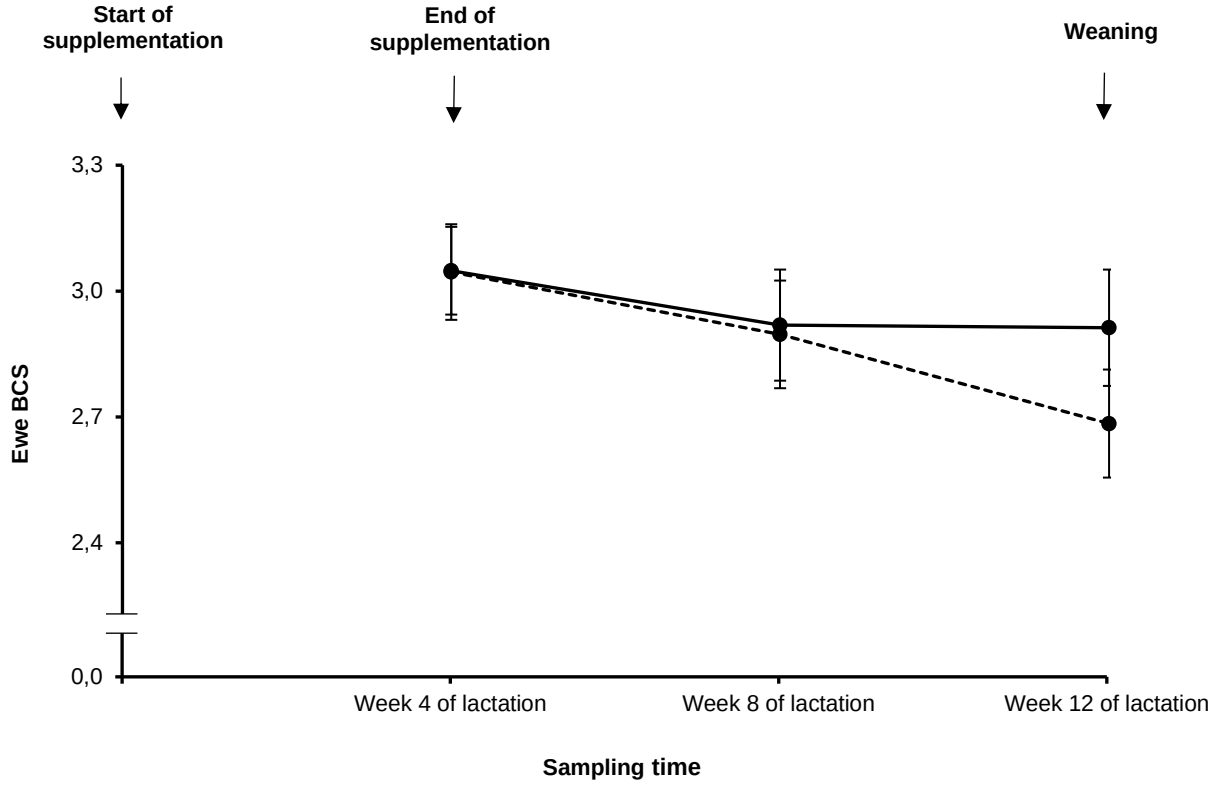


Figure 5.15 Changes of BCS of ewes that were supplemented (solid line) or unsupplemented (dashed line) during the lactation period in 2017.

Live weight (LW) of lamb

Changes in mean LW of lambs reared by supplemented or unsupplemented ewes throughout the lactation period in 2017 are shown in Figure 5.16. Overall, no significant effect of supplementation (P=0.63) nor the interaction between treatment and time (P=0.79) were detected on the LW of lambs from birth until weaning. However, a significant effect of time was observed (P<0.001), demonstrated by a steady increase in lamb LW throughout the lactation period.

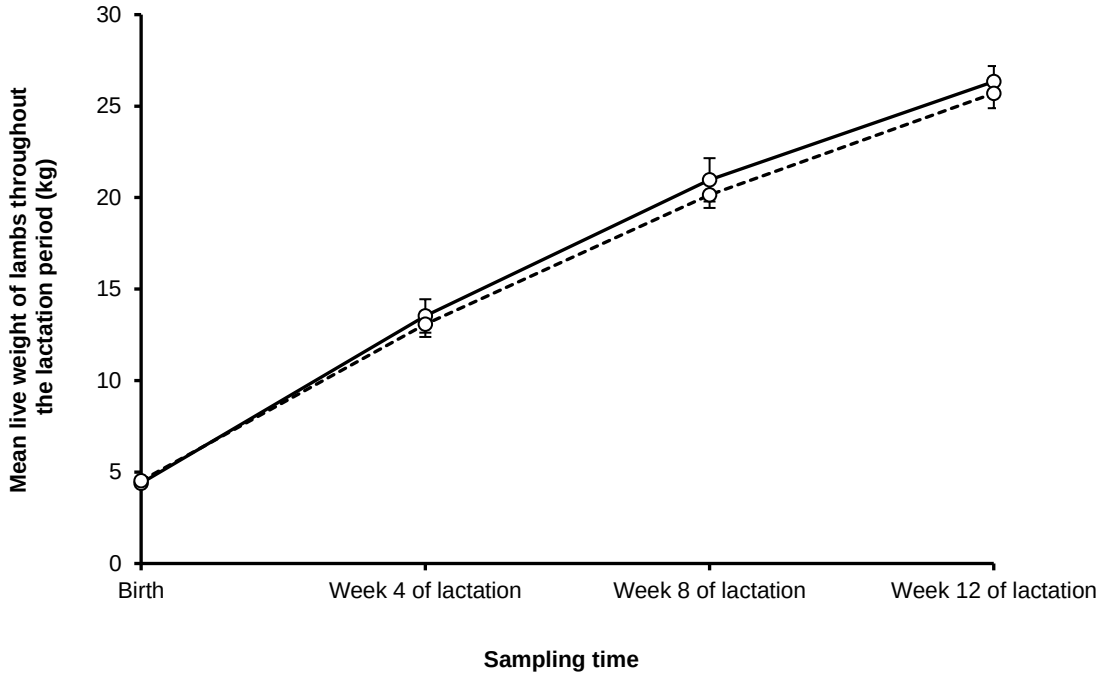


Figure 5.16 Mean live weight changes of lambs reared by supplemented ewes (solid line) or unsupplemented ewes (dashed line) during the lactation period in 2017.

Average daily gain (ADG) of lamb

The average daily gains of lambs throughout the lactation period from supplemented and unsupplemented groups in 2017 are given in Figure 5.17. No treatment difference (P=0.64) nor the interaction between treatment and time (P=0.26) were observed on mean ADG of lambs raised by supplemented or unsupplemented ewes throughout the lactation period. However, a significant effect of time (P=0.001) was detected, reflected by a decrease in ADG over time. Mean ADG of lamb raised by supplemented ewes was 259.00±4.77, 264.50±9.31, and 218.40±6.66 g/d at week four, week eight and week 12 of lactation, respectively, compared with those reared by unsupplemented ewes 245.10±11.19, 252.20±2.14, and 228.40±17.74 g/d, respectively.

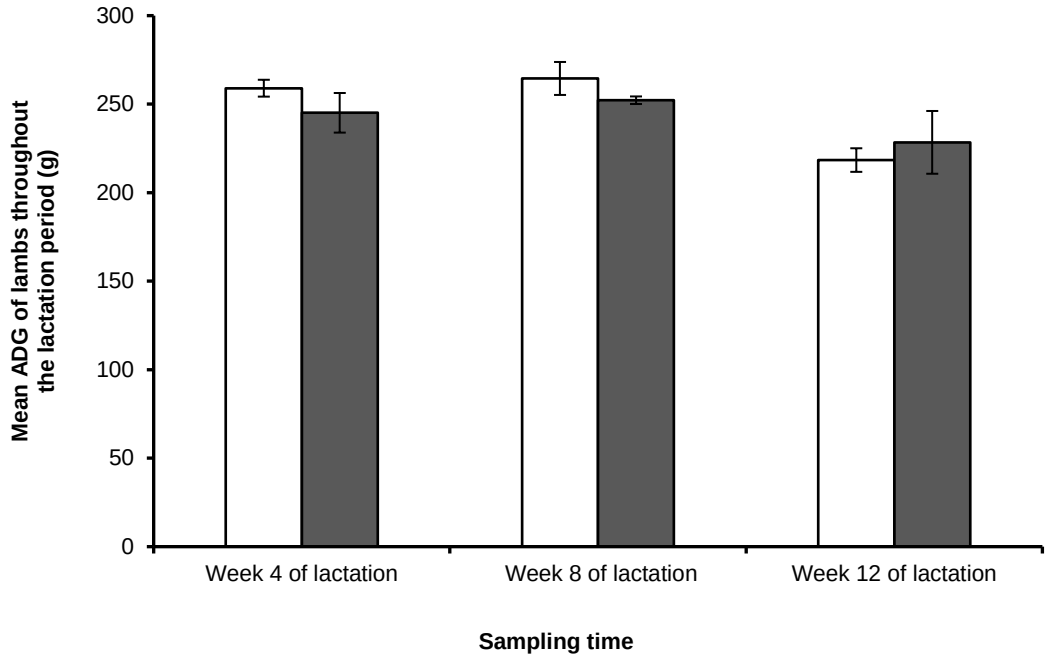


Figure 5.17 Changes in the average daily gain (g) of lambs reared by supplemented ewes (open bars) or unsupplemented ewes (closed bars) during the lactation period in 2017.

Weight of lamb weaned per ewe

The weights of lamb weaned per ewe (WLWE) of supplemented and unsupplemented groups are given in Figure 5.18. Ewe supplementation did not affect the WLWE. The average value was 47.13±2.70 kg for lambs reared by supplemented ewes and 49.49±2.73 kg for lambs raised by unsupplemented cohorts (P=0.56).

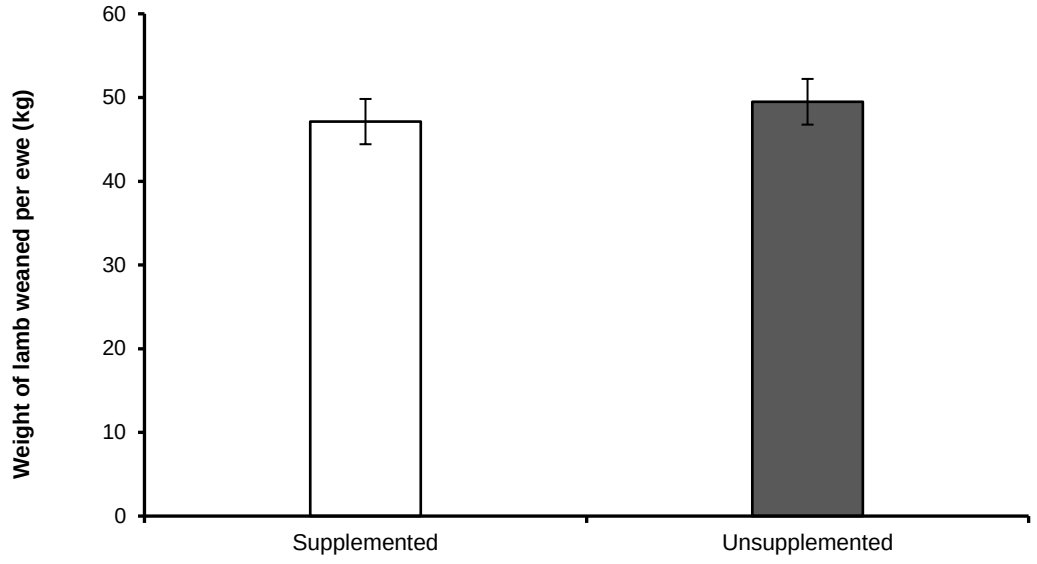


Figure 5.18 Effect of supplementation on the weight of lamb weaned per ewe throughout the lactation period in 2017.

5.4.1.6 Lamb performance after weaning

LWG, the numbers of drench, and LWG/drench

The performance of lambs after weaning that grazed areas where ewes had been supplemented or remained unsupplemented in 2017 are shown in Table 5.3. Overall, there were no differences (P>0.05 for all) on lambs' performance after weaning between the groups.

Table 5.3 The effect of supplementing ewes on pasture on the performance of their offspring after weaning.

Parameter	Sentinel lambs			TST lambs		
	Supplemented	Unsupplemented	P value	Supplemented	Unsupplemented	P value
LWG (g/d)	206.61±15.32	218.78±17.37	0.10	196.78±14.26	183.57±14.30	0.16
Number of drench	-	-	-	1.89±0.16	1.72±0.06	0.37
LWG/drench (g/d)	-	-	-	106.10±11.80	106.92±5.69	0.95

Live weight (LW)

Changes in mean LW of lambs after weaning in 2017 are presented in Figure 5.19. The mean LW of weaned lambs grazed in areas where ewes had been supplemented was similar to the mean LW of lambs grazed in areas where ewes had been unsupplemented, both in sentinel and TST groups ($P>0.05$ for all). A significant interaction between treatment and time ($P=0.01$) was detected in sentinel lambs, but not in TST lambs ($P=0.174$) while TST lambs only observed significant effects of time ($P<0.001$ for all), reflecting a rise in the mean LW of weaned lambs over time.

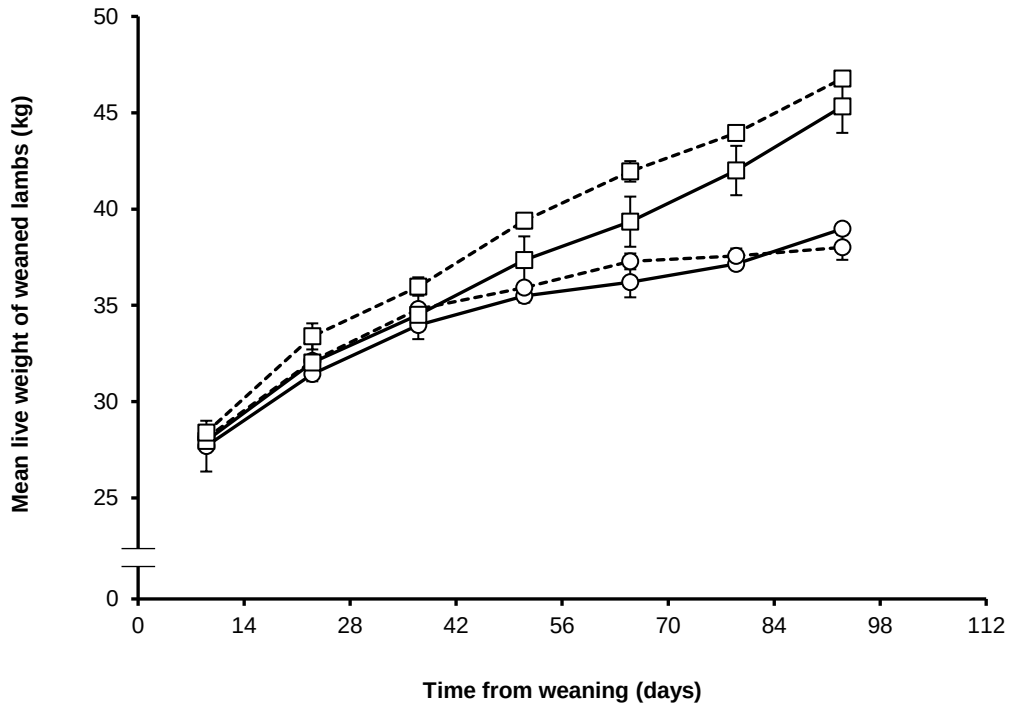


Figure 5.19 Mean live weight changes of weaned lambs that were suppressively drenched (squares) or exposed to a targeted selective treatment anthelmintic regime (circles) that subsequently grazed areas where ewes had been supplemented (solid line) or unsupplemented (dashed line) in 2017. Sentinel lambs with LW above 38 kg remained in the plots throughout the phase of lamb finishing.

Average daily gain (ADG)

The changes in ADG of sentinel and TST lambs that grazed areas where ewes had been supplemented or remained unsupplemented in 2017 are given in Figure 5.20. Overall, no significant treatment differences (P=0.16 for sentinel lambs, P=0.10 for TST lambs) nor supplementation and time interactions (P=0.11 for sentinel lamb, P=0.21 for TST lambs) were observed on mean ADG of TST or sentinel lambs grazed areas where ewes had been supplemented or unsupplemented. However, there were effects of time on mean ADG of weaned lambs (P<0.001 for all), reflected by a decline in mean ADG of all groups throughout the phase of lamb finishing. The mean ADG of sentinel lambs grazed the areas where ewes had been supplemented was 206.6±15.32 g/d while for unsupplemented counterparts was 218.80±17.37 g/d. The means ADG of sentinel lambs were tended to be higher than the TST lambs, the mean ADG of TST lambs grazed the areas where ewes had been supplemented was 196.8±14.26 g/d while for unsupplemented counterparts was 183.60±14.30 g/d.

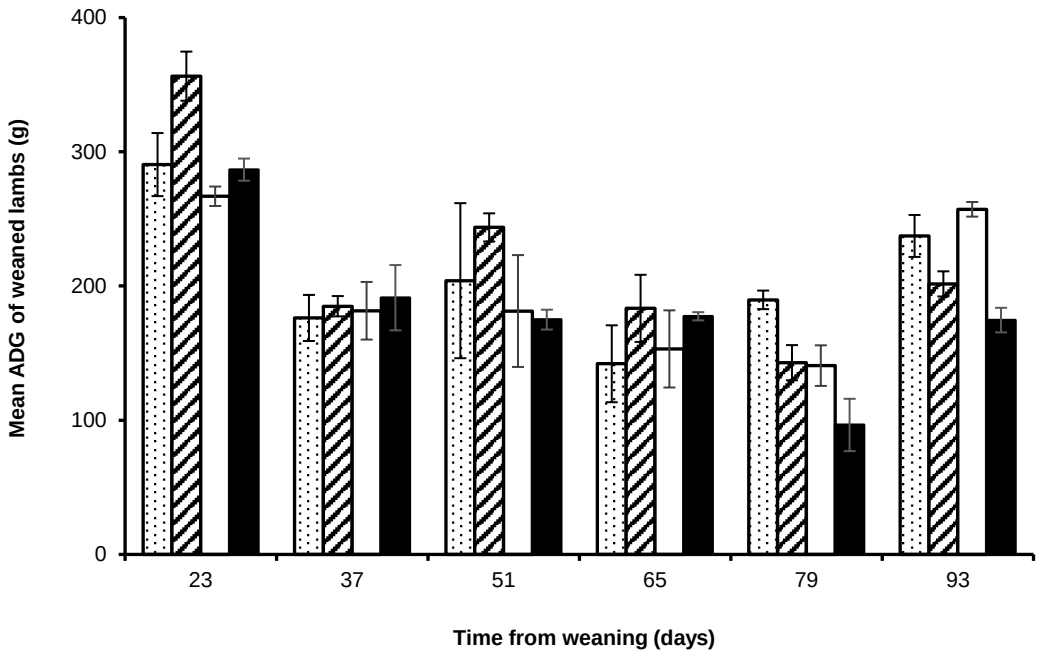


Figure 5.20 Mean average daily gain (g) of sentinel lambs that subsequently grazed areas where ewes had been supplemented (⁘) or unsupplemented (▨), and TST lambs that subsequently grazed areas where ewes had been supplemented (□) or unsupplemented (■) in 2017. Sentinel lambs with LW above 38 kg remained in the plots throughout the phase of lamb finishing.

Cumulative percentage of lambs reached slaughter weight

The cumulative percentages of sentinel and TST lambs that reached slaughter weight exceeded 38 kg in 2017 are shown in Figure 5.21. Overall, the cumulative percentage of sentinel and TST lambs grazed in areas where ewes had been supplemented was similar to those fed in areas where ewes had been unsupplemented (P=0.29 for sentinel lambs, P=0.12 for TST lambs). No supplementation and time interactions were detected (P=0.08 for sentinel lambs, P=0.51 for TST lambs). Nevertheless, a significant time effect was observed (P<0.001 for all), reflecting a rise in the cumulative percentage of sentinel and TST lambs, reaching slaughter weight above 38 kg over lamb finishing. At 93 days after weaning, the cumulative percentage of sentinel lambs that grazed areas where ewes had been supplemented reaching the slaughter weight was 100% while for those fed in areas where ewes had been unsupplemented was 94.44%. At the same time, the cumulative percentage of TST lambs grazed areas where ewes had been supplemented, and for those fed in areas where ewes had been unsupplemented, reaching the slaughter weight was 88.89% and 90.28%, respectively.

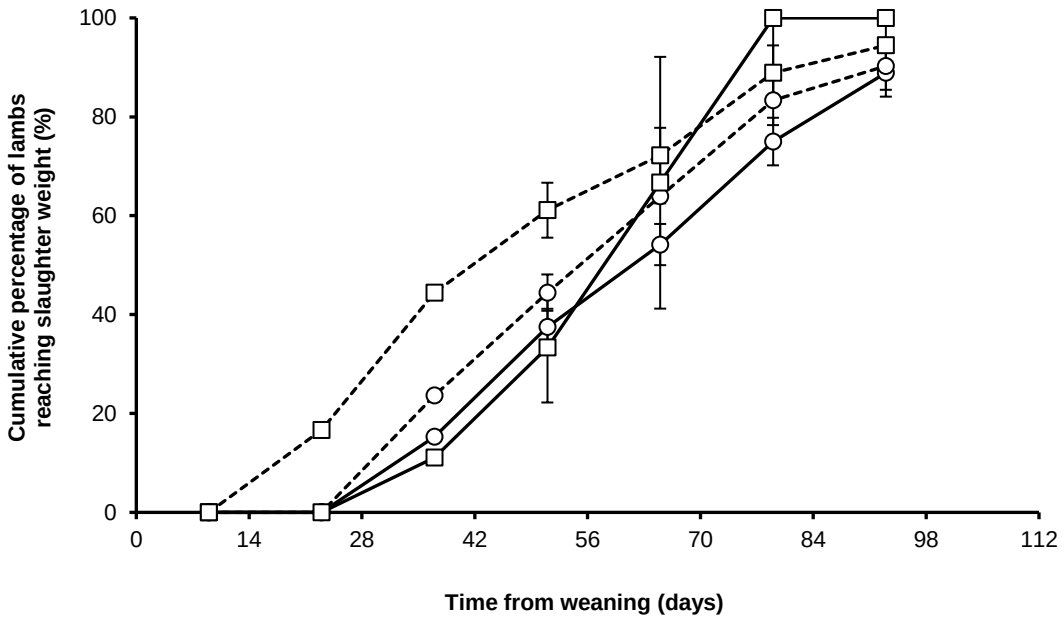


Figure 5.21 Cumulative percentage (%) of weaned lambs that were suppressively drenched (squares) or exposed to a targeted selective treatment anthelmintic regime (circles) that subsequently grazed areas where ewes had been supplemented (solid line) or unsupplemented (dashed line) reaching slaughter weight above 38 kg in 2017.

5.4.1.7 Milk production and milk composition

The mean milk production per four hours and milk components over the lactation period are presented in Figure 5.22 until Figure 5.26. Overall, there was no difference in SCC (P=0.41) between treatments. The back-transformed means of SCC for supplemented and unsupplemented ewes were 311,889±1.53 and 201,372±1.24 cell/ml, respectively. No effect of time (P=0.86) nor an interaction between treatment and time (P=0.68) were detected on SCC.

Milk production per four hours

There was no significant effect of supplementation (P=0.99), nor there was a treatment x time interaction (P=0.12) on milk production per four hours. However, a significant effect of time was detected (P<0.001) on milk production per four hours, reflected by a decrease in milk production over the lactation period (Figure 5.22). The average milk yield from supplemented ewes were 1.11±0.06, 0.67±0.07, and 0.46±0.02 l/4 h at week four, week eight, and week 12 of lactation, respectively while those from unsupplemented ewes were 1.04±0.06, 0.65±0.03, and 0.52±0.003 l/4 h at week four, week eight, and week 12 of lactation, respectively.

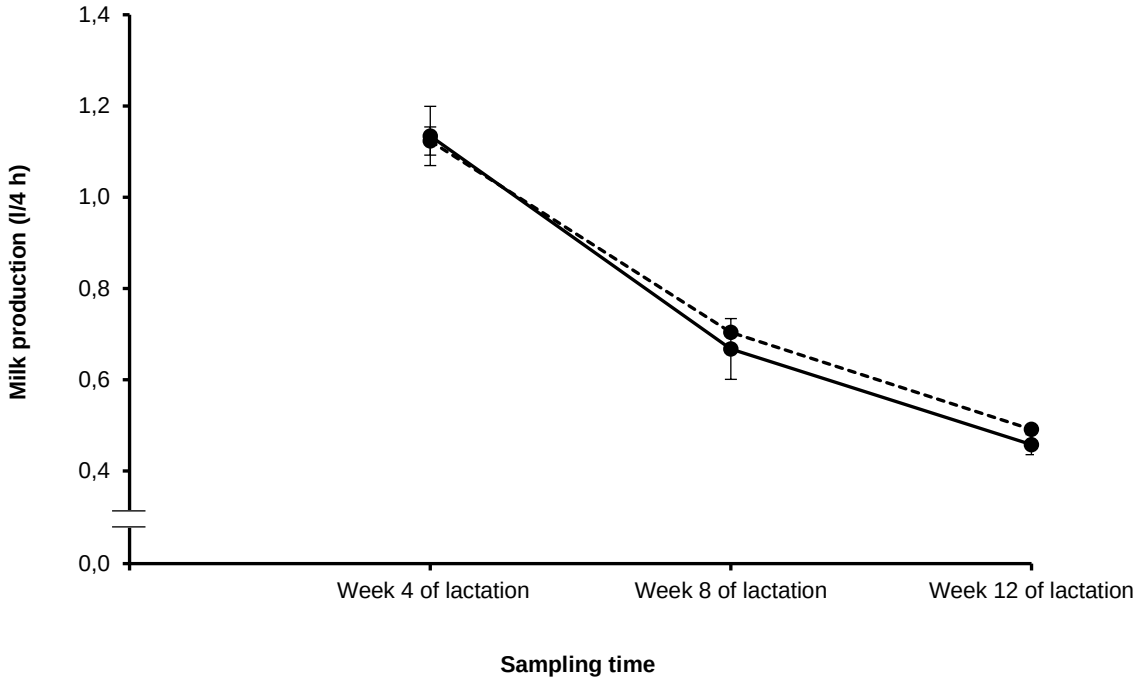


Figure 5.22 Mean milk production per four hours (l/4 h) from supplemented ewes (solid line) or unsupplemented ewes (dashed line) in 2017.

Fat content

The percentage of milk fat of supplemented ewes was similar to milk fat of unsupplemented ewes (P=0.98) with an average of 6.62±0.13 and 6.67±0.14%, respectively. Overall, no supplementation by time interaction (P=0.09) was observed. The milk fat percentage ranged from 5.73 to 7.71% among all sheep (Figure 5.23). In both groups, milk fat increased gradually throughout the lactation period (P<0.001).

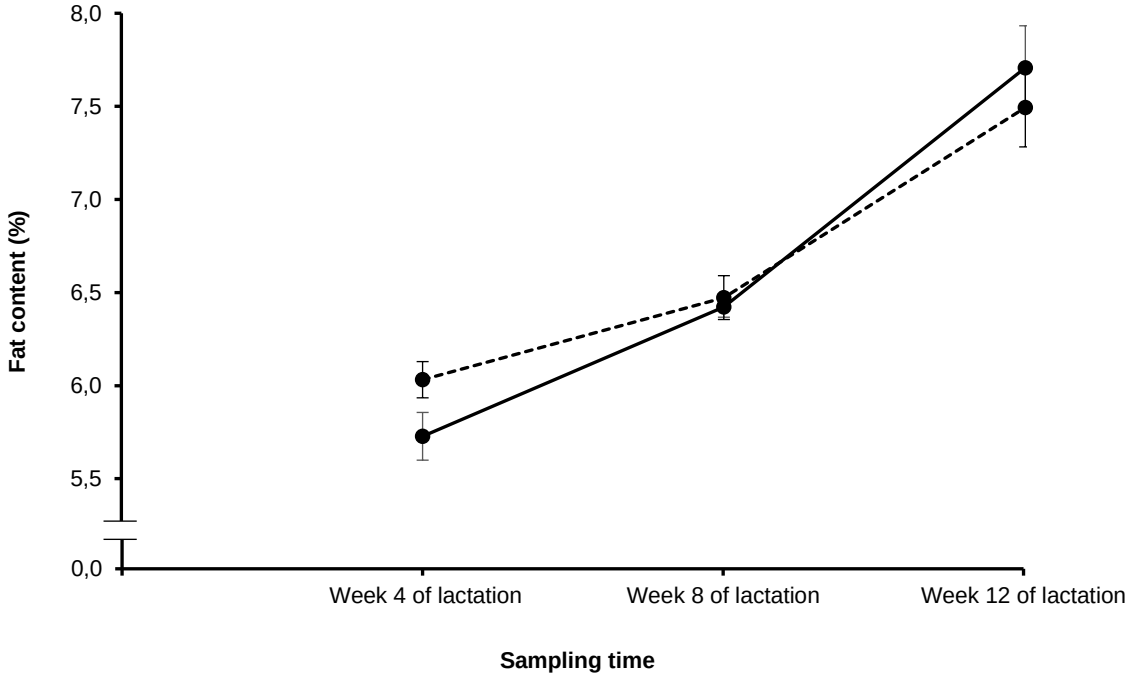


Figure 5.23 Mean fat contents (%) of milk from supplemented ewes (solid line) or unsupplemented ewes (dashed line) in 2017.

Protein content

Protein content (%) of milk is given in Figure 5.24. Overall, the percentage of milk protein in both groups was similar (P=0.17). There was no supplementation x time interaction (P=0.99) on the percentage of milk protein. However, there was an effect of time (P=0.05), reflecting a gradual increase of protein content in both groups over the lactation period.

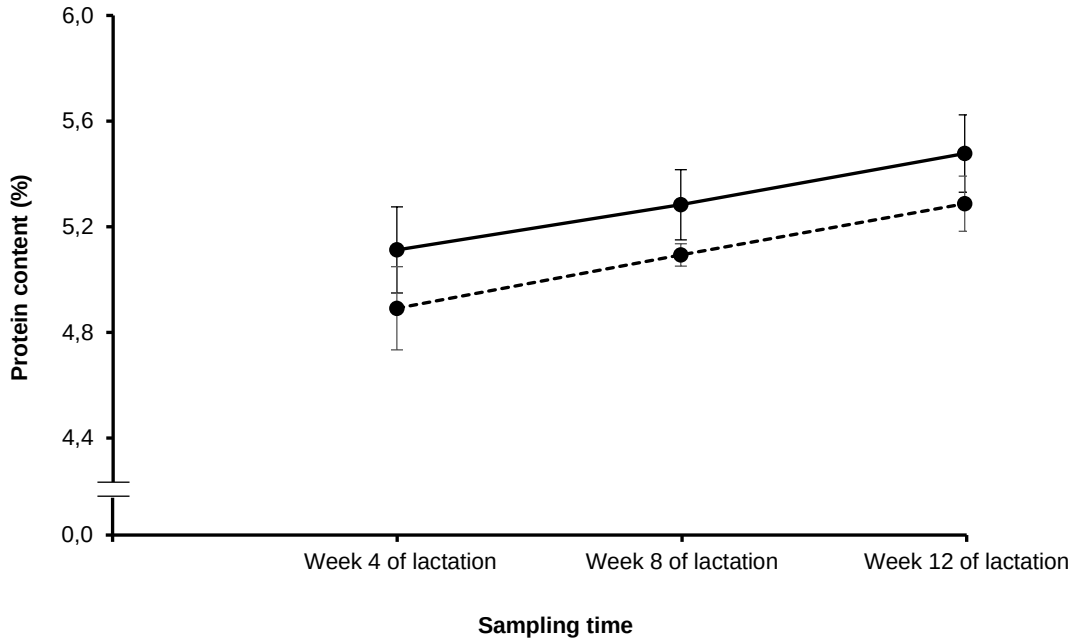


Figure 5.24 Mean protein contents (%) of milk from supplemented ewes (solid line) or unsupplemented ewes (dashed line) in 2017.

Lactose content

There was no significant difference (P=0.87) in lactose contents of the milk from supplemented ewes and unsupplemented ewes, with the average of 5.37±0.06% for supplemented ewes and of 5.38±0.06% for unsupplemented ewes (Figure 5.25). There was no interaction between treatment and time (P=0.82). The lactose content of both groups significantly decreased (P<0.001) over the lactation period.

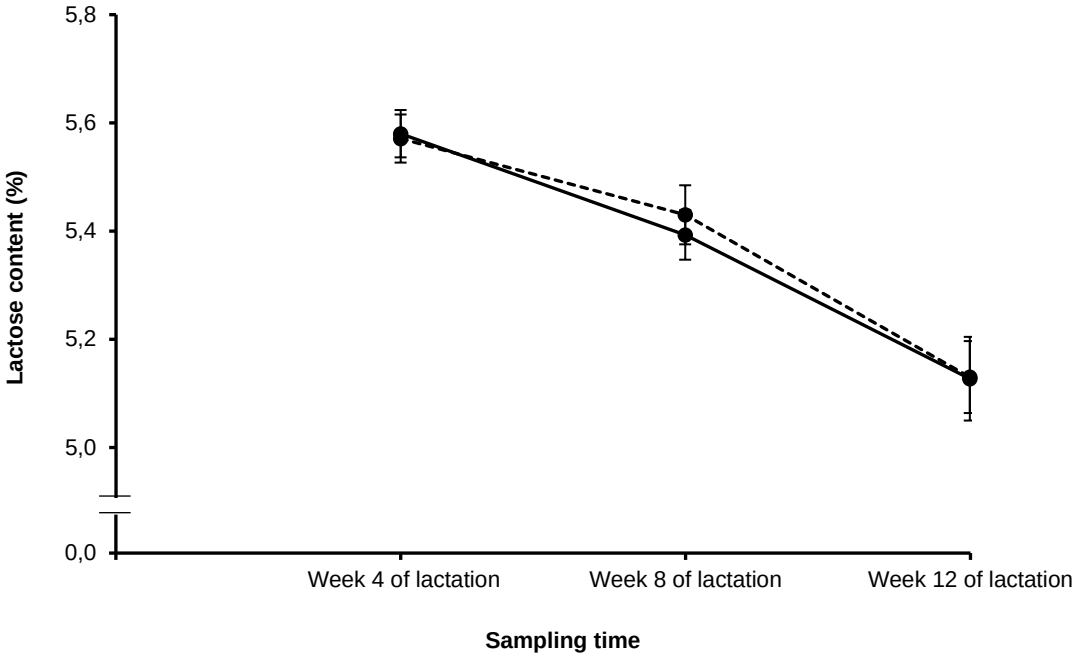


Figure 5.25 Mean lactose contents (%) of milk from supplemented ewes (solid line) or unsupplemented ewes (dashed line) in 2017.

Total solids content

The total solids contents of milk from supplemented ewes were also similar to the total solids' contents of milk from unsupplemented ewes (P=0.44). There was no treatment x time interaction (P=0.32). The mean total solids content for milk from supplemented ewes was 17.85±0.17% while that from unsupplemented ewes was 17.69±0.11%. Total solids content increased steadily in both groups (P<0.001) throughout the lactation period (Figure 5.26).

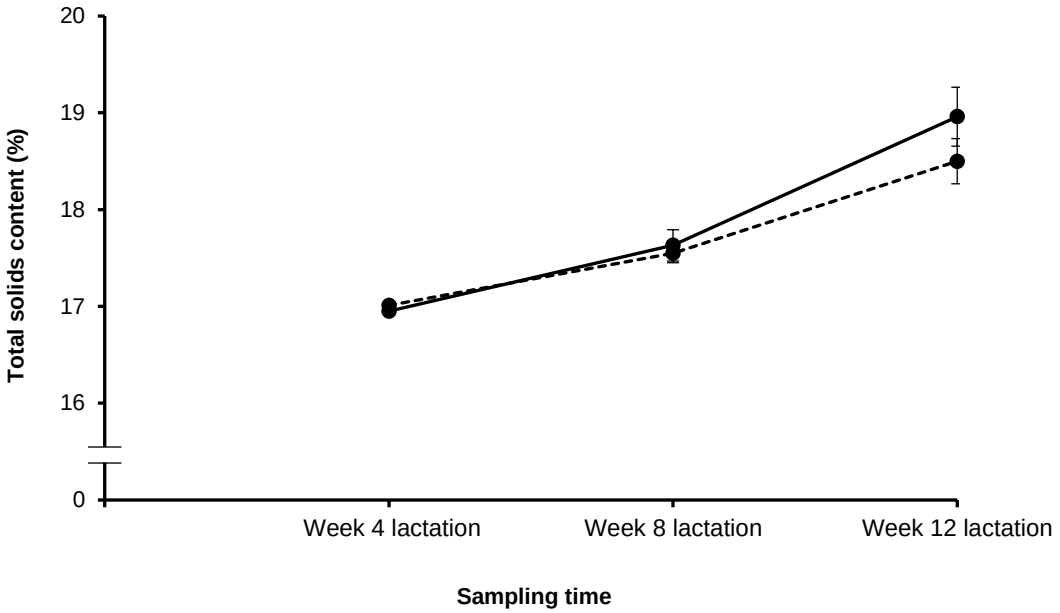


Figure 5.26 Mean total solids contents (%) of milk from supplemented ewes (solid line) or unsupplemented ewes (dashed line) in 2017.

5.4.1.8 The relationships between lamb growth rate and milk production

The relationship between lamb ADG and milk production of ewe per four hours during the lactation period are given in Figure 5.27a until 5.27c. Overall, the relationship between milk production of the ewe (x, l/4 h) and ADG of lamb (y, g/d) for three sampling times, did not differ between supplemented and unsupplemented ewes (P=0.245). Lamb ADG before weaning was affected by sampling time (P<0.001) and ewe milk production/four hours (P=0.001). No interactions between sampling time and supplementation (P=0.150), sampling time and milk production (P=0.059), nor sampling time x supplementation x milk production (P=0.168) were detected. Nonetheless, an interaction between supplementation and milk production (P=0.002) was observed, reflected by significantly increased lamb ADG of the unsupplemented group as milk production increased.

There were very weak associations between lamb growth rate and milk production of ewes at different lactation stages, except for the unsupplemented group in week four (moderate relationship) and only the regression in week four of the unsupplemented group that was statistically significant (P<0.001). At week four, the relationship between lamb ADG and milk production was y = -0.828x + 261.09 (P=0.97, r^2=0.000033) for supplemented group and y = 116.24x + 115.73 (P<0.001, r^2=0.4465) for unsupplemented group. At week eight, the regression equation was y = 12.971x + 248.83 (P=0.62, r^2=0.0051) for supplemented group and y = 17.14x + 246.75 (P=0.68, r^2=0.0043) for unsupplemented cohort. While at week 12, the regression equation was y = 92.336x + 183.36 (P=0.19, r^2=0.0545) for supplemented group and y = 49.019x + 212.42 (P=0.60, r^2=0.0157) for unsupplemented group.

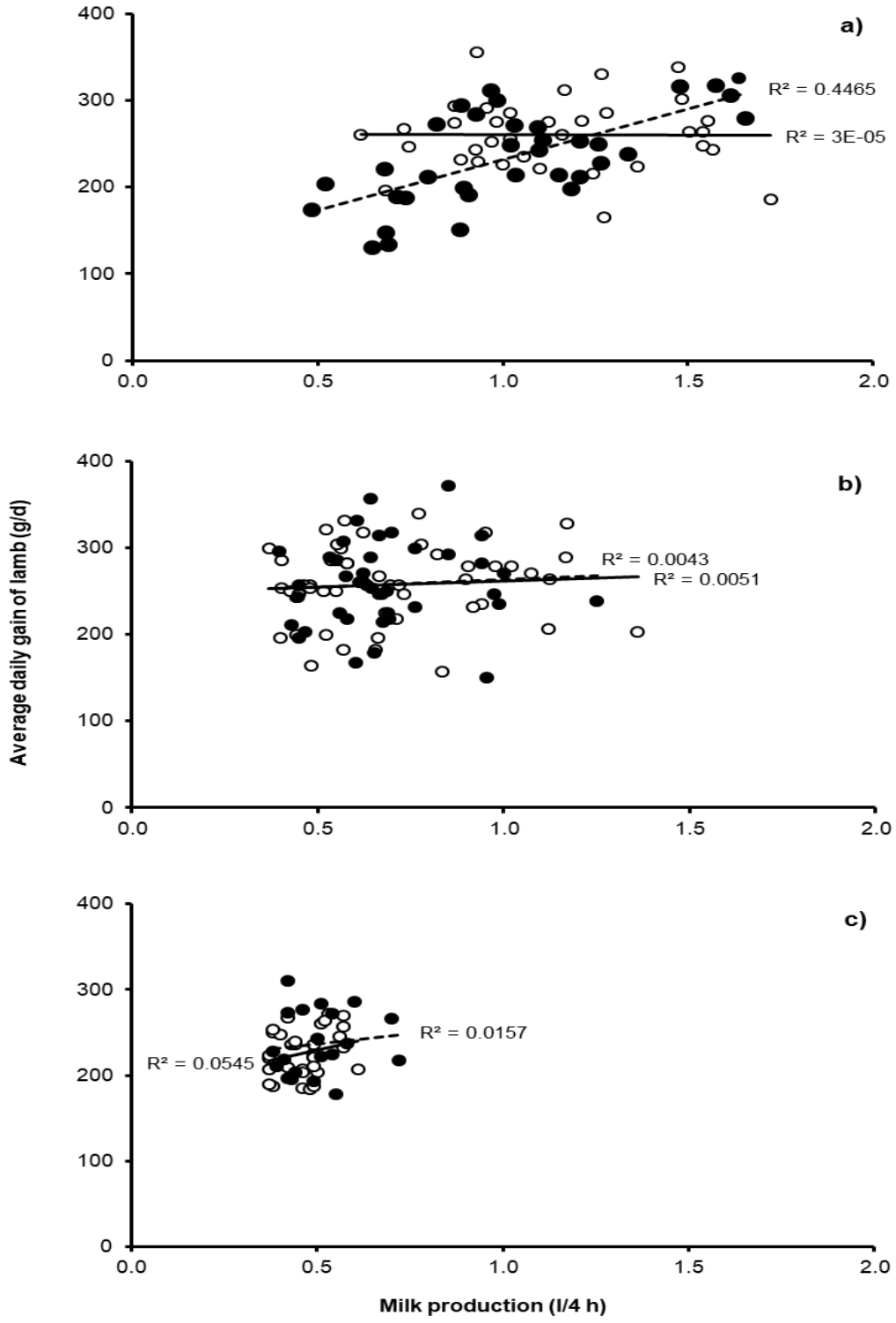


Figure 5.27 **Relationships between average daily gain of lamb (g/d) and milk production of ewe (l/4 h) of the supplemented group (open circles, solid line) or unsupplemented group (closed circles, dashed line) at week 4 of lactation (a), week 8 of lactation (b), and week 12 of lactation (c) in 2017.**

5.4.2 Control groups

5.4.2.1 Faecal egg count (FEC)

Mean back-transformed FEC of ewes is summarised in Figure 5.28. Supplementation of ewes during the first four weeks of lactation did not affect ewe FEC (P=0.99). However, effects of long-acting anthelmintic (P<0.001), time (P<0.001), and drench x time interaction were observed, reflected by gradual increases of FEC of infected ewes throughout the lactation period. Drenched groups were treated with long-acting anthelmintic at the end of supplementation; hence, their FECs gradually decreased to less than 100 epg until week 12 of lactation. The highest egg outputs in the faeces of infected ewes from supplemented and unsupplemented groups were detected at week 12 of lactation (weaning), with mean values of 1,400 epg and 650 epg, respectively.

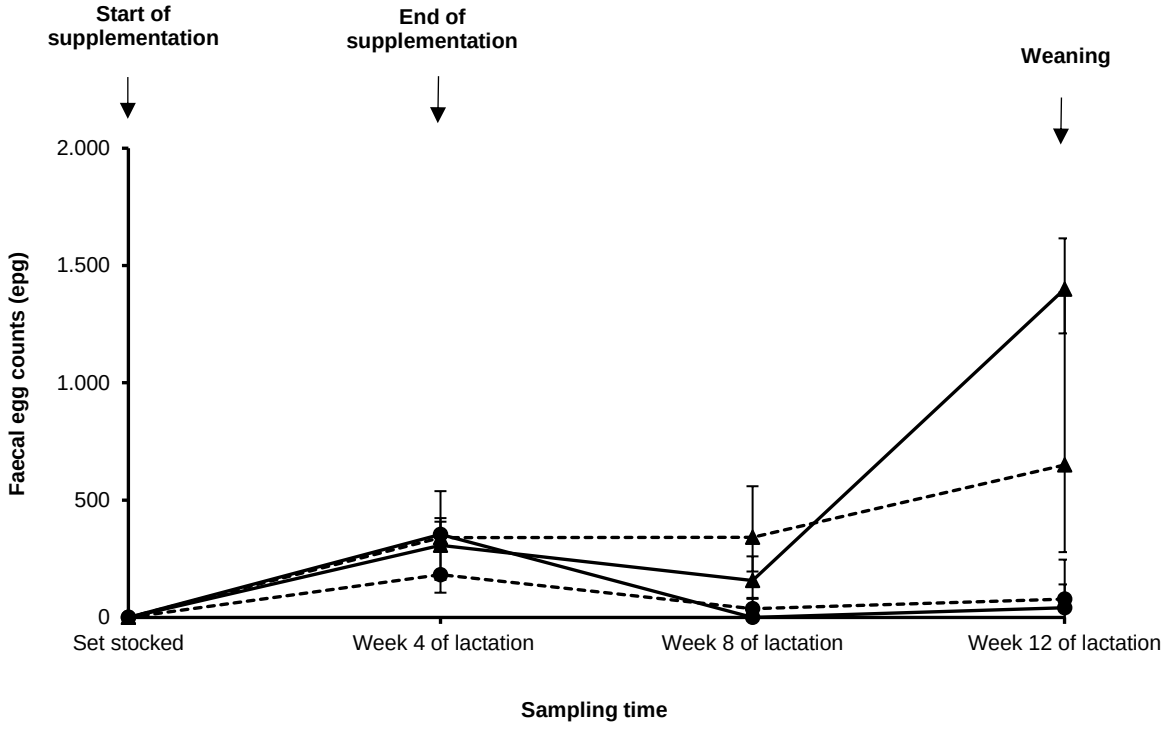


Figure 5.28 Back-transformed (log10 (count + 100)) means of faecal egg count of ewes that were supplemented during the first four weeks of lactation (solid line) or not (dashed line) and undrenched (triangles) or drenched (circles) with long-acting anthelmintic at the end of supplementation in 2017.

5.4.2.2 Serum antibodies

IgA

Mean serum L3 *T. colubriformis*-specific IgA profiles are given in Figure 5.29. There were no effects of supplementation (P=0.35) and drench (P=0.9) on IgA responses. However, there was an effect of time (P<0.001) and an interaction between supplementation x time (P=0.001), reflected by steady declines of *T. colubriformis*-specific IgA absorbance of unsupplemented ewes over the lactation period, while serum IgA absorbance of supplemented groups decreased after week eight of lactation.

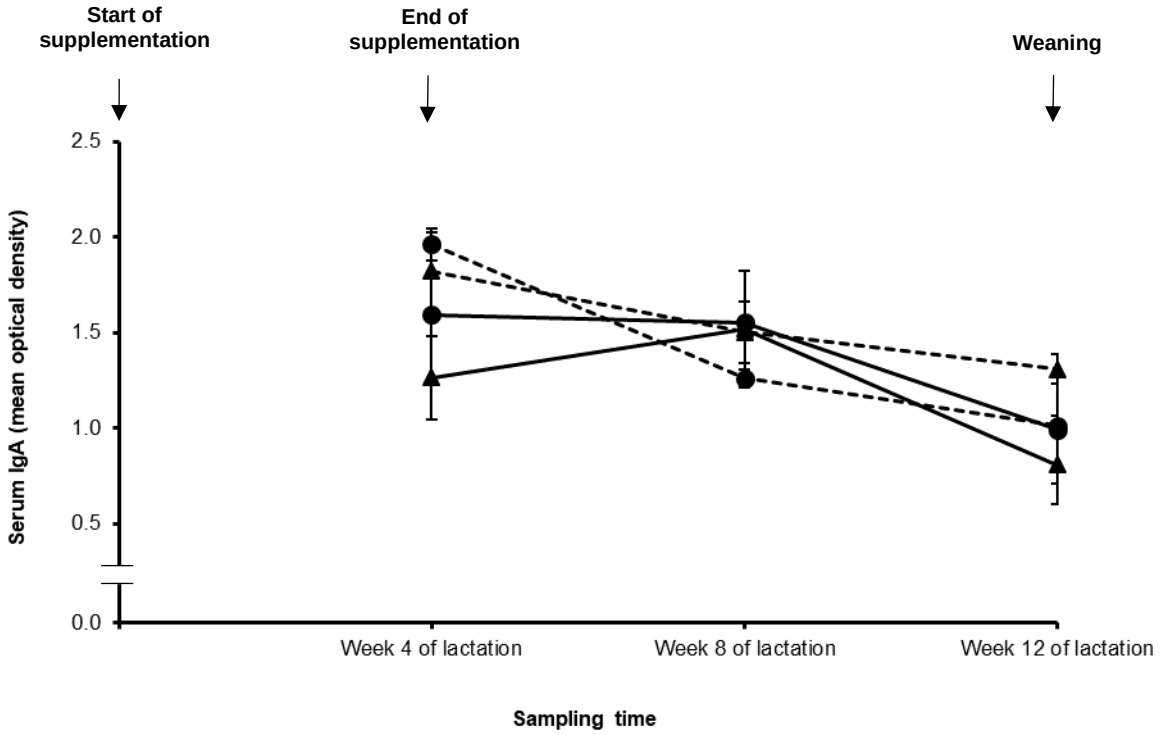


Figure 5.29 Mean optical density for serum specific IgA antibody against L3 *T. colubriformis* of ewes that were supplemented during the first four weeks of lactation (solid line) or not (dashed line) and undrenched (triangles) or drenched (circles) with long-acting anthelmintic at the end of supplementation in 2017.

IgG

Mean serum L3 *T. colubriformis*-specific IgG profiles for supplemented and unsupplemented ewes, drenched or undrenched are given in Figure 5.30. Overall, there was an effect of time (P<0.001) reflecting plateauing IgG absorbance initially in all groups followed by decreasing absorbances from week eight until week 12 of lactation. There was no effect of supplementation (P=0.66) nor drench (P=0.37) in IgG levels, and there were no interactions either between supplementation and drench (P=0.75), supplementation and time (P=0.08), drench and time (P=0.97), or supplementation x drench x time (P=0.99).

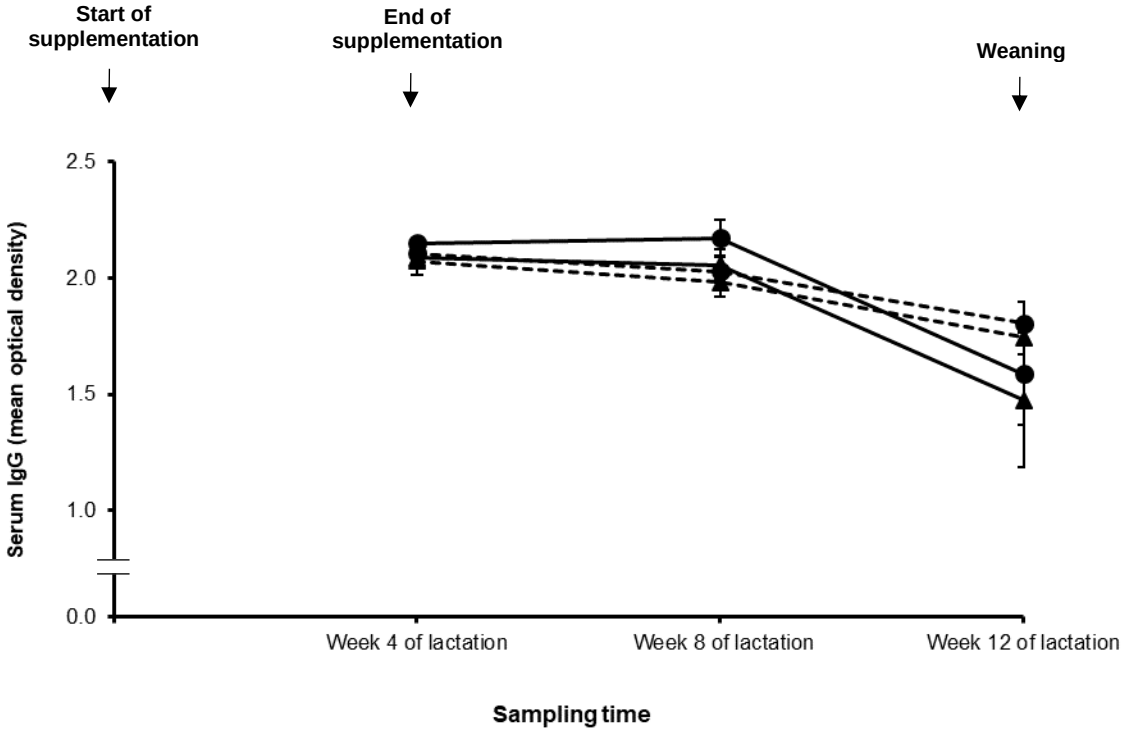


Figure 5.30 Mean optical density for serum specific IgG antibody against L3 *T. colubriformis* of ewes that were supplemented during the first four weeks of lactation (solid line) or not (dashed line) and undrenched (triangles) or drenched (circles) with long-acting anthelmintic at the end of supplementation in 2017.

5.4.2.3 Serum protein concentrations

Serum albumin concentration

Mean serum albumin concentrations are shown in Figure 5.31. There was no difference in serum albumin concentrations between supplemented and unsupplemented groups (P=0.07). Nevertheless, an effect of drench (P=0.03) and time (P=0.002) was detected, reflecting a gradual increase of serum albumin levels of drenched groups until reached a peak at week eight of lactation followed by the decline in concentrations at weaning, except for unsupplemented and undrenched ewes. Serum albumin level of unsupplemented and undrenched ewes slightly decreased over the lactation period.

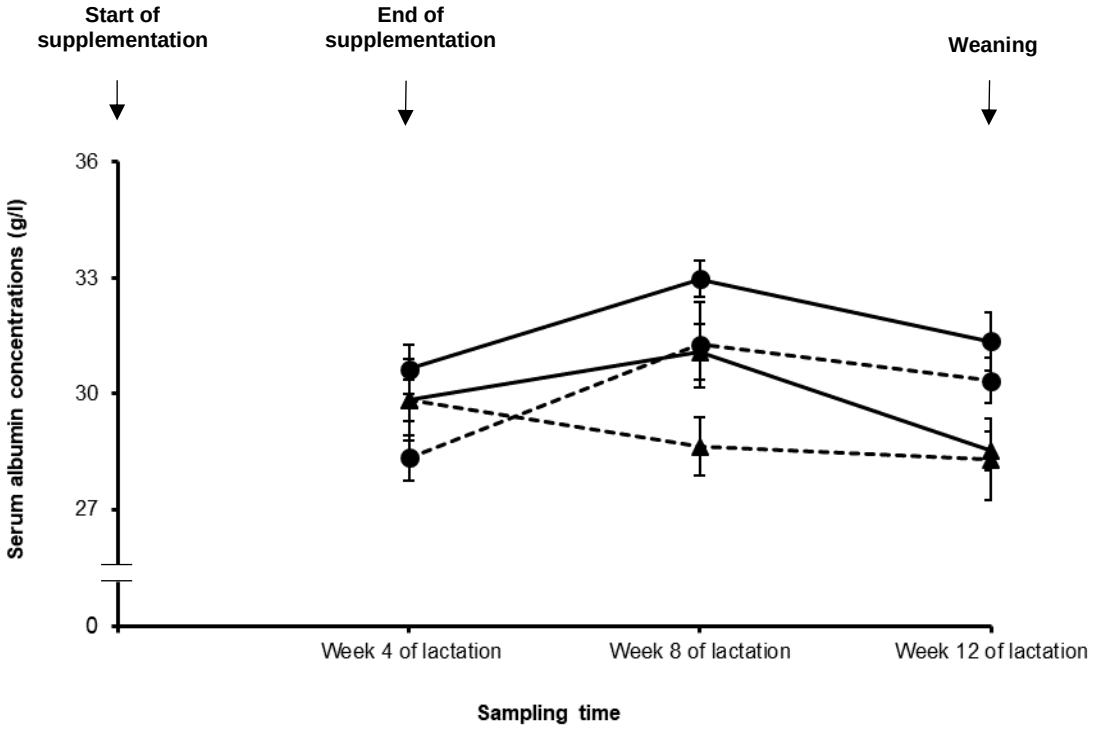


Figure 5.31 Mean serum albumin concentrations (g/l) of ewes that were supplemented during the first four weeks of lactation (solid line) or not (dashed line) and undrenched (triangles) or drenched (circles) with long-acting anthelmintic at the end of supplementation in 2017.

Total serum protein concentration

Mean total serum protein concentrations of all groups are presented in Figure 5.32. Overall, there was no effect of supplementation (P=0.53) nor drench (P=0.8) on total serum protein concentrations. However, there was an effect of time (P=0.01), reflecting initial decreasing concentrations until reached a peak at week eight of lactation followed by gradual reductions at weaning. Conversely, mean total serum protein concentration of unsupplemented and undrenched group decreased initially until week eight of lactation but then slightly increased at weaning.

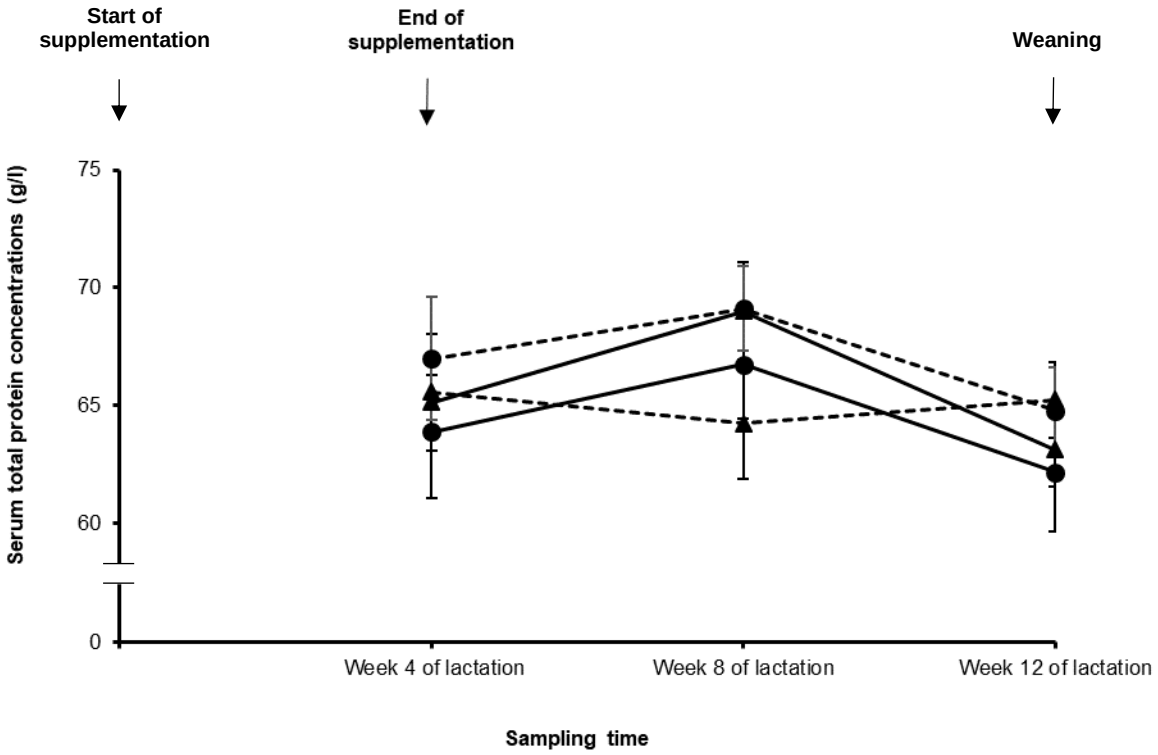


Figure 5.32 Mean total serum protein concentrations (g/l) of ewes that were supplemented during the first four weeks of lactation (solid line) or not (dashed line) and undrenched (triangles) or drenched (circles) with long-acting anthelmintic at the end of supplementation in 2017.

Serum globulin concentration

Changes in mean serum globulin concentrations of all groups are given in Figure 5.33. Overall, there were no effects of supplementation (P=0.17), drench (0.56), nor time (P=0.06) on serum globulin concentrations. The mean serum globulin concentrations for supplemented-undrenched, supplemented- drenched, unsupplemented-undrenched, and unsupplemented-drenched ewes were 35.95±0.75, 32.62±1.19, 36.09±0.92, and 36.98±1.37 g/l, respectively.

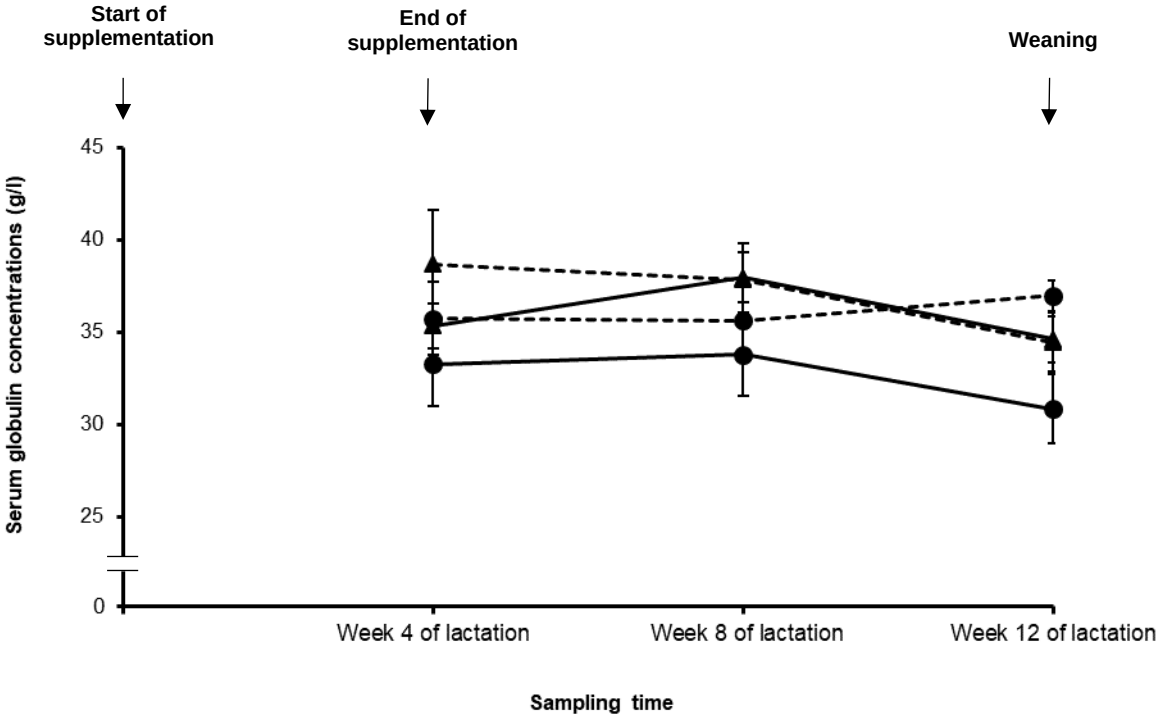


Figure 5.33 Mean serum globulin concentrations (g/l) of ewes that were supplemented during the first four weeks of lactation (solid line) or not (dashed line) and undrenched (triangles) or drenched (circles) with long-acting anthelmintic at the end of supplementation in 2017.

Serum urea concentration

Mean serum urea concentrations of supplemented and unsupplemented ewes, drenched or undrenched are shown in Figure 5.34. There was an effect of time (P<0.001) on serum urea concentrations and an interaction between supplementation and time (P=0.01), reflecting a gradual decrease of the concentrations of all groups but being higher in supplemented groups, followed by a plateau at weaning. However, serum urea concentrations of supplemented groups at weaning were lower than unsupplemented cohorts. No effects of supplementation (P=0.6) nor drench (P=0.93) were observed on serum urea concentrations.

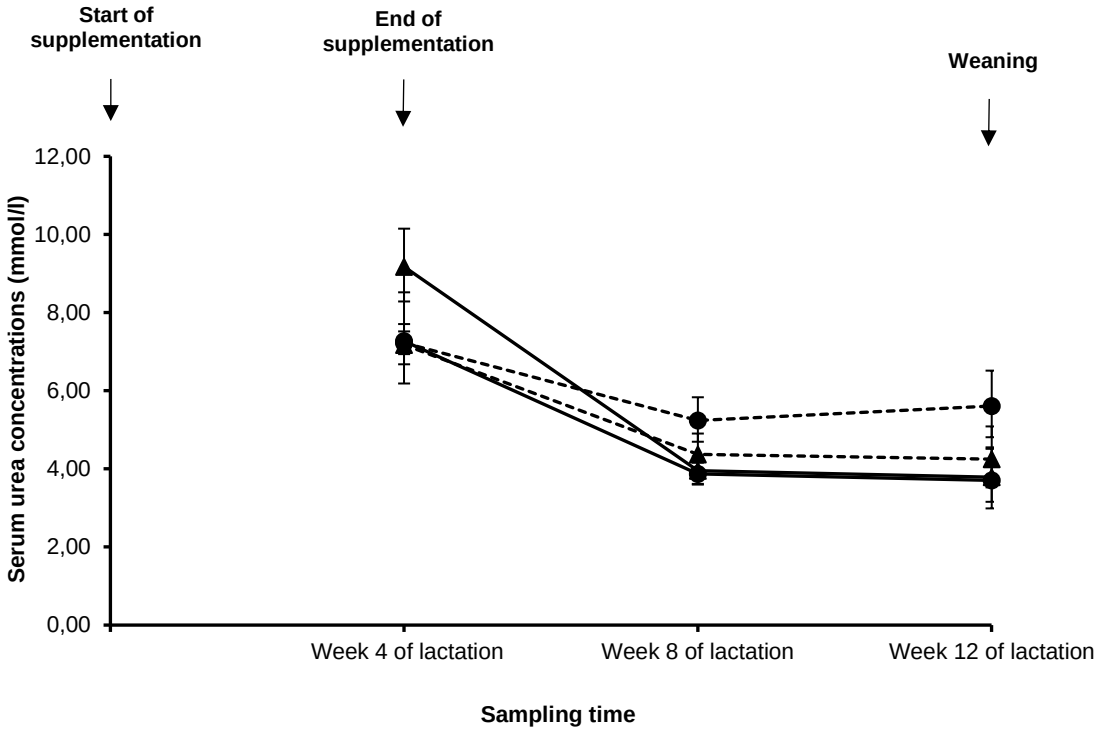


Figure 5.34 Mean serum urea concentrations (mmol/l) of ewes that were supplemented during the first four weeks of lactation (solid line) or not (dashed line) and undrenched (triangles) or drenched (circles) with long-acting anthelmintic at the end of supplementation in 2017.

Serum phosphorus concentration

Mean serum phosphorus concentrations are given in Figure 5.35. Overall, there were no effects of supplementation (P=0.7), drench (P=0.57), nor time (P=0.29) on serum phosphorus concentrations. No interactions between supplementation and drench (P=0.60), supplementation x time (P=0.25), drench x time (P=0.79), nor supplementation x drench x time (P=0.36) were detected on serum phosphorus levels.

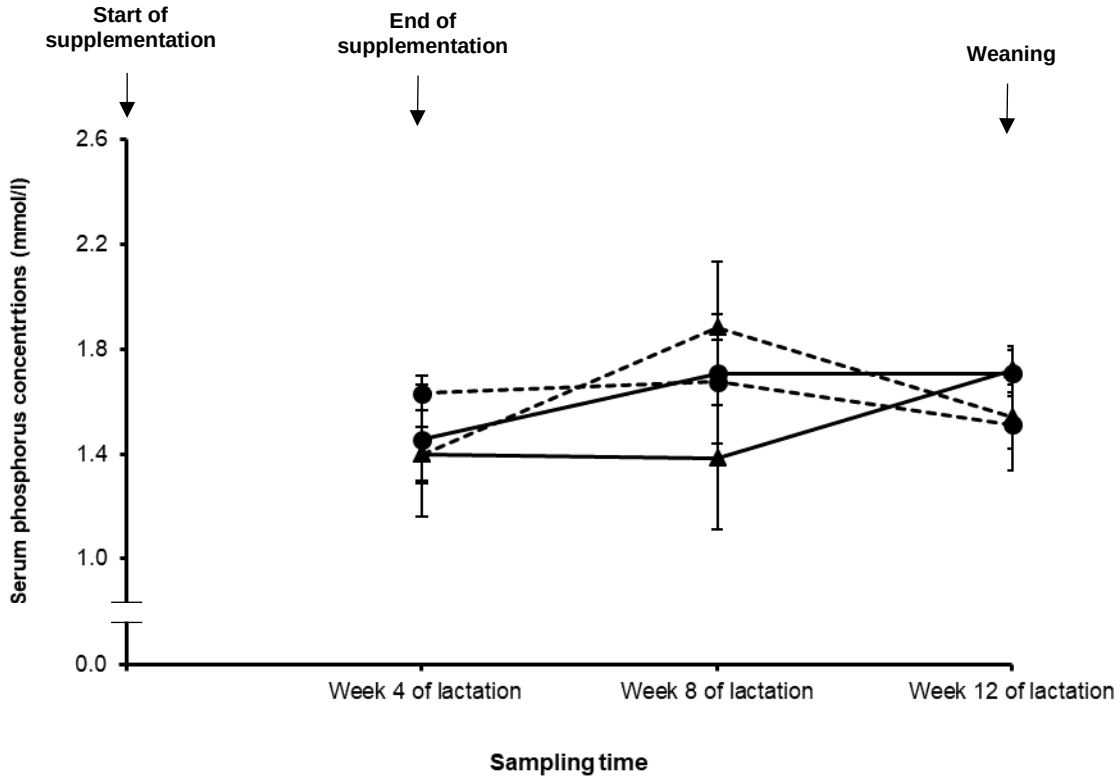


Figure 5.35 Mean serum phosphorus concentrations (mmol/l) of ewes that were supplemented during the first four weeks of lactation (solid line) or not (dashed line) and undrenched (triangles) or drenched (circles) with long-acting anthelmintic at the end of supplementation in 2017.

5.4.2.4 Ewes and lambs performance throughout lactation

Live weight (LW) of ewes

Mean LW changes of ewes from all treatments are given in Figure 5.36. There were no differences between LW of supplemented and unsupplemented groups (P=0.27) and between drenched and undrenched groups (P=0.08). However, there was an effect of time (P=0.003) and an interaction between drench and time (P=0.01), reflecting decreased LW of undrenched groups from week eight to week 12 of lactation.

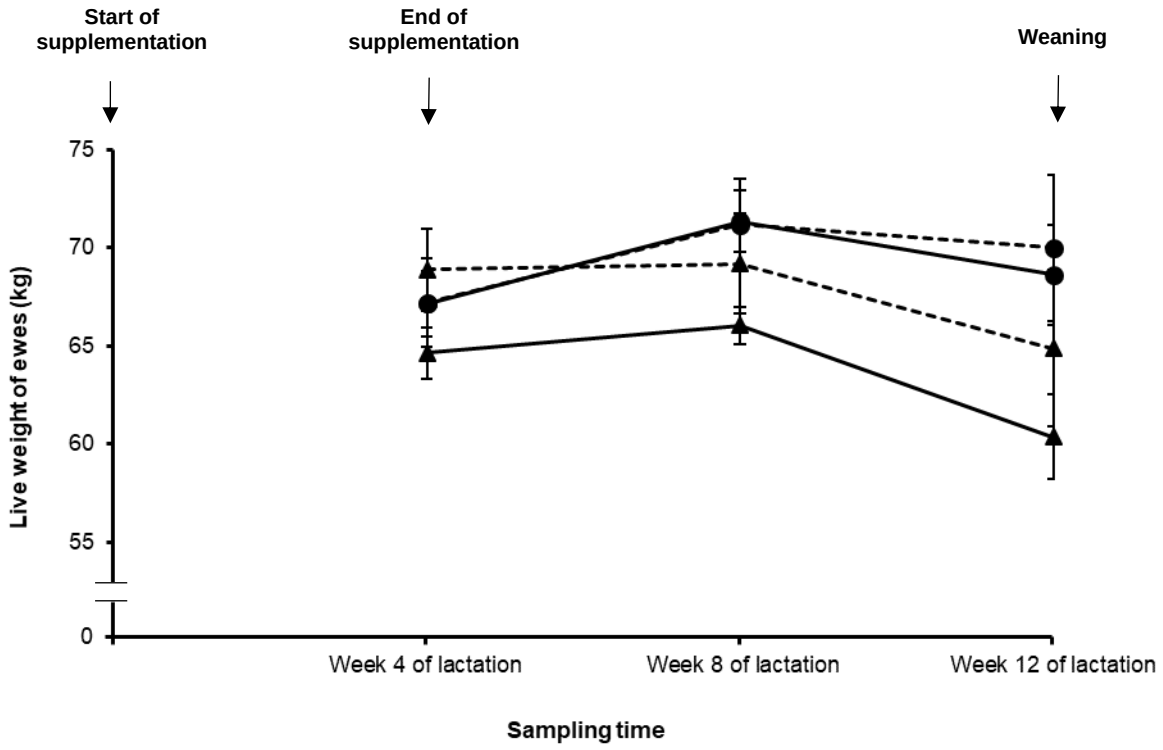


Figure 5.36 Mean live weight changes of ewes that were supplemented during the first four weeks of lactation (solid line) or not (dashed line) and undrenched (triangles) or drenched (circles) with long-acting anthelmintic at the end of supplementation in 2017.

Body condition score (BCS) of ewes

Mean BCS changes of ewes for all groups are presented in Figure 5.37. There were effects of supplementation during the first four weeks of lactation (P=0.05) and time (P=0.05) on ewe BCS, reflecting a decline in ewe BCS throughout the lactation period, which was higher in unsupplemented ewes than supplemented counterparts. However, no effect of drench (P=0.91) was observed. BCS of unsupplemented-undrenched ewes and unsupplemented-drenched ewes was reduced by 19% and 15%, respectively, from week four to week 12 of lactation. However, the BCS of both supplemented groups was slightly increased after week eight of lactation.

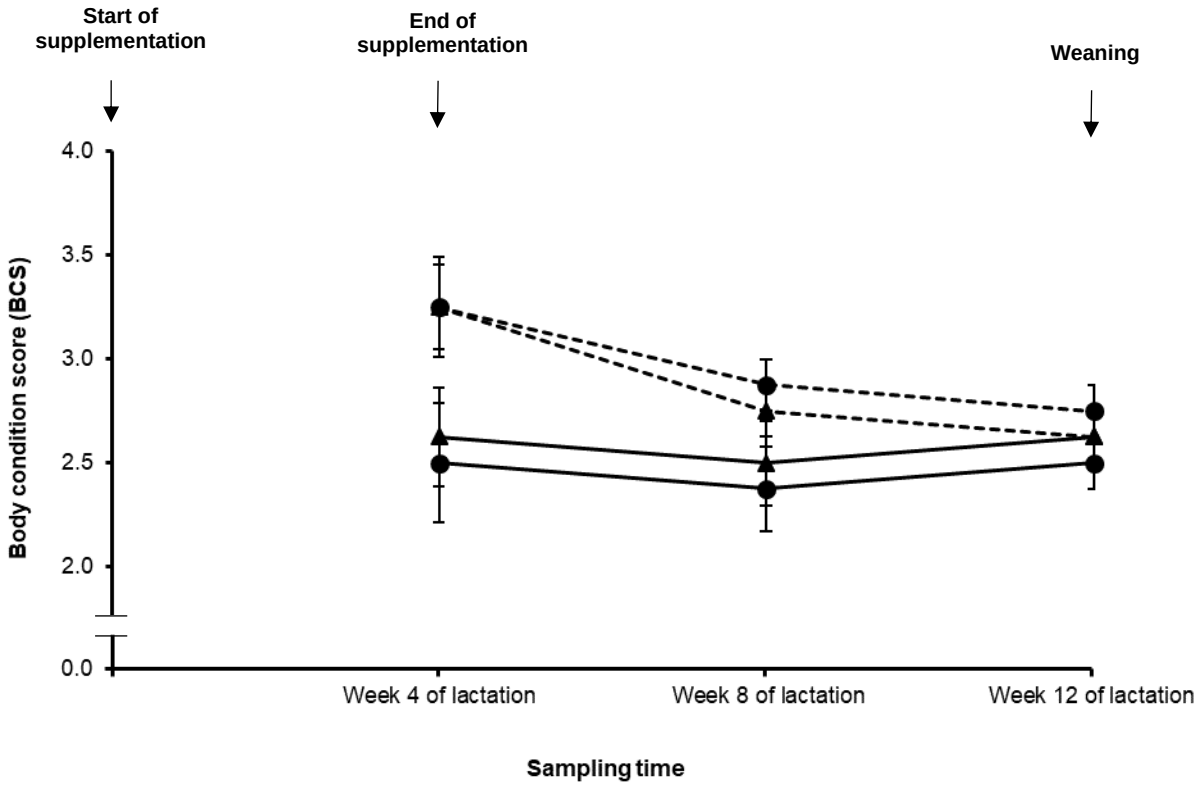


Figure 5.37 Mean body condition score of ewes that were supplemented during the first four weeks of lactation (solid line) or not (dashed line) and undrenched (triangles) or drenched (circles) with long-acting anthelmintic at the end of supplementation in 2017.

Live weight (LW) of lamb

Changes in mean LW of lambs reared by supplemented or unsupplemented ewes, drenched or undrenched, are shown in Figure 5.38. Overall, no effects of supplementation (P=0.55) nor drench (P=0.66) were detected on the LW of lambs from birth until weaning. However, a significant effect of time was observed (P<0.001), reflecting a steady increase in lamb LW of all groups throughout the lactation period. No evidence of interactions among factors was detected (P>0.05 for all) on lamb LW throughout the lactation period.

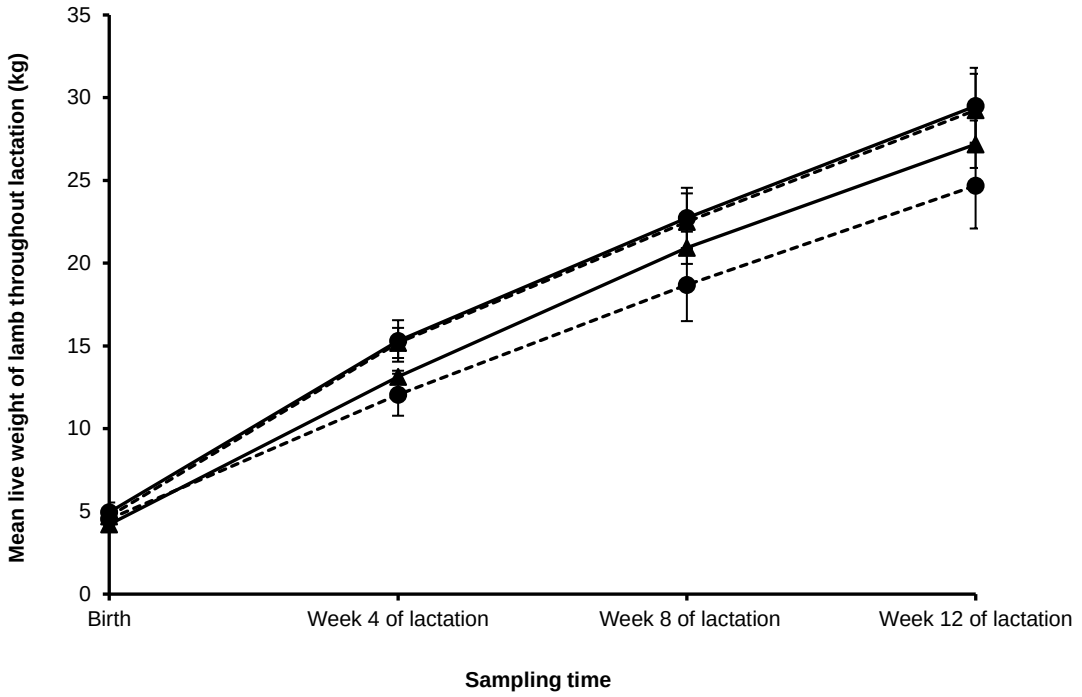


Figure 5.38 Mean live weight changes of lambs reared by supplemented ewes that were supplemented during the first four weeks of lactation (solid line) or not (dashed line) and undrenched (triangles) or drenched (circles) with long-acting anthelmintic at the end of supplementation in 2017.

Average daily gain (ADG) of lamb

The average daily gain of lambs throughout the lactation period are given in Figure 5.39. No effects of supplementation (P=0.58) nor drench (P=0.43) were observed on mean ADG of lambs raised by supplemented or unsupplemented ewes, drenched or undrenched. However, a significant effect of time (P=0.05) was detected, reflected by a decrease in ADG over the lactation period. Mean ADG of lamb raised by supplemented and undrenched ewes was 256.17±12.21 g/d while ADG of lamb raised by supplemented and drenched ewes was 268.57±14.45 g/d. Mean ADG of lamb raised by unsupplemented ewes, undrenched or drenched 273.89±19.93 was and 226.60±14.12 g/d, respectively.

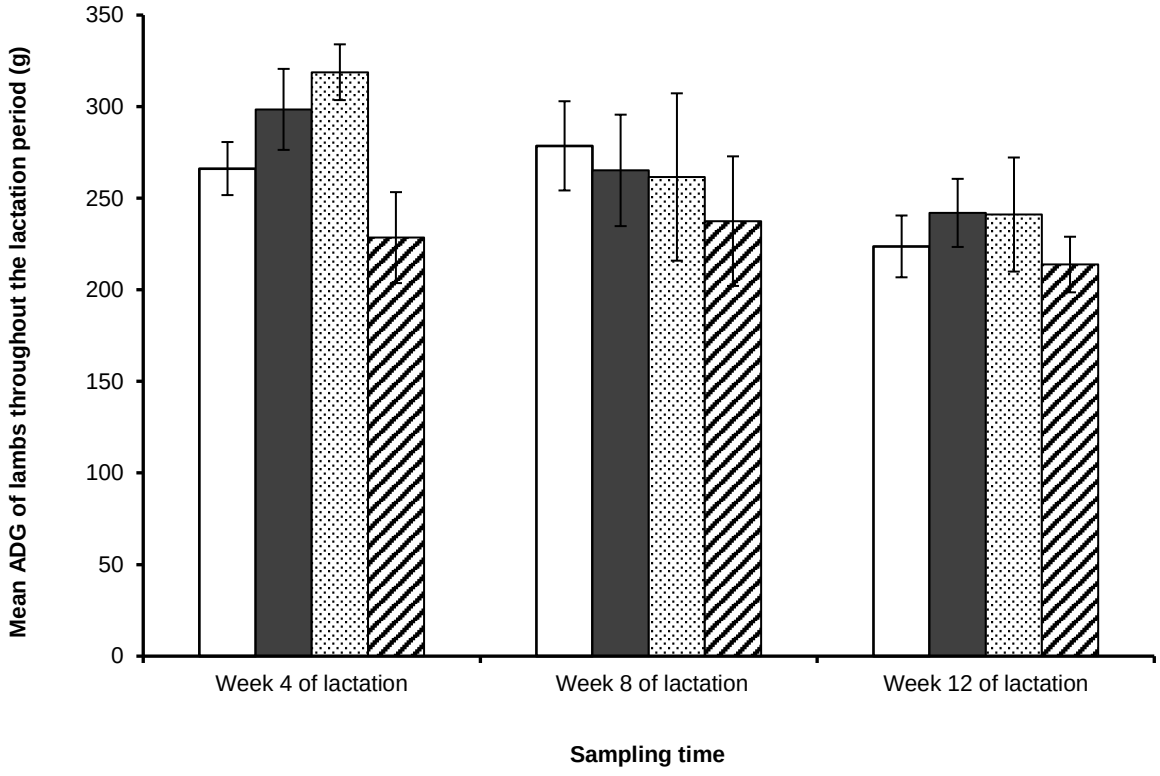


Figure 5.39 Changes in the average daily gain (g) of lambs reared by ewes that were supplemented during the first four weeks of lactation and undrenched (□), by supplemented and drenched ewes (■), by unsupplemented and undrenched ewes (▩), or by unsupplemented and drenched ewes (▨) in 2017.

Weight of lamb weaned per ewe

The weight of lamb weaned per ewe (WLWE) of the supplemented group and unsupplemented groups, drenched or undrenched are shown in Figure 5.40. Overall, supplementation (P=0.16) and drench (P=0.19) did not affect the WLWE. The average WLWE for lambs reared by supplemented and undrenched ewes, supplemented and drenched ewes, unsupplemented and undrenched ewes, or unsupplemented and drenched ewes were 54.38±2.87, 59.0±5.19, 49.38±4.61, and 49.38±5.19 kg, respectively.

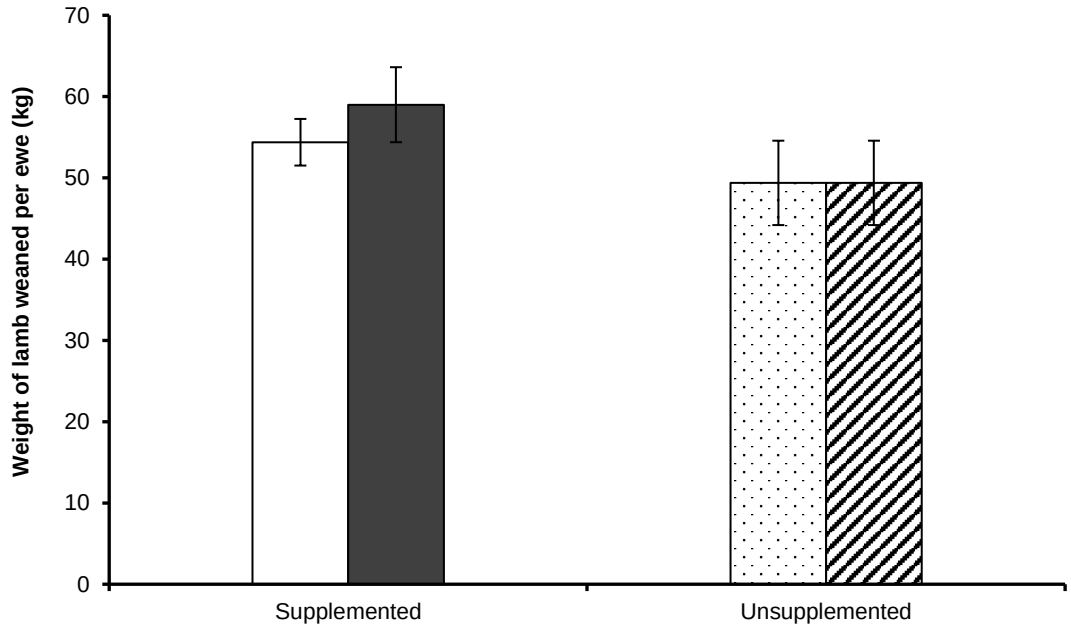


Figure 5.40 Mean weight of lamb weaned per ewe of supplemented and undrenched ewes (□), supplemented and drenched ewes (■), unsupplemented and undrenched ewes (▨), or unsupplemented and drenched ewes (▨) in 2017.

5.5 Discussion

The current study aimed to evaluate the effect of supplementation of ewes during the first four weeks of lactation following a short-acting drench pre-lambing and its epidemiological benefit to grazing lambs after weaning. The results showed the benefits of ewe supplementation when the existing population is removed, such as the difference in FEC, the greater serum albumin concentrations, and the consistently higher milk protein contents. However, these benefits were not sufficient to provide a measurable and consistent epidemiological benefit to the grazing lambs.

Based on the observation on each mob, supplementation did change the profile of FEC within each mob, suggesting that supplemented ewes were better able to withstand the PPR than unsupplemented counterparts. Results of the current study showed that the FEC of ewes increased during the first four weeks of lactation following a short-acting drench at set stocking. Similar to the previous trials (Chapter 4), the FEC of ewes at week eight of lactation in the current study decreased after reaching a peak at week four of lactation with the mean reduction of 110 epg for supplemented ewes and 34 epg for their counterparts. The low egg counts observed in week eight of lactation of the supplemented group occurred in 74% of ewes (consisted of 45% of ewes with 0 epg and 55% of ewes with FEC more than 100 epg). On the other hand, in the unsupplemented group, from 60 ewes with faecal samples collected, 81.67% of them had FEC more than 100 epg. The increase of FEC at week four of lactation in all groups is not surprising since the anthelmintic used in this study was a short-acting drench administered orally, having a transient effect (Sutherland & Scott, 2010) so that animals grazed on pasture were susceptible to reinfection shortly after treatment. The finding in the current study was in accordance with recent works which have shown that the relaxation of the established immunity of the ewe in the periparturient period can be prevented by protein supplementation (Donaldson et al., 1998, 2001; Houdijk et al., 2000).

It is speculated that the small reduction in FEC of the supplemented ewe in the current study was due to nutrient partitioning, where the majority of nutrients from supplementation during early lactation until peak lactation (week four) were used by the ewe to increase milk production, and perhaps not enough nutrient from supplementation was given to increase immunity. As the age of the lambs increased, the demand for nutrients for milk production decreased, which allowed more nutrients available to increase ewe immunity. This interpretation would be in line with the nutrient partitioning framework proposed by Coop and Kyriazakis (1999) that the majority of scarce nutrients would be allocated to the prioritised reproductive effort (pregnancy and lactation) rather than to immune functions. Although not statistically significant, the average daily gain (ADG) of lambs raised by supplemented ewes tended to be higher than those reared by unsupplemented ewes (Figure

5.17). At week 4 of lactation, where milk production at the highest yield, the mean ADG of lambs raised by supplemented ewes was 7% higher than those reared by unsupplemented ewes, and there was a positive association between the milk yield of ewe and the growth of lamb. This result demonstrates the importance of milk in lambs' diet in their early life (Geenty, Clarke, & Wright, 1985). The mean growth rate of lambs reared by supplemented ewes at week 12 of lactation was 17% lower than that of the values at week four of lactation, while those raised by unsupplemented ewes was 12% lower than that of the ADG at week four of lactation. The reductions in the lamb growth rate of both groups during week 12 of lactation may be due to the decline of milk yield to more than 50% at weaning and the resultant decrease in the ratio of protein relative to energy supplied to the lambs.

Although not statistically significant, the reduction in FEC of ewes did appear to have a short-term benefit in terms of pasture larval levels. Pastures that had been grazed by supplemented ewes tended to have lower pasture larval (threefold lower on sampling date December 2017 and January 2018, Figure 5.6) than unsupplemented paddocks, which is reflected in the tendency for fewer L4 in the tracer lambs grazed areas where ewes had been supplemented (Table 5.1 and Table 5.2). This lower pasture contamination then resulted in a slight difference in the FEC profile of lambs, effectively delaying the increase in FEC in the lambs throughout the summer (Figure 5.3b). FEC of lambs after weaning increased significantly throughout the grazing season for both groups, although tended to be delayed in TST lambs grazing the areas where ewes had been supplemented, which reached a peak of 65 days after weaning compared with 51 days after weaning in TST lambs grazed unsupplemented areas. Coop and Jackson (2000) and Sargison, Bartram, and Wilson (2012) reported the benefit of anti-parasitic treatment of ewes during late pregnancy, including the elimination of worms, prevention of built-up parasitic burdens at pastures and consequently the reduced infection of their lambs during the periparturient period. Southcott (1971) recommended drenching ewe as a major source of infection to the lamb before or about lambing time in conjunction with the supply of uncontaminated herbage.

In comparison with sentinel lambs, the mean ADG of TST lambs which grazed the areas where ewes had been supplemented was 5% lower than sentinel lambs grazed the same areas. On the other hand, for lambs grazed the areas where ewes had been unsupplemented, the difference between the mean ADG of TST lambs and sentinel lambs was 16% (Figure 5.20). The difference in the growth rate of the sentinel lambs and TST lambs clearly shows that there was a substantial larval challenge, with the benefits of supplementation possibly masked due to the TST regime, although there was no difference in the number of anthelmintic treatments administered (Table 5.3). These results were supported by the previous studies that reported significant production losses caused by GIN

parasites, as the performance of grazing lambs is inversely correlated with the intensity of nematode parasites (Brunsdon & Vlassoff, 1982; Coop et al., 1985).

The reduction in FEC following supplementation in the present study was not accompanied by a rise in *T. colubriformis*-specific L3 IgA absorbance; instead, the values decreased over time. The absence of nutritional influences on serum *T. colubriformis*-specific L3 IgA absorbance probably indicates that the immune response to ingested larvae was not affected by supplementation, an observation which is in agreement with other studies that found no dietary effect on IgA of periparturient ewes (Houdijk, Kyriazakis, Coop, et al., 2001; Houdijk et al., 2005). Houdijk et al. (2005) observed that feeding treatment and the reproductive status of ewes did not significantly influence the optical density of plasma *T. circumcincta*-specific L3 IgA, which did not concur with a higher degree of parasitism in lactating ewes than barren ewes. In contrast with the findings in 2016, where serum *T. colubriformis*-specific L3 IgG absorbance slightly increased in both groups as the experiment progressed, in the current study, IgG absorbance in both groups decreased with time, with the decline being greater in unsupplemented ewes. The decrease of IgG in the current study was supported by Houdijk et al. (2003) that plasma antibodies (IgA, IgG, and IgE) of lactating ewes were generally reduced over time since those antibodies can all be secreted into the milk.

The higher serum albumin concentrations were observed in supplemented ewes (Figure 5.9), although not statistically significant, indicating that supplementation increased MP supply to the ewes rather than complete substitution. However, MP supply was not measured in the current study. This finding is in accordance with Abbott, Parkins, and Holmes (1988), who noted that increased dietary protein intake might improve the production of albumin, exceeding the concentration required for the replacement of protein loss through the gastrointestinal mucosa as a consequence of parasite-induced pathology.

For the control groups, overall, ewes' and lambs' performances throughout the lactation period were not affected by supplementation of ewes during the first four weeks of lactation, except for the BCS of ewes. Treatment with moxidectin significantly reduced ewe FEC and improved ewe LW. Serum protein concentrations were also not affected by supplementation and long-acting moxidectin injection, except for serum albumin levels. Results in the current study indicated that supplementation and treatment with moxidectin at the end of supplementation (week four of lactation) appeared to assist the ability of the ewes to limit egg excretion. FEC of supplemented-undrenched and unsupplemented-undrenched groups was significantly increased throughout the lactation period, except at week eight of lactation, where FEC of all groups decreased. Surprisingly, the FEC of supplemented-undrenched ewes rapidly elevated to 1,400 epg at week 12 of lactation,

compared to 650 epg in unsupplemented-undrenched ewes. The reason for the speedy elevation of FEC in the first group was probably associated with a decrease in ewes' immunity, as indicated by lower *T. colubriformis*-specific L3 IgA and *T. colubriformis*-specific L3 IgG levels, although the effect on these values was not significant. This reason was supported by Sutherland, Leathwick, Green, Brown, and Miller (1999), who found that the increase in FEC might be due to a decline in host immunity during the period when moxidectin prevents infection resulting in increased susceptibility once the persistent effect of the drug ends. Reinfection may then stimulate immunity, leading to a declined nematode egg output.

The decline in FEC followed by relatively constant serum *T. colubriformis*-specific L3 IgA levels of supplemented, both in drenched and undrenched groups at week eight of lactation, could be an indication of better immune capacity due to additional MP supply during the supplementation period, which was evidenced by greater serum albumin concentrations (Figure 6.4) and total serum protein concentrations (Figure 6.5) in supplemented groups compared with unsupplemented groups. Additionally, both undrenched groups had significantly lower serum albumin concentrations, indicating there was damage to the mucosa of the GI tract of ewes by GIN parasites, which resulted in body protein loss. Kyriazakis, Anderson, Coop, and Jackson (1996) revealed that sheep infected with *T. colubriformis* and received a low-protein diet had significantly lower concentrations of total protein, albumin and urea in their serum throughout the trial than the animals received a high-protein diet or animals selected between low and high protein diets. Several studies reported that reductions in serum albumin concentrations are usually due to plasma protein leakage into the GI tract caused by nematode infections of the small intestine and decreased synthesis of albumin in the liver (Coop, Sykes, & Angus, 1976; Steel et al., 1980; Sykes & Coop, 1976). Sykes and Field (1973) reported that the percentage of change in serum albumin content is related to body protein loss. Serum albumin is reduced with the loss of protein, reflecting the inability of this to be replaced, presumably due to the high protein requirement for lactation.

There was a lack of effect of supplementation on the performance of ewes and lambs throughout the lactation period, except for ewe BCS. As expected, long-acting injection of moxidectin at the end of the supplementation period resulted in a consistent trend of higher LW than undrenched groups due to lower parasitic load. Drenched ewes in the supplemented group had a mean LW of 5.35 kg heavier than the untreated cohort. While in the unsupplemented group, drenched ewes had a mean LW 1.81 kg heavier than untreated ewes. BCS of ewes from supplemented-drenched and supplemented-undrenched groups was slightly increased after week eight of lactation to weaning. From week four to week 12 of lactation, BCS of unsupplemented-undrenched ewes and unsupplemented-drenched ewes was reduced by 19% and 15%, respectively, while in supplemented

groups, no changes in BCS were observed. In parasitised animals, it is expected that increased protein levels may be deflected from production functions related to growth into plasma protein synthesis and the repair of the gastrointestinal tract (Bown, Poppi, & Sykes, 1986; Symons, Steel, & Jones, 1981), which in agreement with the priority of nutrient partitioning framework suggested by Coop and Kyriazakis (1999).

Similar with the results from previous trials, supplementation of ewes and administration of long-acting anthelmintic at the end of the supplementation period did not provide clear benefits to their lambs before weaning. However, lambs raised by supplemented and drenched ewes tended to have higher LW, LWG, and weight of lamb weaned per ewe (WLWE) than those raised by unsupplemented groups. The higher LWG of lambs raised by supplemented ewes was probably due to higher milk production and composition. It may be possible that a greater nutrient supply provided to the treated ewes during the persistent period of moxidectin was sufficient to produce a higher level of milk production for the remainder of the lactation interval. However, due to laboratory error, no milk data at week four of lactation were available for statistical analysis to support this reason. Another reason for higher LW and LWG in lambs raised by supplemented and drenched ewes was probably due to the elimination of parasitic infection of their dam, which resulted in their reduced infection (Coop & Jackson, 2000). Van Emon et al. (2014) showed that the ADG of lambs from birth to weaning has also been positively affected by maternal nutrition during late pregnancy. An alteration in the milk components of ewes fed increased MP during late pregnancy may partly explain the improvement in ADG.

The highest milk yield/four hours during week four of lactation in the current study was as expected. However, the reduction of milk production per four hours to more than 50% in both groups at week 12 of lactation was not anticipated; such a decline resulted in an insufficient sample being collected. Based on regression analyses, even though there were positive correlations between the growth rate of lamb and the milk yield of ewe, the associations over the lactation period were mostly very weak ($r^2 < 0.2$) in both supplemented and unsupplemented groups. Weak correlations in the current study were probably indicating that other factors besides milk production had an additional influence on the lamb growth rate. There are two possible explanations for the weak correlation between lamb ADG and ewe milk yield from week four to week 12 of lactation in the current study. Firstly, the potential milk production being measured using the oxytocin method does not show the actual consumption of milk by the lambs because the level of milk consumption may be affected by the behavioural interactions between the ewe and their lamb(s) (Muir, Smith, Wallace, Fugle, & Bown, 2000). Secondly, as lambs' age, they can consume herbage as a substitute for decreasing milk supply (Geenty, 1979; Geenty & Dyson, 1986; Muir et al., 2000; Spedding, Brown, & Large, 1963).

Accordingly, in a condition of limited feed, the competition for high-quality herbage between ewes and lambs is likely to inhibit the growth rate of lamb (Muir et al., 2000).

The consistently greater milk protein concentrations of supplemented ewes were also observed in the current study, which is required for lamb growth. Despite its function for the growth of lamb, milk protein also has a direct effect associated with milk proteins on the motility of nematode larvae (Zeng, Brown, Przemeck, & Simpson, 2003). Moreover, there was an overall increase in milk components, except for lactose content, over the lactation period. The increase in percentages of milk fat, milk protein, and total solids and the decrease in lactose contents over the lactation stage in the present study are in agreement with the observations of Morgan, Fogarty, Nielsen, and Gilmour (2006). The nutritional compositions of milk from this study were in the ranges of New Zealand sheep milk composition observed by Day, Broadhurst, and Samuelsson (2016).

In summary, the results of the current experiment show that there were no observed consistent epidemiological impacts on gastrointestinal parasitism of supplementation of twin-bearing ewes treated with an anthelmintic pre-lambing. The reduction in the FEC of lactating ewes is not sufficient under these conditions to result in an epidemiological advantage to their lamb, as shown by results on FEC, worm burden, and growth performance from birth to reach slaughter weight while grazing pastures that infected naturally by GIN parasites. Twin-bearing ewes supplemented on pasture during the periparturient period treated with an anthelmintic pre-lambing had similar *T. colubriformis*-specific L3 IgG concentrations with unsupplemented ewes. Moreover, no significant effects of supplementation on serum albumin, serum urea, total serum protein, serum globulin, serum phosphorus, and milk production and compositions.

Ewes' and lambs' performances throughout the lactation period of the control groups were not affected by supplementation of ewes during the first four weeks of lactation, except for BCS of ewes. However, the FEC of the ewe was affected by the administration of long-acting anthelmintic at the end of the supplementation period. The decline in FEC followed by relatively constant serum *T. colubriformis*-specific L3 IgA levels of supplemented, both in drenched and undrenched groups at week eight of lactation, could be an indication of better immune capacity due to an additional supply of MP during the supplementation period. Serum protein concentrations were also not affected by supplementation and long-acting moxidectin injection, except for serum albumin levels and the interaction between supplementation and time in serum urea concentrations.

Chapter 6

General Summary, Conclusions and Future Research Prospects

Many indoor studies in sheep revealed evidence that manipulation of the diet through increasing dietary protein levels or providing an enhanced plane of nutrition resulted in an improvement in the resistance and resilience of sheep against GIN infection. Nevertheless, outdoor studies on the epidemiological benefits of protein supplementation of ewes against GIN parasites and the impact on lambs for the rest of the grazing season are limited. Therefore, these studies were conducted to determine the effect of targeted supplementation of ewes on pasture during the periparturient period in the long term and determine whether the targeted supplementation can provide long-term benefits to the lambs.

Sollenberger and Burns (2001) suggested that the interactions between plants and animals on pastures are very complicated. Grazing experiments can be used to explain input-output relationships that cannot be quantified adequately in the laboratory, greenhouse, or clipping studies. According to them, there are many challenges to conducting appropriate grazing studies, such as (a) availability of sufficient and suitable animals, land, and equipment, (b) difficulties associated with measurement of key variables including herbage mass, diet selection, intake, and changes in animal liveweight, and (c) the resultant limitations in power of statistical tests and ability to establish causal relationships. Indeed, the number of replications used in the current studies was low due to the constraints of the resources available. Each paddock becomes its replication, with only two in year one and four in years two and three. Given resources are always limiting; the number of replications was increased by reducing the number of animals in each replication in years two and three. Although low, the number of replicates in the current study was suggested to be sufficient, with power analysis indicating that three replicates would be adequate to get an 80% power based on a 30% reduction in LWG caused by nematode parasites. However, it is clear a 30% difference in LWG of the lambs between the supplemented and unsupplemented areas did not occur. In part, this lack of expected effect on lamb performance may have been due to the implementation of a TST regime, whereby the true effect of parasitism on lamb performance may have been masked. However, due to ethical concerns, allowing animals to remain untreated was not an option avordable for these experiments.

The value of component-based research resulted from grazing studies can be transferred into practice as grazing experiments provide data that are directly useful to farmers/producers while producing information that is more relevant to natural infections. As much as possible, all trials reported in this thesis were designed to mimic the popular pastoral sheep farming system in New Zealand, which could ease the adoption of the outcomes in commercial lamb production. For example, although supplementation of ewes is not prevalent in New Zealand, the provision of high-protein pellets to the ewes for a short period from around three weeks before lambing until the first four weeks of lactation (periparturient period) was intended as the most cost-effective supplementation regime. The primary purpose of supplementation during this time was to provide additional nutrients at the highest demand, i.e., until peak lactation. Moreover, there was an added benefit of supplementing ewe during this time, as the lambs were unlikely to consume the pellets. By contrast, supplementation later in lactation would require keeping the lambs away from the pellets, then separating the effects of supplementation to specifically break the PPR, with the additional supplementation for lambs would not have been possible. Further, the removal of non-sentinel lambs from the study once their body weight exceeded 38 kg in simulating on-farm conditions where lambs may be sent to slaughter. While on the one hand, these actions provided a more real-world context for the results, equally, they may have sufficiently disrupted the parasite epidemiology to alter the size of the effect and, therefore, the number of replicates required to achieve sufficient experimental power.

The results of the experiments reported here have demonstrated that dietary supplementation with 500 g/head/d of high-protein pellets to twin-bearing ewes during the first four weeks of lactation, whether without anthelmintic treatment (Study 1, Chapter 4) or with short-acting drench pre-lambing (Study 2, Chapter 5), could reduce ewe faecal egg counts for an extended period. The reductions in FEC in these studies were in agreement with the indoor study of Donaldson et al. (1998, 2001) and Houdijk et al. (2000). It was anticipated this level of supplementation would increase MP supply supplementation to reduce worm burden by 50%, which, based on FEC, was largely achieved. However, the reductions of FEC were insufficient to provide a measurable and consistent epidemiological benefit to the grazing lambs. Lamb's performance before and after weaning was similar between supplemented and unsupplemented cohorts. Moreover, ewe supplementation did not affect systemic immunological parameters such as serum L3 *T. colubriformis*-specific IgA, serum L3 *T. colubriformis*-specific IgG, or serum protein concentrations.

There are several possibilities for the lack of effect of supplementation on ewes in the current studies. Firstly, low pasture availability due to climatic conditions may have resulted in unintentional nutritional stress to the sheep and may have resulted in an increased concentration of eggs in the

faeces. Donaldson (1998) reported that when pastures are limited, sheep will graze to a low residual pasture mass, consequently potentially being exposed to a higher larval challenge due to the higher concentration of L3 on the lower 2 cm of the grass. Secondly, in the design of all studies, weaned lambs and ewes within each treatment and replication were rotationally grazed for the remainder of the grazing season, with ewes following the lambs. This design may have contributed to the lack of effect due to the net removal of parasites by grazed non-lactating ewes (Leathwick et al., 2008). Thirdly, the lack of benefit may be due to the nutrition partitioning (Coop & Kyriazakis, 1999), where most of the nutrients supplied by supplementation during early lactation until peak lactation were used by the ewes to increase milk production. Perhaps insufficient nutrition from the supplementation was given to improve immunity. The demand for nutrients for milk production decreases as the lambs get older because they can consume herbage to substitute milk, which allows the more available nutrients to increase the immune capacity of the ewes. However, if this was the case, it may have been expected differences in ewe milk production or lamb live weight gain would have been apparent.

In all studies, although not statistically significant, pasture larval concentrations on supplemented paddocks generally reduced during lactation despite the ewe FEC indicating a reasonable number of nematode eggs were being deposited to the pastures. Lower pasture contamination during the periparturient period then effectively delayed the increase in FEC in the lambs throughout the summer. Kahn, Knox, Walkden-Brown, et al. (2003) reported that the most significant period of pasture larval contamination is during the periparturient phase, as the acquired immunity of ewes disrupted (Williams, Greeff, Vercoe, Dobson, & Karlsson, 2010) and it corresponds with the availability of susceptible hosts, which are the young lambs (Sebastiano et al., 2017). However, while the current series of investigations are in agreement with this suggestion, the generally consistently lower ewe FEC, which presumably indicates less contamination, did not substantially and consistently alter the parasitological parameters of either the pasture or the lambs throughout the remainder of the summer.

Alternatively, the lack of effect of lower FEC may suggest that the PPR is not as epidemiologically important as first considered. The importance of the PPR in shedding the pastures for the grazing lamb to consume has always been based on the immunity restoration in the ewe. From this point, it provides negligible contamination. However, it is clear from the current results that the ewes frequently maintained a high FEC throughout most, if not all, of lactation. In part, this concept that the PPR is of lesser epidemiological significance is supported by several studies that have mixed outcomes when comparing the benefits of long-acting anthelmintic studies on twin-bearing ewes (Ridler et al., 2019; Garland & Leathwick, 2015; Leathwick et al., 2020; Miller et al., 2015). The

variation between mobs in the production and economic benefits of long-acting treatment before lambing was recognised in twin-bearing ewes (Garland & Leathwick, 2015) and the context of these results may reflect an altered pattern of egg shedding and pasture contamination, which may be impacted by both the season and the body condition of the ewe. Possibly, the increased level of production and then the nutritional burden on sheep may induce a change in the excretion patterns of sheep, leading to a more prolonged period of egg excretion, which extends into warmer months, which may favour larval development. With this in mind, it is conceivable a higher epidemiological benefit may be achieved through improving nutrition in late lactation, either through supplementation or the provision of specialist forages, to help ensure restoration of immunity following peak lactation.

Considerable debate has been discussed by many workers on the cost-benefit, sustainability, and relative impact of various anthelmintic treatment or parasite control options administered to ewes around lambing (Cook, 2009). Several studies have determined the production benefits of using short-acting or long-acting anthelmintics in ewes around lambing time (Bingham, Hodge, & Mariadass, 2017; Miller et al., 2015). They revealed that there were no consistent benefits to be obtained from these treatments. Therefore, anthelmintic treatment should be focused on those animals within a mob where the most benefit will be gained. Supplying reliable, good nutrition and having animals with a high BCS will decrease the requirement for anthelmintics. It should be noted from the results of all trials that FEC was favourably associated with BCS of the ewe (Table 6.1). Ewes with better BCS before lambing are likely to have lower egg counts than lower BCS ewes (Chapter 4). Moreover, ewes in poorer body condition are more likely to benefit from anthelmintic treatment than their better-conditioned counterparts (Chapter 5). This result was in accordance with Cornelius, Jacobson, and Besier (2014). They recommended drenching ewes with lower BCS and allowing a proportion of the higher body condition ewes undrenched in a TST programme to delay the development of resistance by providing a population of non-resistant worms. They also suggested that BCS can be used as a selection index under commercial farming conditions for determining which ewes should be left undrenched to provide a source of refugia without compromising the productivity of the flock. Similarly, protein supplementation needs to be effectively targeted as it could be costly if operated for an extended period. The use of protein supplements for sheep is not necessarily a continuing expenditure, but rather to be provided to the animals strategically in times when they are likely to be severely challenged, such as to periparturient ewes with poorer BCS, growing lambs or during times of low pasture availability and or quality to balance nutrient deficiencies. Under a TST strategy, pre-lambing anthelmintic should be given to ewes in the lowest BCS. Undrenched ewes with better body conditions (BCS > 3.0) may be used as refugia for GIN parasites. Table 6.1 shows the possibility of targeting a higher amount of supplementation to just

the poor BCS ewes with the evidence that high BCS ewes had lower FEC. As such, targeting supplementation to poor BCS ewes may have provided a more cost-effective approach and may be an avenue for future studies.

Despite supplementation with additional nutrients, the cumulative percentages of non-sentinel lambs that reached slaughter weight above 38 kg in both groups in all trials were similar. However, the time required for animals to achieve this target weight differed between trials. While care should always be exerted when comparing between years, the time 50% of lambs reached targeted slaughter weight in 2015, 2016, and 2017 took 111, 105, and 65 days after weaning, respectively. Even though the results between treatments were not significant, treated ewes with short-acting drench pre-lambing resulted in lower ewe FEC, lower pasture larval contamination during the lactation period, and lower FEC of weaned lambs (Chapter 5) compared with undrenched ewes (Chapter 4). It appears that lower FEC and pasture contamination allowed weaned lambs to grow better than those with higher FEC and offered more contaminated pastures, hence reaching targeted slaughter weight faster than their counterparts. In 2017, the mean ADG of non-sentinel lambs' grazed areas where ewes had been supplemented was 196.8 g/d, while their unsupplemented counterpart was 183.6 g/d compared with 127.7 and 143.8 g/d, respectively for non-sentinel lambs in 2015 and 134.1 and 131.5 g/d, respectively for weaned lambs in 2016. Miller et al. (2012) stated that the opportunity to harvest more lambs with targeted slaughter weight and sell them sooner would give additional benefits to the farmer, such as more herbage available for the remaining animals, saving pasture, and supporting the introduction of new stock.

The protein status of the ewe may only be one of several factors involved in the periparturient breakdown of resistance to gastrointestinal parasitism. However, results from the present study suggest that protein supplementation may still play a part in reducing larval contamination of the grazing area if appropriately targeted. The practical significance of these findings will depend upon the relative costs of providing supplements and the availability of effective anthelmintic compounds.

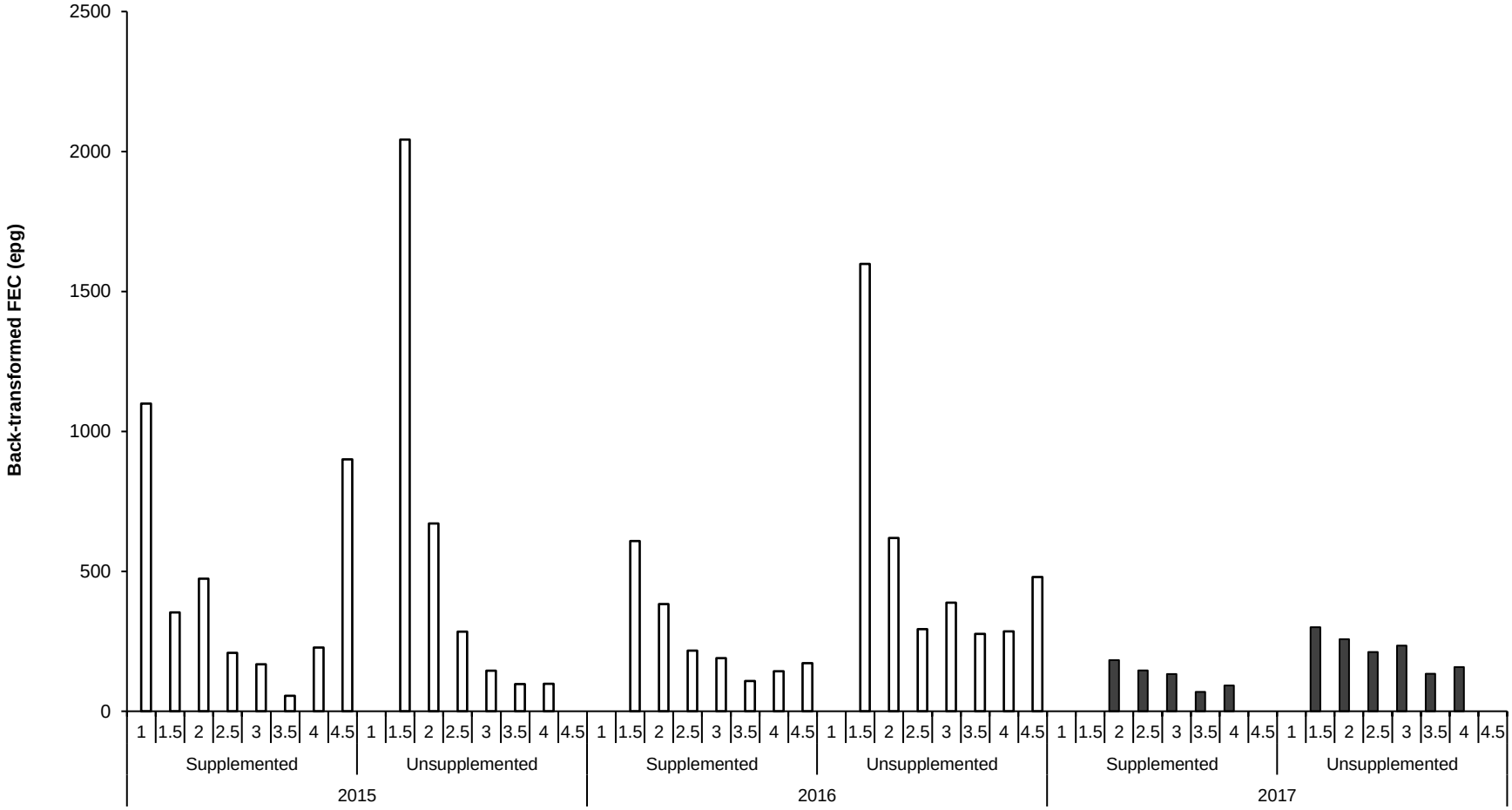


Figure 6.1 The effect of supplementation, BCS, and year of sampling on FEC (epg) of undrenched ewes (open bars) in 2015 and 2016, and drenched ewes (closed bars) in 2017.

Table 6.1 Total sum of faecal egg counts (FEC) based on BCS of ewes from 2015-2017

Treatment	BCS	2015			2016			2017		
		Number of animals	Total sum of FEC	Average FEC	Number of animals	Total sum of FEC	Average FEC	Number of animals	Total sum of FEC	Average FEC
Supplemented	1.0	1	1,200	1,200	-	-	-	-	-	-
	1.5	18	8,156	453	1	707	707	-	-	-
	2.0	24	13,769	574	7	3,382	483	14	3,955	282
	2.5	55	16,971	309	85	26,872	316	62	15,260	246
	3.0	38	10,177	268	95	27,515	290	60	13,964	233
	3.5	28	4,346	155	66	13,737	208	26	4,398	169
	4.0	12	3,941	328	31	7,524	243	24	4,604	192
	4.5	1	1,000	1,000	3	814	271	-	-	-
Unsupplemented	1.0	-	-	-	-	-	-	-	-	-
	1.5	14	29,994	2,142	1	1,700	1,700	1	400	400
	2.0	37	28,548	772	8	5,760	720	22	7,869	358
	2.5	66	25,401	385	87	34,211	393	68	21,203	312
	3.0	47	11,514	245	103	50,215	488	61	20,391	334
	3.5	24	4,733	197	60	22,599	377	21	4,915	234
	4.0	14	2,777	198	44	16,980	386	23	5,929	258
	4.5	-	-	-	10	5,791	579	-	-	-

Future research prospects

1. Study on protein turnover

Several studies indicated that immune responsiveness in parasitised sheep improves due to protein supplementation (Coop et al., 1995; van Houtert, Barger, & Steel, 1995). However, there has been a limited focus on whether this improvement is because of protein supplementation *per se* or an increased supply of specific amino acids (Miller et al., 2000). Le Floc'h et al. (2004) suggested that amino acids requirements may also elevate as a direct effect of metabolic changes associated with inflammation and infection. In normal conditions, the metabolism of AA within the gut and its accessory organs causes an excessive contribution to AA turnover. There is limited data on protein turnover in sheep, especially in grazing lactating ewes infected naturally by the GIN parasite. Because of protein synthesis and turnover importance, much effort has gone into quantifying the rate of these processes, including the use of isotopic labels. MacDonald et al. (2013) suggested using heavy water or deuterium oxide (^2H_2O or D_2O) as a tracer. Many studies showed deuterium oxide had been used safely as a tracer for the determination of protein synthesis and turnover rates in humans and animals (Busch et al., 2006; Kasumov et al., 2011; Liu, Smith, Briegel, Gao, & Peng, 2007; MacDonald et al., 2013). A dose rate of 12 g D_2O/kg body weight is optimal for labelling low-abundant proteins (T. McNeilly; personal communication, July 2019). The incomplete amino acid turnover analysis is a shortcoming of this study. The study in control groups (Chapter 5) was initially designed to determine the effect of supplementation to ewes during the first four weeks of lactation and GIN infection on the protein synthesis rate of lactating ewes on pasture. However, a study for amino acid turnover analyses was previously planned but did not materialise due to circumstances beyond the author's control. It would be valuable and of interest to understand the amino acid turnover in lactating ewes with twin lambs at foot while grazing and being naturally infected by gastrointestinal nematode parasites.

2. Supplementation study on optimal amount of feed and ewe BCS

The current study showed that feed offered at 260 g/head/d MP to graze ewes during the periparturient period was successful in temporarily reducing FEC by around 50%. However, the reduction was insufficient to provide a measurable and consistent epidemiological benefit to the ewes and grazing lambs, so a reduction of ewe FEC by more than 50% is required. Hence, more than 260 g/head/d MP of feed should be given to the ewes to provide more protein available to increase immunity. Further research is needed to determine the optimal amount of feed greater than 260 g/head/d MP that gives a measurable and consistent epidemiological benefit to the ewes and lambs.

Additionally, further research focused on more targeted supplementation on low BCS ewes should be considered to get a better response to FEC reduction. Table 6.1 shows that ewes with poorer BCS (BCS = 2 or lower) got a higher decline in FEC than ewes with better BCS (BCS >2) when supplemented during the parturient period.

3. Supplementation study on different ewe and environmental condition

Another prospect is to target ewes supplementation later into lactation (after peak lactation) when lactation demands are lower and larval survival on pasture may be higher. In the current study, milk production gradually decreased, from approximately 1.13 l/4 h in week 4 of lactation to 0.46 l/4 h in week 12 of lactation on supplemented ewes. Similarly, milk production of unsupplemented ewes decreased from 1.12 l/4 h in week 4 to 0.49 l/4 h in week 12 of lactation (Figure 6.2a). The MP demand for lactation was expected to decrease as the lactation time increased. Furthermore, the current study was conducted from early spring until summer 2017, where a small peak of L3 on herbage was expected during these periods. This number increased in late summer/autumn due to ewes and lambs contamination (Figure 5.6). These will show the potential magnitude of the response to extra MP supply. In addition, ewes FEC in the present study remains elevated (Figure 6.2b) with the expected increase in temperature (Figure 5.2) or more favourable environmental conditions because environmental conditions, especially temperature and humidity, affect species distribution and the presence of parasites in the pasture. For the future study, it is proposed whether the MP supply of more than 260 g/d to twin-rearing ewes after peak lactation will benefit their immune response's restoration to achieve a more pronounced epidemiological benefit. Nevertheless, to acquire this, care will need to be taken to ensure lambs do not have access to the feed supplement.

It is possible, therefore, that combining an understanding of the individual amino acid turnover with the importance of ewes' condition and BCS may assist in targeting a more appropriate supplementation strategy.

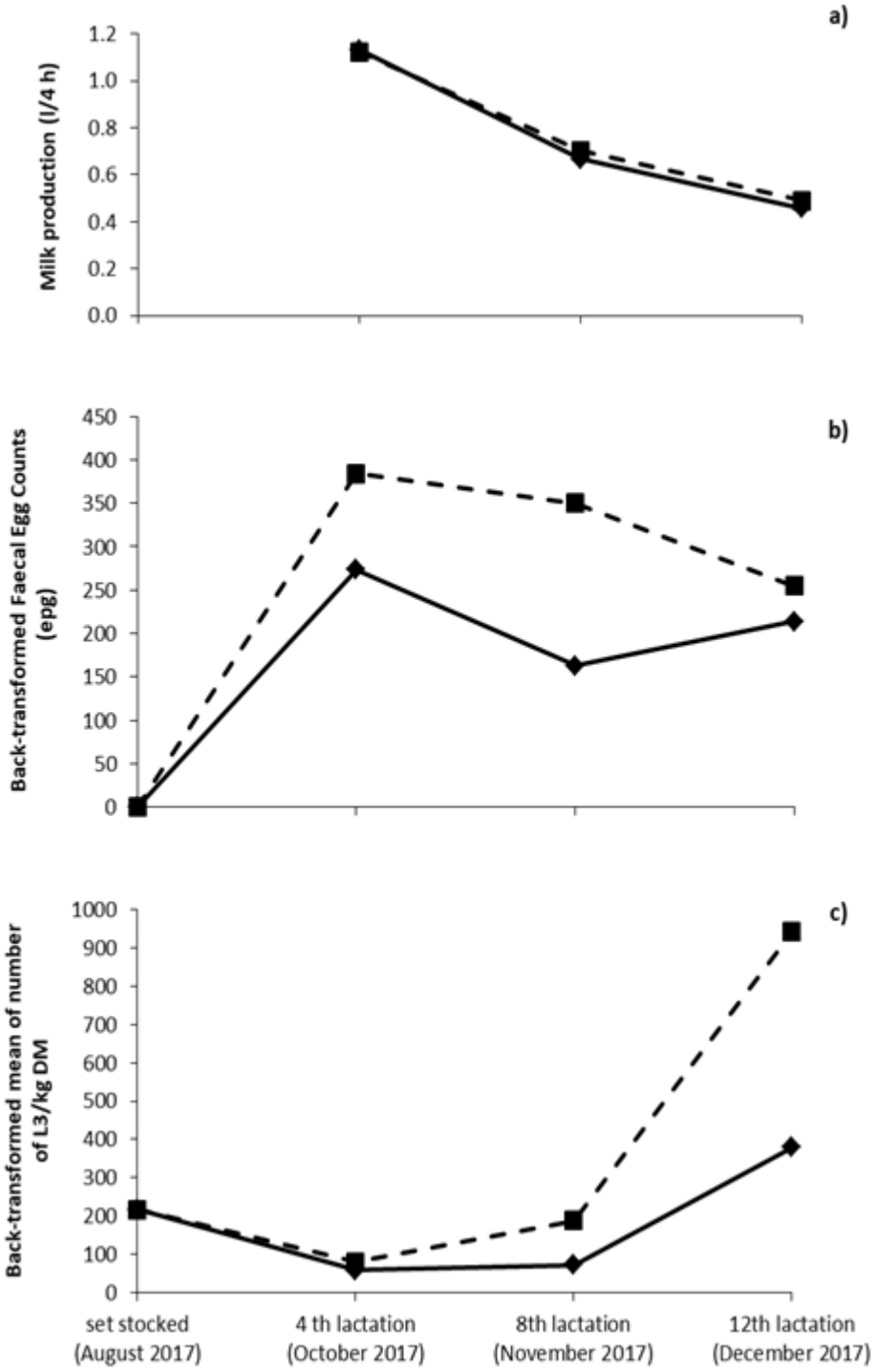


Figure 6.2 Milk production (a), back-transfomed FEC (b), and back-transformed mean of number of L3/kg DM (c) of ewes that were supplemented (solid line) or unsupplemented (dashed line) during the lactation period in 2017.

General conclusions

It was concluded from the series of experiments from this study that supplementation of ewes during the first four weeks of lactation, whether undrenched, drenched pre-lambing or drenched at the end of the supplementation period, did not affect ewe performance. However, it was temporarily successful in reducing faecal egg counts, presumably reflecting better maintenance of immune function through higher nutrient supply, although this was not detected in parasite-specific immunoglobulins. However, the reduction in parasite contamination was insufficient to provide a measurable and consistent epidemiological benefit to the grazing lambs that may assist with parasite control. If the supplementation of ewes during this time is to be employed as a means of reducing the need for anthelmintics to control parasitism, then the refinement, including an understanding of the specific amino acid requirement and any interaction with BCS, needs to be investigated.

References

Abbott, E. M., Parkins, J. J., & Holmes, P. H. (1988). Influence of dietary protein on the pathophysiology of haemonchosis in lambs given continuous infections. *Research in Veterinary Science, 45*(1), 41-49.

Abdelatif, A. M., El-Nageeb, M. E., Makawi, S. E. A., & Fadlalla, A. M. (2009). Blood constituents in cycling, gestating and lactating desert ewes (*Ovis aries*) in relation to dietary supplementation. *Global Veterinaria, 3*(3), 248-259.

Aboshady, H. M., Stear, M. J., Johansson, A., Jonas, E., & Bambou, J.-C. (2020). Immunoglobulins as biomarkers for gastrointestinal nematodes resistance in small ruminants: A systematic review. *Scientific reports, 10*(1), 7765.

Adams, D. B., Anderson, B. H., & Windon, R. G. (1989). Cross-immunity between *Haemonchus contortus* and *Trichostrongylus colubriformis* in sheep. *International Journal for Parasitology, 19*(7), 717-722. doi:10.1016/0020-7519(89)90056-8

Afolayan, R. A., Fogarty, N. M., Morgan, J. E., Gaunt, G. M., Cummins, L. J., Gilmour, A. R., & Nielsen, S. (2009). Genetic analysis of milk production and composition in crossbred ewes from different maternal genotypes. *Animal Production Science, 49*, 24-31. doi:https://doi.org/10.1071/EA08157

Agricultural and Food Research Council. (1993). *Energy and protein requirements of ruminants : An advisory manual*. Wallingford, England: CAB International.

Ahmad, S., Ramzan, F., Aziz-ur-Rahman, M., Hussain, K., Umer, S., Saleem, M. N., & Thekkiniath, J. (2023). Parasite control strategies: Selective breeding. In H. M. Rizwan & M. S. Sajid (Eds.), *Parasitism and parasitic control in animals: Strategies for the developing world* (pp. 168-182): CABI GB.

Albers, G. A. A., Gray, G. D., Piper, L. R., Barker, J. S. F., Le Jambre, L. F., & Barger, I. A. (1987). The genetics of resistance and resilience to *Haemonchus contortus* infection in young Merino sheep. *International Journal for Parasitology, 17*(7), 1355-1363.

Armour, J., & Bruce, R. G. (1974). Inhibited development in *Ostertagia ostertagi* infections-A diapause phenomenon in a nematode. *Parasitology, 69*, 161-174. doi:https://doi.org/10.1017/S0031182000048009

Arsenopoulos, K., Symeonidou, I., & Papadopoulos, E. (2017). Immune and other factors modulating host resistance against gastrointestinal nematode parasites in sheep. *Journal of the Hellenic Veterinary Medical Society, 68*(2), 131-144. Retrieved from <Go to ISI>://WOS:000418953100003

Athanasiadou, S., Githiori, J., & Kyriazakis, I. (2007). Medicinal plants for helminth parasite control: Facts and fiction. *Animal, 1*(9), 1392-1400. doi:10.1017/S1751731107000730

Athanasiadou, S., Gray, D., Younie, D., Tzamaloukas, O., Jackson, F., & Kyriazakis, I. (2007). The use of chicory for parasite control in organic ewes and their lambs. *Parasitology, 134*(2), 299-307. doi:10.1017/S0031182006001363

Athanasiadou, S., Houdijk, J. G. M., & Kyriazakis, I. (2008). Exploiting synergisms and interactions in the nutritional approaches to parasite control in sheep production systems. *Small Ruminant Research, 76*, 2-11. doi:http://dx.doi.org/10.1016/j.smallrumres.2007.12.016

Aumont, G., Coulaud, G., Grude, A., & Gruner, L. (1989). Pasture populations of cattle nematode larvae in Guadeloupe (French West Indies). *International Journal for Parasitology, 19*(5), 547-554.

Baker, R. L., Nagda, S., Rodriguez-Zas, S. L., Southey, B. R., Audho, J. O., Aduda, E. O., & Thorpe, W. (2003). Resistance and resilience to gastro-intestinal nematode parasites and relationships with productivity of Red Maasai, Dorper and Red Maasai× Dorper crossbred lambs in the sub-humid tropics. *Animal Science, 76*(1), 119-136.

Barcelos, C. A., Maeda, R. N., Santa Anna, L. M. M., & Pereira Jr, N. (2016). Sweet sorghum as a whole-crop feedstock for ethanol production. *Biomass and Bioenergy, 94*, 46-56.

Barger. (1993). Influence of sex and reproductive status on susceptibility of ruminants to nematode parasitism. *International Journal for Parasitology, 23*(4), 463-469. doi:http://dx.doi.org/10.1016/0020-7519(93)90034-V

Barger. (1997). Control by management. *Veterinary parasitology, 72*(3-4), 493-506. doi:https://doi.org/10.1016/S0304-4017(97)00113-1

Barger. (1999). The role of epidemiological knowledge and grazing management for helminth control in small ruminants. *International Journal for Parasitology, 29*(1), 41-47. doi:https://doi.org/10.1016/S0020-7519(98)00176-3

Bassetto, C. C., Almeida, F. A., Newlands, G. F. J., Smith, W. D., Castilhos, A. M., Fernandes, S., . . . Amarante, A. F. T. (2018). Trials with the Haemonchus vaccine, Barbervax®, in ewes and lambs in a tropical environment: Nutrient supplementation improves protection in periparturient ewes. *Veterinary parasitology, 264*, 52-57. doi:https://doi.org/10.1016/j.vetpar.2018.11.006

Beasley, A. M., Kahn, L. P., & Windon, R. G. (2010). The periparturient relaxation of immunity in Merino ewes infected with *Trichostrongylus colubriformis*: Parasitological and immunological responses. *Veterinary parasitology, 168*, 60-70. doi:http://dx.doi.org/10.1016/j.vetpar.2009.08.028

Beasley, A. M., Kahn, L. P., & Windon, R. G. (2012). The influence of reproductive physiology and nutrient supply on the periparturient relaxation of immunity to the gastrointestinal nematode *Trichostrongylus colubriformis* in Merino ewes. *Veterinary parasitology, 188*, 306-324. doi:http://dx.doi.org/10.1016/j.vetpar.2012.03.022

Beraldi, D., Craig, B. H., Bishop, S. C., Hopkins, J., & Pemberton, J. M. (2008). Phenotypic analysis of host-parasite interactions in lambs infected with *Teladorsagia circumcincta*. *International Journal for Parasitology, 38*, 1567-1577. doi:10.1016/j.ijpara.2008.04.011

Bingham, C., Hodge, A., & Mariadass, B. (2017). Comparison of two long acting pre-lambing anthelmintic treatments on the productivity of ewes in low body condition. *New Zealand Veterinary Journal, 65*(3), 152-155. doi:https://doi.org/10.1080/00480169.2016.1249528

Bishop, S. C., & Stear, M. J. (2001). Inheritance of faecal egg counts during early lactation in Scottish Blackface ewes facing mixed, natural nematode infections. *Animal Science, 73*(3), 389-395. doi:10.1017/S1357729800058355

Bishop, S. C., & Stear, M. J. (2003). Modeling of host genetics and resistance to infectious diseases: understanding and controlling nematode infections. *Veterinary Parasitology, 115*(2), 147-166.

Bisset, S. A., Vlassoff, A., Douch, P. G. C., Jonas, W. E., West, C. J., & Green, R. S. (1996). Nematode burdens and immunological responses following natural challenge in Romney lambs selectively bred for low or high faecal worm egg count. *Veterinary parasitology, 61*(3), 249-263. doi:10.1016/0304-4017(95)00836-5

Blackie, S. (2014). A review of the epidemiology of gastrointestinal nematode infections in sheep and goats in Ghana. *Journal of Agricultural Science, 6*(4), 109-118. doi:10.5539/jas.v6n4p109

Bouix, J., Krupinski, J., Rzepecki, R., Nowosad, B., Skrzyzala, I., Roborzynski, M., . . . Gruner, L. (1998). Genetic resistance to gastrointestinal nematode parasites in Polish long-wool sheep. *International Journal for Parasitology, 28*, 1797-1804. doi:https://doi.org/10.1016/S0020-7519(98)00147-7

Bowdridge, S., MacKinnon, K. M., McCann, J. C., Zajac, A. M., & Notter, D. R. (2013). Hair-type sheep generate an accelerated and longer-lived humoral immune response to *Haemonchus contortus* infection. *Veterinary Parasitology, 196*(1-2), 172-178.

Bown, M. D., Poppi, D. P., & Sykes, A. R. (1986). The effect of post-ruminal infusion of protein or energy on the pathology of *Trichostrongylus colubriformis* infection and body composition in lambs. *Proceedings of the New Zealand Society of Animal Production, 46*, 27-30.

Bown, M. D., Poppi, D. P., & Sykes, A. R. (1991). Nitrogen transactions along the digestive tract of lambs concurrently infected with *Trichostrongylus colubriformis* and *Ostertagia circumcincta*. *British Journal of Nutrition, 66*, 237-249. doi:https://doi.org/10.1079/BJN19910028

Bricarello, P. A., Amarante, A. F. T., Rocha, R. A., Cabral Filho, S. L., Huntley, J. F., Houdijk, J. G. M., . . . Gennari, S. M. (2005). Influence of dietary protein supply on resistance to experimental infections with *Haemonchus contortus* in Ile de France and Santa Ines lambs. *Veterinary parasitology, 134*, 99-109. doi:https://doi.org/10.1016/j.vetpar.2005.05.068

Brookes, I. M., & Nicol, A. M. (2007). The protein requirements of grazing livestock. In P. V. Rattray, I. M. Brookes, & A. M. Nicol (Eds.), *Pasture and supplements for grazing animals* (pp. 173-187). Hamilton, New Zealand: New Zealand Society of Animal Production.

Brown, D. J., & Fogarty, N. M. (2016). Genetic relationships between internal parasite resistance and production traits in Merino sheep. *Animal Production Science, 57*(2), 209-215.

Brunsdon, R. V. (1967). The significance of *Nematodirus* in New Zealand. *New Zealand Veterinary Journal, 15*(6), 105-108. doi:https://doi.org/10.1080/00480169.1967.33704

Brunsdon, R. V. (1982). Host/parasite interrelationship in Trichostrongylid infections. In A. D. Ross (Ed.), *Control of internal parasites in sheep: Animal Industries Workshop, July 1982* (pp. 21-24). Lincoln, New Zealand: Lincoln College.

Brunsdon, R. V., Kissling, R., & Hosking, B. C. (1983). A survey of anthelmintic usage for sheep: A time for change? *New Zealand Veterinary Journal, 31*(3), 24-29. doi:https://doi.org/10.1080/00480169.1983.34953

Brunsdon, R. V., & Vlassoff, A. (1982). Production and parasitological responses of lambs exposed to differing low levels of trichostrongylid larvae on pasture. *New Zealand journal of experimental agriculture, 10*(4), 391-394. doi:10.1080/03015521.1982.10427905

Brunsdon, R. V., Vlassoff, A., & West, C. J. (1986). Effect of natural trichostrongylid larval challenge on breeding ewes. *New Zealand Journal of Experimental Agriculture, 14*(1), 37-41. doi:http://dx.doi.org/10.1080/03015521.1986.10426122

Burke, J. M., Miller, J. E., & Terrill, T. H. (2009). Impact of rotational grazing on management of gastrointestinal nematodes in weaned lambs. *Veterinary parasitology, 163*(1), 67-72. doi:https://doi.org/10.1016/j.vetpar.2009.03.054

Busch, R., Kim, Y., Neese, R. A., Schade-Serin, V., Collins, M., Awada, M., . . . Hellerstein, M. K. (2006). Measurement of protein turnover rates by heavy water labeling of nonessential amino acids. *Biochimica et Biophysica Acta (BBA), 1760*(5), 730-744. doi:http://dx.doi.org/10.1016/j.bbagen.2005.12.023

Callinan, A. P. L., & Westcott, J. M. (1986). Vertical distribution of trichostrongylid larvae on herbage and in soil. *International Journal for Parasitology, 16*(3), 241-244.

Cardia, D. F. F., Rocha-Oliveira, R. A., Tsunemi, M. H., & Amarante, A. F. T. (2011). Immune response and performance of growing Santa Ines lambs to artificial *Trichostrongylus colubriformis* infections. *Veterinary parasitology, 182*, 248-258. doi:10.1016/j.vetpar.2011.05.017

Caroprese, M., Giannenas, I., & Fthenakis, G. C. (2015). Interactions between nutritional approaches and defences against microbial diseases in small ruminants. *Veterinary Microbiology, 181*, 8-14. doi:http://dx.doi.org/10.1016/j.vetmic.2015.07.014

Chandrawathani, P., Jamnah, O., Adnan, M., Waller, P. J., Larsen, M., & Gillespie, A. T. (2004). Field studies on the biological control of nematode parasites of sheep in the tropics, using the microfungus *Duddingtonia flagrans*. *Veterinary parasitology, 120*, 177-187. doi:https://doi.org/10.1016/j.vetpar.2003.12.014

Chay-Canul, A., Ayala-Burgos, A., Kú-Vera, J., Magaña-Monforte, J., & Ferrell, C. (2011). Metabolizable energy intake and changes in body weight and body condition of pelibuey ewes fed three levels of roughage diets under tropical conditions. *Tropical and subtropical agroecosystems, 14*(3), 777-786.

Colvin, A. F., Walkden-Brown, S. W., Knox, M. R., & Scott, J. M. (2008). Intensive rotational grazing assists control of gastrointestinal nematodosis of sheep in a cool temperate environment with summer-dominant rainfall. *Veterinary parasitology, 153*, 108-120. doi:https://doi.org/10.1016/j.vetpar.2008.01.014

Commonwealth Scientific and Industrial Research Organisation. (2007). *Nutrient requirements of domesticated ruminants*. Collingwood, Australia: CSIRO Publishing.

Cook, T. (2009). Pre-lamb drenching of ewes–What result do you expect? *Proceedings of the Sheep and Beef Cattle Veterinarians of the New Zealand Veterinary Association, 39*, 125-127.

Coop, R. L., Graham, R. B., Jackson, F., Wright, S. E., & Angus, K. W. (1985). Effect of experimental *Ostertagia circumcincta* infection on the performance of grazing lambs. *Research in Veterinary Science, 38*(3), 282-287.

Coop, R. L., & Holmes, P. H. (1996). Nutrition and parasite interaction. *International Journal for Parasitology, 26*, 951-962. doi:http://dx.doi.org/10.1016/S0020-7519(96)80070-1

Coop, R. L., Huntley, J. F., & Smith, W. D. (1995). Effect of dietary protein supplementation on the development of immunity to *Ostertagia circumcincta* in growing lambs. *Research in Veterinary Science, 59*, 24-29. doi:http://dx.doi.org/10.1016/0034-5288(95)90025-X

Coop, R. L., & Jackson, F. (2000). Gastrointestinal helminthosis. In W. B. Martin & I. D. Aitken (Eds.), *Diseases of sheep* (3rd ed., pp. 159-167). Oxford: Blackwell Science.

Coop, R. L., & Kyriazakis, I. (1999). Nutrition–parasite interaction. *Veterinary parasitology, 84*, 187-204. doi:http://dx.doi.org/10.1016/S0304-4017(99)00070-9

Coop, R. L., & Kyriazakis, I. (2001). Influence of host nutrition on the development and consequences of nematode parasitism in ruminants. *TRENDS in Parasitology, 17*(7), 325-330. doi:https://doi.org/10.1016/S1471-4922(01)01900-6

Coop, R. L., Mellor, D. J., Jackson, E., Jackson, F., Flint, D. J., & Vernon, R. G. (1990). *Teladorsagia circumcincta* egg output at the onset of natural and induced lactation in ewes. *Veterinary parasitology, 35*, 295-305. doi:https://doi.org/10.1016/0304-4017(90)90135-X

Coop, R. L., & Sykes, A. R. (2002). Interactions between gastrointestinal parasites and nutrients. In M. Freer & H. Dove (Eds.), *Sheep nutrition* (pp. 313-331). Canberra, Australia: CABI Publishing in association with CSIRO Publishing.

Coop, R. L., Sykes, A. R., & Angus, K. W. (1976). Subclinical trichostrongylosis in growing lambs produced by continuous larval dosing. The effect on performance and certain plasma constituents. *Research in Veterinary Science, 21*(3), 253-258. doi:https://doi.org/10.1016/S0034-5288(18)33332-0

Coop, R. L., Sykes, A. R., & Angus, K. W. (1977). The effect of a daily intake of *Ostertagia circumcincta* larvae on body weight, food intake and concentration of serum constituents in sheep. *Research in Veterinary Science, 23*(1), 76-83. doi:https://doi.org/10.1016/S0034-5288(18)33229-6

Coop, R. L., Sykes, A. R., & Angus, K. W. (1982). The effect of three levels of intake of *Ostertagia circumcincta* larvae on growth rate, food intake and body composition of growing lambs. *The Journal of Agricultural Science, 98*, 247-255. doi:10.1017/S0021859600041782

Cornelius, M. P., Jacobson, C., & Besier, R. B. (2014). Body condition score as a selection tool for targeted selective treatment-based nematode control strategies in Merino ewes. *Veterinary parasitology, 206*(3), 173-181. doi:https://doi.org/10.1016/j.vetpar.2014.10.031

Corner-Thomas, R. A., Hickson, R. E., Morris, S. T., Back, P. J., Ridler, A. L., Stafford, K. J., & Kenyon, P. R. (2015). Effects of body condition score and nutrition in lactation on twin-bearing ewe and lamb performance to weaning. *New Zealand Journal of Agricultural Research, 58*(2), 156-169. doi:10.1080/00288233.2014.987401

Courtney, C. H., Gessner, R., Sholz, S. R., & Loggins, P. E. (1986). The periparturient rise in fecal egg counts in three strains of Florida Native ewes and its value in predicting resistance of lambs to *Haemonchus contortus*. *International Journal for Parasitology, 16*(3), 185-189. doi:https://doi.org/10.1016/0020-7519(86)90042-1

CSIRO. (2007). *Nutrient requirements of domesticated ruminants*: CSIRO Publishing.

Dalton, C. (2006). *Internal parasites of sheep and their control-now and in the future* (2nd ed.). Hamilton, New Zealand: Reward Publishing.

Day, L., Broadhurst, M., & Samuelsson, L. (2016). New Zealand sheep milk-nutritional composition. *Food New Zealand, 16*(4), 20-21.

De Barbieri, I., Navajas, E., Douhard, F., Conington, J., Ramos, Z., & Ciappesoni, G. (2023). PL-8 A review of sheep resilience. *Animal - science proceedings, 14*(1), 11-12. doi:10.1016/j.anscip.2023.01.009

Donald, A. D., & Waller, P. J. (1973). Gastro-intestinal nematode parasite populations in ewes and lambs and the origin and time course of infective larval availability in pastures. *International Journal for Parasitology, 3*, 219-233. doi:https://doi.org/10.1016/0020-7519(73)90027-1

Donaldson, J. (1997). The effect of dietary protein on the establishment and maturation of nematode populations in adult sheep. In G. K. Barrell (Ed.), *Sustainable control of internal parasites in ruminants* (pp. 193-201). Lincoln, New Zealand: Lincoln University.

Donaldson, J. (1998). *The effect of nutrition on the periparturient parasite status of sheep.* (Doctor of Philosophy Unpublished doctoral thesis). Lincoln University, Lincoln, New Zealand.

Donaldson, J., van Houtert, M. F. J., & Sykes, A. R. (1998). The effect of nutrition on the periparturient parasite status of mature ewes. *Animal Science, 67*, 523-533. doi:10.1017/S1357729800032951

Donaldson, J., van Houtert, M. F. J., & Sykes, A. R. (2001). The effect of dietary fish-meal supplementation on parasite burdens of periparturient sheep. *Animal Science, 72*, 149-158. doi:https://doi.org/10.1017/S1357729800055648

Donaldson, J., van Houtert, M. F. J., & Sykes, A. R. (2010). The effect of nutrition on the periparturient parasite status of mature ewes. *Animal Science, 67*(3), 523-533. doi:10.1017/s1357729800032951

Douch, P. G. C., Green, R. S., Morris, C. A., & Hickey, S. M. (1995). Genetic factors affecting antibody responses to four species of nematode parasite in Romney ewe lambs. *International Journal for Parasitology, 25*(7), 823-828. doi:https://doi.org/10.1016/0020-7519(94)00213-8

Douch, P. G. C., Green, R. S., & Risdon, P. L. (1994). Antibody responses of sheep to challenge with *Trichostrongylus colubriformis* and the effect of dexamethasone treatment. *International Journal for Parasitology, 24*(7), 921-928. doi:https://doi.org/10.1016/0020-7519(94)90155-4

Duncan, J. L., Smith, W. D., & Dargie, J. D. (1978). Possible relationship of levels of mucosal IgA and serum IgG to immune unresponsiveness of lambs to *Haemonchus contortus*. *Veterinary parasitology, 4*, 21-27. doi:10.1016/0304-4017(78)90032-8

Dunsmore, J. D. (1965). *Ostertagia* spp. in lambs and pregnant ewes. *Journal of Helminthology, 39*(2-3), 159-184. doi:https://doi.org/10.1017/S0022149X00020575

Eady, S. J., Woolaston, R. R., & Barger, I. A. (2003). Comparison of genetic and nongenetic strategies for control of gastrointestinal nematodes of sheep. *Livestock Production Science, 81*(1), 11-23. doi:https://doi.org/10.1016/S0301-6226(02)00197-5

Eysker, M. (1993). The role of inhibited development in the epidemiology of *Ostertagia* infections. *Veterinary parasitology, 46*, 259-269. doi:http://dx.doi.org/10.1016/0304-4017(93)90063-S

Falzon, L. C., Menzies, P. I., Shakya, K. P., Jones-Bitton, A., Vanleeuwen, J., Avula, J., . . . Peregrine, A. S. (2013). A longitudinal study on the effect of lambing season on the periparturient egg rise in Ontario sheep flocks. *Preventive Veterinary Medicine, 110*, 467-480. doi:http://dx.doi.org/10.1016/j.prevetmed.2012.12.007

Familton, A. S. (1991). Re-examination of gastrointestinal parasite control-The contribution of the ewe. *Proceedings of the 21st Sheep and Beef Cattle Society Annual Seminar, New Zealand Veterinary Association (NZVA)*, 25-35.

Freer, M., Moore, A. D., & Donnelly, J. R. (1997). GRAZPLAN: Decision support systems for Australian grazing enterprises-II. The animal biology model for feed intake, production and reproduction and the GrazFeed DSS. *Agricultural Systems, 54*(1), 77-126.

Garland, C. B., & Leathwick, D. M. (2015). A cost-benefit analysis of pre-and post-lambing anthelmintic treatments to twin-bearing ewes on commercial farms in the southern North Island of New Zealand. *New Zealand Veterinary Journal, 63*(4), 220-226. doi:http://dx.doi.org/10.1080/00480169.2015.1012133

Gauly, M., & Erhardt, G. (2001). Genetic resistance to gastrointestinal nematode parasites in Rhön sheep following natural infection. *Veterinary parasitology, 102*, 253-259. doi:https://doi.org/10.1016/S0304-4017(01)00530-1

Gazda, T. L., Piazzetta, R. G., Dittrich, J. R., Monteiro, A. L. G., & Thomaz-Soccol, V. (2009). Distribution of nematode larvae of sheep in tropical pasture plants. *Small Ruminant Research, 82*, 94-98. doi:10.1016/j.smallrumres.2009.02.004

Geenty, K. G. (1979). Lactation performance, growth, and carcass composition of sheep: I. Milk production, milk composition, and live weights of Romney, Corriedale, Dorset, Romney×Dorset, and Dorset×Romney ewes in relation to the growth of their lambs. *New Zealand Journal of Agricultural Research, 22*(2), 241-250. doi:https://doi.org/10.1080/00288233.1979.10430743

Geenty, K. G., Clarke, J. N., & Wright, D. E. (1985). Lactation performance, growth, and carcass composition of sheep: 2. Relationships between ewe milk production, lamb water turnover, and lamb growth in Romney, Dorset, and crossbred sheep. *New Zealand Journal of Agricultural Research, 28*(2), 249-255. doi:https://doi.org/10.1080/00288233.1985.10420935

Geenty, K. G., & Dyson, C. B. (1986). The effects of various factors on the relationship between lamb growth rate and ewe milk production. *Proceedings of the New Zealand Society for Animal Production, 46*, 265-269.

Gill, H. S., Gray, G. D., Watson, D. L., & Husband, A. J. (1993). Isotype-specific antibody-responses to *Haemonchus contortus* in geneticall resistant sheep. *Parasite Immunology, 15*, 61-67. doi:10.1111/j.1365-3024.1993.tb00585.x

Gray, G. D. (1997). The use of genetically resistant sheep to control nematode parasitism. *Veterinary parasitology, 72*, 345-366. doi:https://doi.org/10.1016/S0304-4017(97)00105-2

Greer, A. W. (2005). *Estimates of the nutritional cost of the development of immunity to gastrointestinal parasites in sheep.* (Doctor of Philosophy Unpublished doctoral thesis). Lincoln University, Lincoln, New Zealand.

Greer, A. W., Kenyon, F., Bartley, D. J., Jackson, E. B., Gordon, Y., Donnan, A. A., . . . Jackson, F. (2009). Development and field evaluation of a decision support model for anthelmintic treatments as part of a targeted selective treatment (TST) regime in lambs. *Veterinary parasitology, 164*, 12-20. doi:http://dx.doi.org/10.1016/j.vetpar.2009.04.017

Greer, A. W., McAnulty, R. W., Logan, C. M., & Hoskin, S. O. (2010). Suitability of the Happy Factor decision support model as part of targeted selective anthelmintic treatment in Coopworth sheep. *Proceedings of the New Zealand Society of Animal Production, 70*, 212-216.

Greer, A. W., Sedcole, R. J., Jay, N. P., McAnulty, R. W., Green, R. S., Stankiewicz, M., & Sykes, A. R. (2009). Protein supply influences the nutritional penalty associated with the development of immunity in lambs infected with *Trichostrongylus colubriformis*. *animal, 3*(3), 437-445. doi:10.1017/S1751731108003534

Gronvold, J., & Hogh-Schmidt, K. (1989). Factors influencing rain splash dispersal of infective larvae of *Ostertagia ostertagi* (Trichostrongylidae) from cow pats to the surroundings. *Veterinary Parasitology, 31*(1), 57-70.

Haile, A., Tembely, S., Anindo, D. O., Mukasa-Mugerwa, E., Rege, J. E. O., Yami, A., & Baker, R. L. (2002). Effects of breed and dietary protein supplementation on the responses to gastrointestinal nematode infections in Ethiopian sheep. *Small Ruminant Research, 44*, 247-261. doi:https://doi.org/10.1016/S0921-4488(02)00080-9

Halliday, A. M., Routledge, C. M., Smith, S. K., Matthews, J. B., & Smith, W. D. (2007). Parasite loss and inhibited development of *Teladorsagia circumcincta* in relation to the kinetics of the local IgA response in sheep. *Parasite Immunology, 29*, 425-434. doi:10.1111/j.1365-3024.2007.00959.x

Heckler, R. P., & Borges, F. d. A. (2016). Climate variations and the environmental population of gastrointestinal nematodes of ruminants. *Nematoda, 3*, e012016.

Hein, W. R., Pernthaner, A., Piedrafita, D., & Meeusen, E. N. (2010). Immune mechanisms of resistance to gastrointestinal nematode infections in sheep. *Parasite Immunology, 32*, 541-548. doi:10.1111/j.1365-3024.2010.01213.x

Herlich, H. (1956). A digestion method for post-mortem recovery of nematodes from ruminants. *Proceedings of the Helminthological Society of Washington, 23*(2), 102-103.

Hine, B., Acton, G., Elks, D., Niemeyer, D., Bell, A., Colditz, I., . . . Smith, J. (2022). Targeting improved resilience in Merino sheep–Correlations between immune competence and health and fitness traits. *animal, 16*(7), 100544.

Holasová, E., Pavlasek, I., & Kotrlá, B. (1989). Migration of the infective larvae of sheep gastrointestinal nematodes. *Acta Veterinaria Brno, 58*(4), 369-378.

Hoste, H., & Torres-Acosta, J. F. J. (2011). Non chemical control of helminths in ruminants: Adapting solutions for changing worms in a changing world. *Veterinary parasitology, 180*(1), 144-154. doi:https://doi.org/10.1016/j.vetpar.2011.05.035

Houdijk, J. G. M. (2008). Influence of periparturient nutritional demand on resistance to parasites in livestock. *Parasite Immunology, 30*, 113-121. doi:https://doi.org/10.1111/j.1365-3024.2008.00992.x

Houdijk, J. G. M., Jackson, F., Coop, R. L., & Kyriazakis, I. (2006). Rapid improvement of immunity to *Teladorsagia circumcincta* is achieved through a reduction in the demand for protein in lactating ewes. *International Journal for Parasitology, 36*, 219-227. doi:https://doi.org/10.1016/j.ijpara.2005.09.014

Houdijk, J. G. M., Jackson, F., & Kyriazakis, I. (2009). Nutritional sensitivity of resistance to *Trichostrongylus colubriformis* in lactating ewes. *Veterinary parasitology, 160*, 258-266. doi:http://dx.doi.org/10.1016/j.vetpar.2008.11.013

Houdijk, J. G. M., Jessop, N. S., & Kyriazakis, I. (2001). Nutrient partitioning between reproductive and immune functions in animals. *Proceedings of the Nutrition Society, 60*, 515-525. doi:10.1079/PNS2001114

Houdijk, J. G. M., Kyriazakis, I., Coop, R. l., & Jackson, F. (2001). The expression of immunity to *Teladorsagia circumcincta* in ewes and its relationship to protein nutrition depend on body protein reserves. *Parasitology, 122*, 661-672. doi:https://doi.org/10.1017/S0031182001007922

Houdijk, J. G. M., Kyriazakis, I., Jackson, F., & Coop, R. L. (2001). The relationship between protein nutrition, reproductive effort and breakdown in immunity to *Teladorsagia circumcincta* in periparturient ewes. *Animal Science, 72*, 595-606. doi:https://doi.org/10.1017/S1357729800052127

Houdijk, J. G. M., Kyriazakis, I., Jackson, F., Huntley, J. F., & Coop, R. L. (2000). Can an increased intake of metabolizable protein affect the periparturient relaxation in immunity against *Teladorsagia circumcincta* in sheep? *Veterinary parasitology, 91*, 43-62. doi:http://dx.doi.org/10.1016/S0304-4017(00)00255-7

Houdijk, J. G. M., Kyriazakis, I., Jackson, F., Huntley, J. F., & Coop, R. L. (2003). Is the allocation of metabolisable protein prioritised to milk production rather than to immune functions in *Teladorsagia circumcincta*-infected lactating ewes? *International Journal for Parasitology, 33*, 327-338. doi:http://dx.doi.org/10.1016/S0020-7519(02)00284-9

Houdijk, J. G. M., Kyriazakis, I., Jackson, F., Huntley, J. F., & Coop, R. L. (2005). Effects of protein supply and reproductive status on local and systemic immune responses to *Teladorsagia circumcincta* in sheep. *Veterinary parasitology, 129*, 105-117. doi:10.1016/j.vetpar.2004.12.023

Houdijk, J. G. M., Kyriazakis, I., Kidane, A., & Athanasiadou, S. (2012). Manipulating small ruminant parasite epidemiology through the combination of nutritional strategies. *Veterinary parasitology, 186*, 38-50. doi:10.1016/j.vetpar.2011.11.044

Hunt, P. W., McEwan, J. C., & Miller, J. E. (2008). Future perspectives for the implementation of genetic markers for parasite resistance in sheep. *Tropical Biomedicine, 25*(1), 18-33.

Hunter, T. E., Suster, D., DiGiacomo, K., Dunshea, F. R., Cummins, L. J., Egan, A. R., & Leury, B. J. (2015). Milk production and body composition of single-bearing East Friesian×Romney and Border Leicester×Merino ewes. *Small Ruminant Research, 131*, 123-129. doi:https://doi.org/10.1016/j.smallrumres.2015.08.006

Huntley, J. F., Jackson, F., Coop, R. L., Macaldowie, C., Houdijk, J. G. M., Familton, A. S., . . . Sykes, A. R. (2004). The sequential analysis of local inflammatory cells during abomasal nematode infection in periparturient sheep. *Veterinary Immunology and Immunopathology, 97*, 163-176. doi:http://dx.doi.org/10.1016/j.vetimm.2003.09.002

Iposu, S. O., Greer, A. W., McAnulty, R. W., Stankiewicz, M., & Sykes, A. R. (2010). Does milk supply have long-term benefits for resistance and resilience to nematode parasites in sheep? *Small Ruminant Research, 94*(1-3), 142-149.

Jackson, F., & Coop, R. L. (2000). The development of anthelmintic resistance in sheep nematodes. *Parasitology, 120*, S95-S107. doi:https://doi.org/10.1017/S0031182099005740

Jackson, F., Greer, A. W., Huntley, J., McAnulty, R. W., Bartley, D. J., Stanley, A., . . . Sykes, A. R. (2004). Studies using *Teladorsagia circumcincta* in an in vitro direct challenge method using abomasal tissue explants. *Veterinary parasitology, 124*(1), 73-89. doi:https://doi.org/10.1016/j.vetpar.2004.06.025

Jackson, F., Jackson, E., & Williams, J. T. (1988). Susceptibility of the pre-parturient ewe to infection with *Trichostrongylus vitrinus* and *Ostertagia circumcincta*. *Research in Veterinary Science, 45*(2), 213-218.

Jackson, F., & Miller, J. (2006). Alternative approaches to control—Quo vadit? *Veterinary parasitology, 139*(4), 371-384. doi:https://doi.org/10.1016/j.vetpar.2006.04.025

Jacobs, H. J., Wiltshire, C., Ashman, K., & Meeusen, E. N. T. (1999). Vaccination against the gastrointestinal nematode, *Haemonchus contortus*, using a purified larval surface antigen. *Vaccine, 17*, 362-368. doi:https://doi.org/10.1016/S0264-410X(98)00206-0

Jayanegara, A., Ridla, M., Astuti, D., Wiryawan, K., Laconi, E., & Nahrowi, N. (2017). Determination of energy and protein requirements of sheep in Indonesia using a meta-analytical approach. *Media Peternakan, 40*(2), 118-127.

Jeffcoate, I. A., Fishwick, G., Bairden, K., Armour, J., & Holmes, P. H. (1990). Pathophysiology of the periparturient egg rise in sheep: The role of prolactin. *Research in Veterinary Science, 48*(3), 295-300.

Jeffcoate, I. A., Wedrychowicz, H., Fishwick, G., Dunlop, E. M., Duncan, J. L., & Holmes, P. H. (1992). Pathophysiology of the periparturient egg rise in sheep: A possible role for IgA. *Research in Veterinary Science, 53*(2), 212-218. Retrieved from http://www.sciencedirect.com/science/article/pii/003452889290112F

Jørgensen, L. T. (2000). *The effect of host immunity on the development and survival of the free-living stages of common trichostrongylid parasites of sheep.* (Doctor of Philosophy Doctoral thesis, Massey University, 2000). Retrieved from https://mro.massey.ac.nz/bitstream/handle/10179/2261/01_front.pdf?sequence=2&isAllowed=y

Jørgensen, L. T., Leathwick, D. M., Charleston, W. A. G., Godfrey, P. L., Vlassoff, A., & Sutherland, I. A. (1998). Variation between hosts in the developmental success of the free-living stages of trichostrongyle infections of sheep. *International Journal for Parasitology, 28*(9), 1347-1352. doi:10.1016/s0020-7519(98)00092-7

Kahn, L. P. (2003). Regulation of the resistance and resilience of periparturient ewes to infection with gastrointestinal nematode parasites by dietary supplementation. *Australian Journal of Experimental Agriculture, 43*(12), 1477-1485.

Kahn, L. P., Knox, M. R., & Gray, G. D. (1999). Enhancing immunity to nematode parasites in pregnant and lactating sheep through nutrition and genetic selection. *Recent Advances in Animal Nutrition in Australia, 12*, 15-22. Retrieved from https://pdfs.semanticscholar.org/518a/d285b465b150ab0ab6db58b92be33e7f6d96.pdf

Kahn, L. P., Knox, M. R., Gray, G. D., Lea, J. M., & Walkden-Brown, S. W. (2003). Enhancing immunity to nematode parasites in single-bearing Merino ewes through nutrition and genetic selection. *Veterinary Parasitology, 112*(3), 211-225. doi:http://dx.doi.org/10.1016/S0304-4017(02)00438-7

Kahn, L. P., Knox, M. R., Walkden-Brown, S. W., & Lea, J. M. (2003). Regulation of the resistance to nematode parasites of single- and twin-bearing Merino ewes through nutrition and genetic selection. *Veterinary parasitology, 114*(1), 15-31. doi:https://doi.org/10.1016/S0304-4017(03)00099-2

Kahn, L. P., Kyriazakis, I., Jackson, F., & Coop, R. L. (2000). Temporal effects of protein nutrition on the growth and immunity of lambs infected with *Trichostrongylus colubriformis*. *International Journal for Parasitology, 30*(2), 193-205. doi:http://dx.doi.org/10.1016/S0020-7519(99)00192-7

Kambara, T., & McFarlane, R. G. (1996). Changes in T cell subpopulations of sheep due to age and dietary protein intake; association with protective immunity to *Trichostrongylus colubriformis*. *Veterinary Immunology and Immunopathology, 51*, 127-135. doi:http://dx.doi.org/10.1016/0165-2427(95)05513-4

Kasumov, T., Ilchenko, S., Li, L., Rachdaoui, N., Sadygov, R. G., Willard, B., . . . Previs, S. (2011). Measuring protein synthesis using metabolic ^{2}H labeling, high-resolution mass spectrometry, and an algorithm. *Analytical Biochemistry, 412*(1), 47-55. doi:10.1016/j.ab.2011.01.021

Ketzis, J. K., Vercruysse, J., Stromberg, B. E., Larsen, M., Athanasiadou, S., & Houdijk, J. G. M. (2006). Evaluation of efficacy expectations for novel and non-chemical helminth control strategies in ruminants. *Veterinary parasitology, 139*(4), 321-335. doi:https://doi.org/10.1016/j.vetpar.2006.04.022

Khadijah, S., Kahn, L. P., Walkden-Brown, S. W., Bailey, J. N., & Bowers, S. F. (2013). Effect of simulated rainfall timing on faecal moisture and development of *Haemonchus contortus* and *Trichostrongylus colubriformis* eggs to infective larvae. *Veterinary Parasitology, 192*(1-3), 199-210.

Kidane, A., Houdijk, J. G. M., Tolkamp, B. J., Athanasiadou, S., & Kyriazakis, I. (2009). Consequences of infection pressure and protein nutrition on periparturient resistance to *Teladorsagia circumcincta* and performance in ewes. *Veterinary parasitology, 165*, 78-87. doi:http://dx.doi.org/10.1016/j.vetpar.2009.06.039

Kimambo, A. E., MacRae, J. C., Walker, A., Watt, C. F., & Coop, R. L. (1988). Effect of prolonged subclinical infection with *Trichostrongylus colubriformis* on the performance and nitrogen metabolism of growing lambs. *Veterinary parasitology, 28*, 191-203. doi:https://doi.org/10.1016/0304-4017(88)90107-0

Knox, & Faedo, M. (2001). Biological control of field infections of nematode parasites of young sheep with *Duddingtonia flagrans* and effects of spore intake on efficacy. *Veterinary parasitology, 101*(2), 155-160. doi:https://doi.org/10.1016/S0304-4017(01)00504-0

Knox, & Jones, D. G. (1990). Studies on the presence and release of proteolytic enzymes (proteinases) in gastro-intestinal nematodes of ruminants. *International Journal for Parasitology, 20*(2), 243-249. doi:https://doi.org/10.1016/0020-7519(90)90106-W

Knox, & Smith, W. D. (2001). Vaccination against gastrointestinal nematode parasites of ruminants using gut-expressed antigens. *Veterinary parasitology, 100*(1), 21-32. doi:https://doi.org/10.1016/S0304-4017(01)00480-0

Knox, Torres-Acosta, J. F., & Aguilar-Caballero, A. J. (2006). Exploiting the effect of dietary supplementation of small ruminants on resilience and resistance against gastrointestinal nematodes. *Veterinary Parasitology, 139*(4), 385-393. doi:10.1016/j.vetpar.2006.04.026

Köhler, P. (2001). The biochemical basis of anthelmintic action and resistance. *International Journal for Parasitology, 31*(4), 336-345. doi:https://doi.org/10.1016/S0020-7519(01)00131-X

Kyriazakis, I. (2010). Is anorexia during infection in animals affected by food composition? *Animal Feed Science and Technology, 156*, 1-9. doi:10.1016/j.anifeedsci.2010.01.001

Kyriazakis, I., Anderson, D. H., Coop, R. L., & Jackson, F. (1996). The pathophysiology and development of immunity during long-term subclinical infection with *Trichostrongylus colubriformis* of sheep receiving different nutritional treatments. *Veterinary parasitology, 65*(1), 41-54. doi:https://doi.org/10.1016/0304-4017(96)00947-8

Kyriazakis, I., Anderson, D. H., Oldham, J. D., Coop, R. L., & Jackson, F. (1996). Long-term subclinical infection with *Trichostrongylus colubriformis*: Effects on food intake, diet selection and performance of growing lambs. *Veterinary parasitology, 61*, 297-313. doi:https://doi.org/10.1016/0304-4017(95)00824-1

Kyriazakis, I., & Houdijk, J. G. M. (2006). Immunonutrition: Nutritional control of parasites. *Small Ruminant Research, 62*, 79-82. doi:10.1016/j.smallrumres.2005.07.036

Kyriazakis, I., Tolkamp, B. J., & Hutchings, M. R. (1998). Towards a functional explanation for the occurrence of anorexia during parasitic infections. *Animal Behaviour, 56*(2), 265-274. doi:https://doi.org/10.1006/anbe.1998.0761

Lawrence, K. E., Leathwick, D. M., Rhodes, A. P., Jackson, R., Heuer, C., Pomroy, W. E., . . . Moffat, J. R. (2007). Management of gastrointestinal nematode parasites on sheep farms in New Zealand. *New Zealand Veterinary Journal, 55*(5), 228-234. doi:10.1080/00480169.2015.1012133

Le Floc'h, N., Melchior, D., & Obled, C. (2004). Modifications of protein and amino acid metabolism during inflammation and immune system activation. *Livestock Production Science, 87*, 37-45. doi:10.1016/j.livprodsci.2003.09.005

Leathwick, D. M. (2013). The influence of temperature on the development and survival of the pre-infective free-living stages of nematode parasites of sheep. *New Zealand Veterinary Journal, 61*(1), 32-40. doi:https://doi.org/10.1080/00480169.2012.712092

Leathwick, D. M., Miller, C. M., Atkinson, D. S., Haack, N. A., Alexander, R. A., Oliver, A. M., . . . Sutherland, I. A. (2006). Drenching adult ewes: Implications of anthelmintic treatments pre- and post-lambing on the development of anthelmintic resistance. *New Zealand Veterinary Journal, 54*(6), 297-304. doi:10.1080/00480169.2006.36714

Leathwick, D. M., Miller, C. M., Atkinson, D. S., Haack, N. A., Waghorn, T. S., & Oliver, A. M. (2008). Managing anthelmintic resistance: Untreated adult ewes as a source of unselected parasites, and their role in reducing parasite populations. *New Zealand Veterinary Journal, 56*(4), 184-195. doi:10.1080/00480169.2008.36832

Leathwick, D. M., Miller, C. M., & Waghorn, T. S. (2011). Development and spatial distribution of the free-living stages of Teladorsagia circumcincta and Trichostrongylus colubriformis on pasture: A pilot study. *New Zealand Veterinary Journal, 59*(6), 272-278. doi:10.1080/00480169.2011.610273

Leathwick, D. M., Pomroy, W. E., & Heath, A. C. G. (2001). Anthelmintic resistance in New Zealand. *New Zealand Veterinary Journal, 49*(6), 227-235. doi:10.1080/00480169.2001.36237

Leathwick, D. M., Vlassoff, A., & Barlow, N. D. (1995). A model for nematodiasis in New Zealand lambs: The effect of drenching regime and grazing management on the development of anthelmintic resistance. *International Journal for Parasitology, 25*(12), 1479-1490. doi:https://doi.org/10.1016/0020-7519(95)00059-3

Lee, C. Y., Munyard, K. A., Gregg, K., Wetherall, J. D., Stear, M. J., & Groth, D. M. (2011). The influence of MHC and Immunoglobulins A and E on host resistance to gastrointestinal nematodes in sheep. *Journal of Parasitology Research, 2011*, 1-11. doi:10.1155/2011/101848

Leyva, V., Henderson, A. E., & Sykes, A. R. (1981). The effect of daily infection with *O. circumcincta* larvae on the performance of pregnant and lactating sheep. *Proceedings of the New Zealand Society of Animal Production, 41*, 279-282.

Leyva, V., Henderson, A. E., & Sykes, A. R. (1982). Effect of daily infection with *Ostertagia circumcincta* larvae on food intake, milk production and wool growth in sheep. *The Journal of Agricultural Science, 99*, 249-259. doi:https://doi.org/10.1017/S0021859600030008

Liu, S. M., Smith, T. L., Briegel, J., Gao, S. B., & Peng, W. K. (2007). Fractional protein synthesis rate and polyamine concentrations in tissues of Merino sheep selected for gastrointestinal nematode resistance. *Livestock Science, 106*(1), 65-75. doi:https://doi.org/10.1016/j.livsci.2006.07.004

Louvandini, H., Veloso, C. F. M., Paludo, G. R., Dell'Porto, A., Gennari, S. M., & McManus, C. M. (2006). Influence of protein supplementation on the resistance and resilience on young hair sheep naturally infected with gastrointestinal nematodes during rainy and dry seasons. *Veterinary parasitology, 137*, 103-111. doi:10.1016/j.vetpar.2006.01.004

Lützelschwab, C. M., Fiel, C. A., Pedonesse, S. I., Najle, R., Rodríguez, E., Steffan, P. E., . . . Iglesias, L. (2005). Arrested development of *Ostertagia ostertagi*: Effect of the exposure of infective larvae to natural spring conditions of the Humid Pampa (Argentina). *Veterinary parasitology, 127*, 253-262. doi:http://dx.doi.org/10.1016/j.vetpar.2004.10.006

MacDonald, A. J., Small, A. C., Greig, C. A., Husi, H., Ross, J. A., Stephens, N. A., . . . Preston, T. (2013). A novel oral tracer procedure for measurement of habitual myofibrillar protein synthesis. *Rapid Communications in Mass Spectrometry, 27*, 1769-1777. doi:10.1002/rcm.6622

MacKinnon, K. M., Zajac, A. M., Kooyman, F. N. J., & Notter, D. R. (2010). Differences in immune parameters are associated with resistance to *Haemonchus contortus* in Caribbean hair sheep. *Parasite Immunology, 32*(7), 484-493.

McAnulty, R. W. (1990). *Subsceptibility of the breeding ewe to parasitism.* (Master of Applied Science Unpublish Master Thesis). Lincoln College, University of Canterbury, Lincoln, New Zealand.

McBean, D., Nath, M., Kenyon, F., Zile, K., Bartley, D. J., & Jackson, F. (2016). Faecal egg counts and immune markers in a line of Scottish Cashmere goats selected for resistance to gastrointestinal nematode parasite infection. *Veterinary Parasitology, 229*, 1-8.

McClure, S. J. (2009). Mucosal delivery of native and recombinant protein vaccines against *Trichostrongylus colubriformis*. *International Journal for Parasitology, 39*(5), 599-606. doi:https://doi.org/10.1016/j.ijpara.2008.09.010

McFarlane, R. G. (1997). Immunity to nematodes in ruminants. In G. K. Barell (Ed.), *Sustainable control of internal parasites in ruminants* (pp. 149-159). Lincoln, New Zealand: Lincoln University.

McRae, K. M., Stear, M. J., Good, B., & Keane, O. M. (2015). The host immune response to gastrointestinal nematode infection in sheep. *Parasite Immunology, 37*(12), 605-613. doi:10.1111/pim.12290

Michel, J. F. (1985). Strategies for the use of anthelmintics in livestock and their implications for the development of drug resistance. *Parasitology, 90*(4), 621-628. doi:10.1017/S0031182000052276

Miller, Blair, H. T., Birtles, M. J., Reynolds, G. W., Gill, H. S., & Revell, D. K. (2000). Cysteine may play a role in the immune response to internal parasites in sheep. *Australian Journal of Agricultural Research, 51*, 793-799. doi:https://doi.org/10.1071/AR99189

Miller, Ganesh, S., Garland, C. B., & Leathwick, D. M. (2015). Production benefits from pre-and post-lambing anthelmintic treatment of ewes on commercial farms in the southern North Island of New Zealand. *New Zealand Veterinary Journal, 63*(4), 211-219. doi:http://dx.doi.org/10.1080/00480169.2015.1007108

Miller, Waghorn, T. S., Leathwick, D. M., Candy, P. M., Oliver, A. M. B., & Watson, T. G. (2012). The production cost of anthelmintic resistance in lambs. *Veterinary parasitology, 186*(3-4), 376-381.

Ministry of Agriculture Fisheries and Food. (1986). *Manual of veterinary parasitological laboratory techniques* (3rd ed.). London, UK: Her Majesty's Stationary Office (HMSO).

Morgan, Fogarty, N. M., Nielsen, S., & Gilmour, A. R. (2006). Milk yield and milk composition from grazing primiparous non-dairy crossbred ewes. *Australian Journal of Agricultural Research, 57*(4), 377-387. doi:https://doi.org/10.1071/AR05180

Morgan, & van Dijk, J. (2012). Climate and the epidemiology of gastrointestinal nematode infections of sheep in Europe. *Veterinary parasitology, 189*, 8-14. doi:http://dx.doi.org/10.1016/j.vetpar.2012.03.028

Morris, C. A., Bisset, S. A., Vlassoff, A., Wheeler, M., West, C. J., Devantier, B. P., & Mackay, A. D. (2010). Selecting for resilience in Romney sheep under nematode parasite challenge, 1994–2007. *New Zealand journal of agricultural research, 53*(3), 245-261.

Muir, P. D., Smith, N. B., Wallace, G. J., Fugle, C. J., & Bown, M. D. (2000). Maximising lamb growth rates. *Proceedings of the New Zealand Grassland Association, 62*, 55-58.

Muñoz-Guzmán, M. A., Cuéllar-Ordaz, J. A., Valdivia-Anda, A. G., Buendía-Jiménez, J. A., & Alba-Hurtado, F. (2006). Correlation of parasitological and immunological parameters in sheep with high and low resistance to haemonchosis. *Canadian Journal of Animal Science, 86*(3), 363-371. doi:https://doi.org/10.4141/A06-010

Ng'ang'a, C. J., Munyua, W. K., Maingi, N., & Kanyari, P. W. N. (2004). Occurrence of peri-parturient rise in trichostrongylid nematode egg output in Dorper ewes in a semi-arid area of Kajiado District of Kenya. *Acta Tropica, 92*(3), 213-218. doi:http://dx.doi.org/10.1016/j.actatropica.2004.05.016

Nicol, A. M., & Brookes, I. M. (2007). The metabolisable energy requirements of grazing livestock. In P. V. Rattray, I. M. Brookes, & A. M. Nicol (Eds.), *Pasture and supplement for grazing animals* (pp. 151-172). Hamilton, New Zealand: New Zealand Society of Animal Production.

Nisbet, A. J., McNeilly, T. N., Greer, A. W., Bartley, Y., Oliver, E. M., Smith, S. K., . . . Matthews, J. B. (2016). Protection of ewes against *Teladorsagia circumcincta* infection in the periparturient period by vaccination with recombinant antigens. *Veterinary parasitology, 228*, 130-136. doi:https://doi.org/10.1016/j.vetpar.2016.09.002

Nunns, V. J., Rawes, D. A., & Shearer, G. C. (1965). Strategic anthelmintic medication of ewes. *Veterinary Record, 77*(12), 328-332.
O'Sullivan, B. M., & Donald, A. D. (1970). A field study of nematode parasite populations in the lactating ewe. *Parasitology, 61*, 301-315. doi:https://doi.org/10.1017/S0031182000041135
O'Sullivan, B. M., & Donald, A. D. (1973). Responses to infection with *Haemonchus contortus* and *Trichostrongylus colubriformis* in ewes of different reproductive status. *International Journal for Parasitology, 3*(4), 521-530. doi:https://doi.org/10.1016/0020-7519(73)90049-0
O'Connor, L. J., Kahn, L. P., & Walkden-Brown, S. W. (2007). The effects of amount, timing and distribution of simulated rainfall on the development of *Haemonchus contortus* to the infective larval stage. *Veterinary parasitology, 146*, 90-101. doi:http://dx.doi.org/10.1016/j.vetpar.2007.02.002
O'Connor, L. J., Kahn, L. P., & Walkden-Brown, S. W. (2008). Interaction between the effects of evaporation rate and amount of simulated rainfall on development of the free-living stages of *Haemonchus contortus*. *Veterinary parasitology, 155*, 223-234. doi:http://dx.doi.org/10.1016/j.vetpar.2008.05.010
O'Connor, L. J., Walkden-Brown, S. W., & Kahn, L. P. (2006). Ecology of the free-living stages of major trichostrongylid parasites of sheep. *Veterinary parasitology, 142*, 1-15. doi:http://dx.doi.org/10.1016/j.vetpar.2006.08.035
Parkins, J. J., & Holmes, P. H. (1989). Effects of gastrointestinal helminth parasites on ruminant nutrition. *Nutrition Research Reviews, 2*(1), 227-246. doi:https://doi.org/10.1079/NRR19890016
Poppi, D. P., MacRae, J. C., Brewer, A., & Coop, R. L. (1986). Nitrogen transactions in the digestive tract of lambs exposed to the intestinal parasite, *Trichostrongylus colubriformis*. *British Journal of Nutrition, 55*, 593-602. doi:https://doi.org/10.1079/BJN19860064
Råberg, L. (2014). How to live with the enemy: Understanding tolerance to parasites. *Plos Biology, 12*(11), e1001989-e1001989.
Rattray, P. V. (1978). Pasture constraints to sheep production. *Proceedings of Agronomy Society of New Zealand, 8*, 103-108.
Reid, J. F. S., & Armour, J. (1975). Seasonal variations in the gastro-intestinal nematode populations of Scottish hill sheep. *Research in Veterinary Science, 18*(3), 307-313. doi:https://doi.org/10.1016/S0034-5288(18)33583-5
Robertson, T. G., & Elliott, D. C. (1966). The laboratory assessment of worm parasite populations in sheep. *New Zealand Journal of Agricultural Research, 9*(2), 350-358. doi:https://doi.org/10.1080/00288233.1966.10420787
Rocha, R. A., Bricarello, P. A., Silva, M. B., Houdijk, J. G. M., Almeida, F. A., Cardia, D. F. F., & Amarante, A. F. T. (2011). Influence of protein supplementation during late pregnancy and lactation on the resistance of Santa Ines and Ile de France ewes to *Haemonchus contortus*. *Veterinary parasitology, 181*, 229-238. doi:10.1016/j.vetpar.2011.03.055
Romjali, E., Dorny, P., Batubara, A., Pandey, V. S., & Gatenby, R. M. (1997). Peri-parturient rise in faecal strongyle egg counts of different genotypes of sheep in North Sumatra, Indonesia. *Veterinary parasitology, 68*, 191-196. doi:http://dx.doi.org/10.1016/S0304-4017(96)01008-4
Rowe, J. B., Nolan, J. V., De Chaneet, G., Teleni, E., & Holmes, P. H. (1988). The effect of haemonchosis and blood loss into the abomasum on digestion in sheep. *British Journal of Nutrition, 59*(1), 125-139. doi:https://doi.org/10.1079/BJN19880016
Russel, A. J. F., Doney, J. M., & Gunn, R. G. (1969). Subjective assessment of body fat in live sheep. *The Journal of Agricultural Science, 72*(3), 451-454. doi:https://doi.org/10.1017/S0021859600024874
Sakkas, P., Houdijk, J. G. M., Athanasiadou, S., & Kyriazakis, I. (2012). Sensitivity of periparturient breakdown of immunity to parasites to dietary protein source. *Journal of Animal Science, 90*(11), 3954-3962. doi:10.2527/jas.2011-4829
Salisbury, J. R., & Arundel, J. H. (1970). Peri-parturient deposition of nematode eggs by ewes and residual pasture contamination as sources of infection for lambs. *Australian Veterinary Journal, 46*, 523-529. doi:https://doi-org.ezproxy.lincoln.ac.nz/10.1111/j.1751-0813.1970.tb06637.x

Santos, M. C., Silva, B. F., & Amarante, A. F. (2012). Environmental factors influencing the transmission of Haemonchus contortus. *Veterinary Parasitology, 188*(3-4), 277-284.

Sargison, N. D., Bartram, D. J., & Wilson, D. J. (2012). Use of a long acting injectable formulation of moxidectin to control the periparturient rise in faecal *Teladorsagia circumcincta* egg output of ewes. *Veterinary parasitology, 189*, 274-283. doi:10.1016/j.vetpar.2012.04.020

Sayers, G., & Sweeney, T. (2007). Gastrointestinal nematode infection in sheep – A review of the alternatives to anthelmintics in parasite control. *Animal Health Research Reviews, 6*(2), 159-171. doi:10.1079/AHR2005108

Schallig, H. D. F. H. (2000). Immunological responses of sheep to *Haemonchus contortus*. *Parasitology, 120*, S63-S72.

Sebastiano, R. S., Sweeney, T., Keady, T. W. J., Hanrahan, J. P., & Good, B. (2017). Can the amount of digestible undegraded protein offered to ewes in late pregnancy affect the periparturient change in resistance to gastrointestinal nematodes? *Veterinary parasitology, 235*, 8-16. doi:https://doi.org/10.1016/j.vetpar.2016.12.019

Shaw, R. J., McNeill, M. M., Gatehouse, T. K., & Douch, P. G. C. (1997). Quantification of total sheep IgE concentration using anti-ovine IgE monoclonal antibodies in an enzyme immunoassay. *Veterinary Immunology and Immunopathology, 57*(3), 253-265. doi:10.1016/S0165-2427(97)00010-X

Shaw, R. J., Morris, C. A., Wheeler, M., Tate, M., & Sutherland, I. A. (2012). Salivary IgA: A suitable measure of immunity to gastrointestinal nematodes in sheep. *Veterinary Parasitology, 186*(1-2), 109-117. doi:10.1016/j.vetpar.2011.11.051

Sheep Improvement Limited. (2008). Resilience to internal parasites. Part 2. Protocol for measurement & genetic evaluation. Retrieved 22 May 2019 from https://www.sil.co.nz/files/1496280304597.pdf.

Smith, Jackson, F., Jackson, E., & Williams, J. (1985). Age immunity to *Ostertagia circumcincta*: Comparison of the local immune-responses of 41/2-month-old and 10-month-old lambs. *Journal of Comparative Pathology, 95*(2), 235-245. doi:10.1016/0021-9975(85)90010-6

Smith, Mills, A., & Moot, D. (2022). Total annual and seasonal DM production of improved and unimproved resident pastures at three farms in Canterbury. *Journal of New Zealand Grasslands, 84*, 79-94.

Sollenberger, L. E., & Burns, J. C. (2001). The conduct of grazing trials: Rationale. *56th Southern Pasture and Forage Crop Improvement Conference, April 21-22, 2001*.

Southcott, W. H. (1971). Management practices and helminthosis in the lamb. *Australian Veterinary Journal, 47*(4), 170-174. doi:https://doi-org.ezproxy.lincoln.ac.nz/10.1111/j.1751-0813.1971.tb02132.x

Spedding, C. R. W., Brown, T. H., & Large, R. V. (1963). The effect of milk intake on nematode infestation of the lamb. *Proceedings of the Nutrition Society, 22*, 32-41. doi:10.1079/PNS19630009

Stear, M. J., Bairden, K., Innocent, G. T., Mitchell, S., Strain, S., & Bishop, S. C. (2004). The relationship between IgA activity against 4th-stage larvae and density-dependent effects on the number of 4th-stage larvae of *Teladorsagia circumcincta* in naturally infected sheep. *Parasitology, 129*, 363-369. doi:10.1017/s0031182004005736

Stear, M. J., Boag, B., Cattadori, I., & Murphy, L. (2009). Genetic variation in resistance to mixed, predominantly *Teladorsagia circumcincta* nematode infections of sheep: from heritabilities to gene identification. *Parasite Immunology, 31*(5), 274-282. doi:10.1111/j.1365-3024.2009.01105.x

Stear, M. J., Park, M., & Bishop, S. C. (1996). The key components of resistance to *Ostertagia circumcincta* in lambs. *Parasitology Today, 12*(11), 438-441. doi:10.1016/0169-4758(96)10069-7

Stear, M. J., Strain, S., & Bishop, S. C. (1999a). How lambs control infection with *Ostertagia circumcincta*. *Veterinary Immunology and Immunopathology, 72*(1), 213-218. doi:https://doi.org/10.1016/S0165-2427(99)00134-8

Stear, M. J., Strain, S., & Bishop, S. C. (1999b). Mechanisms underlying resistance to nematode infection. *International Journal for Parasitology, 29*(1), 51-56. doi:https://doi.org/10.1016/S0020-7519(98)00179-9

Steel, J. W., & Knox, M. R. (2003). Nutrition-parasite interactions in sheep: Future research priorities. *Recent Advances in Animal Nutrition in Australia, 14*, 103-110.

Steel, J. W., Symons, L. E. A., & Jones, W. O. (1980). Effects of level of larval intake on the productivity and physiological and metabolic responses of lambs infected with *Trichostrongylus colubriformis*. *Australian Journal of Agricultural Research, 31*(4), 821-838. doi:https://doi.org/10.1071/AR9800821

Strain, S. A. J., & Stear, M. J. (2001). The influence of protein supplementation on the immune response to *Haemonchus contortus*. *Parasite Immunology, 23*, 527-531. doi:https://doi.org/10.1046/j.1365-3024.2001.00410.x

Stromberg, B. E. (1997). Environmental factors influencing transmission. *Veterinary parasitology, 72*(3), 247-264. doi:https://doi.org/10.1016/S0304-4017(97)00100-3

Sutherland, Leathwick, D. M., Green, R., Brown, A. E., & Miller, C. M. (1999). The effect of continuous drug exposure on the immune response to *Trichostrongylus colubriformis* in sheep. *Veterinary parasitology, 80*(3), 261-271. doi:https://doi.org/10.1016/S0304-4017(98)00220-9

Sutherland, & Scott, I. (2010). *Gastrointestinal nematodes of sheep and cattle: Biology and control.* Chichester, U.S.A.: Wiley-Blackwell.

Sutherland, Shaw, J., & Shaw, R. J. (2010). The production costs of anthelmintic resistance in sheep managed within a monthly preventive drench program. *Veterinary parasitology, 171*, 300-304. doi:10.1016/j.vetpar.2010.03.035

Sykes, A. R. (1994). Parasitism and production in farm animals. *Animal Production, 59*(2), 155-172. doi:10.1017/S0003356100007649

Sykes, A. R. (1997). Effect of parasitism on ruminant animal performance. In G. K. Barrell (Ed.), *Sustainable control of internal parasites in ruminants* (pp. 81-91). Lincoln, New Zealand: Lincoln University.

Sykes, A. R., & Coop, R. L. (1976). Intake and utilization of food by growing lambs with parasitic damage to the small intestine caused by daily dosing with *Trichostrongylus colubriformi*s larvae. *The Journal of Agricultural Science, 86*(3), 507-515. doi:10.1017/S0021859600061049

Sykes, A. R., & Coop, R. L. (1977). Intake and utilization of food by growing sheep with abomasal damage caused by daily dosing with *Ostertagia circumcincta* larvae. *Journal of Agricultural Science, 88*, 671-677. doi:https://doi.org/10.1017/S0021859600037369

Sykes, A. R., & Coop, R. L. (2001). Interaction between nutrition and gastrointestinal parasitism in sheep. *New Zealand Veterinary Journal, 49*(6), 222-226. doi:10.1080/00480169.2001.36236

Sykes, A. R., & Field, A. C. (1973). Effects of dietary deficiencies of energy, protein and calcium on the pregnant ewe: IV. Serum total protein, albumin, globulin, transferrin and plasma urea levels. *Journal of Agricultural Science, 80*, 29-36. doi:10.1017/S0021859600057026

Sykes, A. R., & Greer, A. W. (2003). Effects of parasitism on the nutrient economy of sheep: An overview. *Australian Journal of Experimental Agriculture, 43*(12), 1393-1398. doi:https://doi.org/10.1071/EA02228

Sykes, A. R., & Kyriazakis, I. (2007). Opportunities to control herbivore nematodes through manipulation of the grazing environment. In Q. X. Ming, L. P. Ren, & Z. J. Gao (Eds.), *Herbivore Nutrition for the Development of Efficient, Safe and Sustainable Livestock Production* (pp. 329-353). Beijing: China Agricultural University Press.

Symons, L. E. A., Steel, J. W., & Jones, W. O. (1981). Effects of level of larval intake on the productivity and physiological and metabolic responses of lambs infected with *Ostertagia circumcincta*. *Australian Journal of Agricultural Research, 32*, 139-148.

Tembely, S., Lahlou-Kassi, A., Rege, J. E. O., Mukasa-Mugerwa, E., Anindo, D., Sovani, S., & Baker, R. L. (1998). Breed and season effects on the peri-parturient rise in nematode egg output in indigenous ewes in a cool tropical environment. *Veterinary Parasitology, 77*, 123-132. doi:http://dx.doi.org/10.1016/S0304-4017(97)00219-7

Thamsborg, S. M., Roepstorff, A., & Larsen, M. (1999). Integrated and biological control of parasites in organic and conventional production systems. *Veterinary parasitology, 84*(3), 169-186. doi:https://doi.org/10.1016/S0304-4017(99)00035-7

Thomas, R. J., & Ali, D. A. (1983). The effect of *Haemonchus contortus* infection on the pregnant and lactating ewe. *International Journal for Parasitology, 13*(4), 393-398. doi:https://doi.org/10.1016/S0020-7519(83)80047-2

Tizard, I. R. (2000). *Veterinary immunology : An introduction* (6th ed.). Philadelphia: W.B. Saunders Company.

Toscan, G., Cadore, G. C., Limana, J. F. T., Weber, A., Palma, H. H., Duarte, M. M. F., . . . Vogel, F. S. F. (2017). Immune response of sheep naturally infected with Haemonchus spp. on pastures with two different nutritional conditions. *Semina-Ciencias Agrarias, 38*(2), 809-820. doi:10.5433/1679-0359.2017v38n2p809

Urquhart, G. M., Armour, J., Duncan, J. L., Dunn, A. M., & Jennings, F. W. (1987). *Veterinary parasitology*. Essex, England: Longman Scientific & Technical.

van Dijk, J., & Morgan, E. R. (2011). The influence of water on the migration of infective trichostrongyloid larvae onto grass. *Parasitology, 138*(6), 780-788.

van Dijk, J., Sargison, N. D., Kenyon, F., & Skuce, P. J. (2010). Climate change and infectious disease: helminthological challenges to farmed ruminants in temperate regions. *Animal, 4*(3), 377-392. doi:10.1017/S1751731109990991

Van Emon, M. L., Schauer, C. S., Lekatz, L. A., Eckerman, S. R., Maddock-Carlin, K., & Vonnahme, K. A. (2014). Supplementing metabolizable protein to ewes during late gestation: I. Effects on ewe performance and offspring performance from birth to weaning. *Journal of Animal Science, 92*(1), 339-348. doi:https://doi.org/10.2527/jas.2013-6851

van Houtert, M. F. J., Barger, I. A., & Steel, J. W. (1995). Dietary protein for young grazing sheep: Interactions with gastrointestinal parasitism. *Veterinary parasitology, 60*, 283-295. doi:http://dx.doi.org/10.1016/0304-4017(95)00864-8

van Houtert, M. F. J., Barger, I. A., Steel, J. W., Windon, R. G., & Emery, D. L. (1995). Effects of dietary protein intake on responses of young sheep to infection with *Trichostrongylus colubriformis*. *Veterinary parasitology, 56*(1), 163-180. doi:https://doi.org/10.1016/0304-4017(94)00668-3

van Houtert, M. F. J., & Sykes, A. R. (1996). Implications of nutrition for the ability of ruminants to withstand gastrointestinal nematode infections. *International Journal for Parasitology, 26*(11), 1151-1167. doi:http://dx.doi.org/10.1016/S0020-7519(96)00120-8

van Wyk, J. A., & Mayhew, E. (2013). Morphological identification of parasitic nematode infective larvae of small ruminants and cattle: A practical lab guide. *Onderstepoort Journal of Veterinary Research, 80*(1), 1-14. doi:http://dx.doi.org/10.4102/ojvr.v80i1.539

Vaughan, A. L., Greer, A. W., McAnulty, R. W., & Sykes, A. R. (2006). Plasma protein loss in lambs during a mixed infection of *Trichostrongylus colubriformis* and *Teladorsagia circumcincta*-a consequence of the immune response? *Proceedings of the New Zealand Society of Animal Production, 66*, 83-87.

Vengesa, P. B., & Leese, H. J. (1979). Sugar absorption by the mouse small intestine following infection with *Schistosoma mansoni*. *Transactions of The Royal Society of Tropical Medicine and Hygiene, 73*(1), 55-60. doi:https://doi.org/10.1016/0035-9203(79)90130-5

Venturina, V. M., Gossner, A. G., & Hopkins, J. (2013). The immunology and genetics of resistance of sheep to *Teladorsagia circumcincta*. *Veterinary Research Communications, 37*(2), 171-181. doi:10.1007/s11259-013-9559-9

Vlassoff, A. (1982). Biology and population dynamics of the free living stages of gastrointestinal nematodes of sheep. In A. D. Ross (Ed.), *Control of Internal Parasites of Sheep – an Animal Industries Workshop* (pp. 39). Lincoln, New Zealand: Lincoln College Press.

Vlassoff, A., & Bisset, S. A. (1991). Basic principles of parasite epidemiology. *Proceedings of the 21st Sheep and Beef Cattle Society Annual Seminar, New Zealand Veterinary Association (NZVA), 134*, 5-12.

Vlassoff, A., Leathwick, D. M., & Heath, A. C. G. (2001). The epidemiology of nematode infections of sheep. *New Zealand Veterinary Journal, 49*(6), 213-221. doi:https://doi.org/10.1080/00480169.2001.36235

Waghorn, T. S., Reynecke, D. P., Oliver, A. M. B., Miller, C. M., Vlassoff, A., Koolaard, J. P., & Leathwick, D. M. (2011). Dynamics of the free-living stages of sheep intestinal parasites on pasture in the North Island of New Zealand. 1. Patterns of seasonal development. *New Zealand Veterinary Journal, 59*(6), 279-286. doi:10.1080/00480169.2011.610279

Walkden-Brown, S. W., & Kahn, L. P. (2002). Nutritional modulation of resistance and resilience to gastrointestinal nematode infection–A review. *Asian-Australasian Journal of Animal Sciences, 15*(6), 912-924.

Waller, P. J. (1997). Nematode parasite control of livestock in the tropics/subtropics: The need for novel approaches. *International Journal for Parasitology, 27*(10), 1193-1201. doi:https://doi.org/10.1016/S0020-7519(97)00117-3

Wang, T., van Wyk, J. A., Morrison, A., & Morgan, E. R. (2014). Moisture requirements for the migration of *Haemonchus contortus* third stage larvae out of faeces. *Veterinary Parasitology, 204*(3-4), 258-264.

Watson, D. L., Colditz, I. G., Andrew, M., Gill, H. S., & Altmann, K. G. (1994). Age-dependent immune response in Merino sheep. *Research in Veterinary Science, 57*(2), 152-158. doi:https://doi.org/10.1016/0034-5288(94)90051-5

Watson, D. L., & Gill, H. S. (1991). Post natal ontogeny of immunological responsiveness in Merino sheep. *Research in Veterinary Science, 51*(1), 88-93. doi:https://doi.org/10.1016/0034-5288(91)90037-O

West, D. M., Bruere, A. N., & Ridler, A. L. (2002). *The Sheep: Health, Disease and Production.* (2nd ed.). Palmerston North, New Zealand: Foundation for Veterinary Continuing Education of the N.Z. Veterinary Association.

Williams, A. R., Greeff, J. C., Vercoe, P. E., Dobson, R. J., & Karlsson, L. J. E. (2010). Merino ewes bred for parasite resistance reduce larval contamination onto pasture during the periparturient period. *Animal, 4*(1), 122-127. doi:10.1017/S1751731109990802

Woof, J. M., & Kerr, M. A. (2004). IgA function – variations on a theme. *Immunology, 113*(2), 175-177. doi:10.1111/j.1365-2567.2004.01958.x

Wright, D. A., McAnulty, R. W., Noonan, M. J., & Stankiewicz, M. (2003). The effect of *Duddingtonia flagrans* on trichostrongyle infections of Saanen goats on pasture. *Veterinary parasitology, 118*(1), 61-69. doi:https://doi.org/10.1016/j.vetpar.2003.10.005

Xie, H. L. (2004). *Nutritional and immunological interrelationships in response to nematode infections in periparturient ewes* (Doctor of Philosophy Unpublished doctoral thesis). Lincoln University, Lincoln, New Zealand.

Xie, H. L., Stankiewicz, M., Huntley, J. F., Sedcole, J. R., McAnulty, R. W., Green, R. S., & Sykes, A. R. (2004). The effects of cold exposure, food allowance and litter size on immunity of periparturient sheep to *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. *Animal Science, 78*, 149-158. doi:https://doi.org/10.1017/S1357729800053935

Zaralis, K., Tolkamp, B. J., Houdijk, J. G. M., Wylie, A. R. G., & Kyriazakis, I. (2009). Consequences of protein supplementation for anorexia, expression of immunity and plasma leptin concentrations in parasitized ewes of two breeds. *British Journal of Nutrition, 101*, 499-509. doi:10.1017/S000711450802401X

Zeng, S., Brown, S., Przemeck, S. M. C., & Simpson, H. V. (2003). Milk and milk components reduce the motility of *Ostertagia circumcincta* larvae in vitro. *New Zealand veterinary journal, 51*(4), 174-178. doi:10.1080/00480169.2003.36360

Supplementation of ewes on pasture to provide an epidemiological benefit for gastrointestinal parasitism

RD Tambunan[1,2*], CM Logan[1], AC Bywater[1] and AW Greer[1]

[1]*Faculty of Agriculture and Life Sciences, Lincoln University, PO Box 85084, Lincoln 7647, Christchurch, New Zealand*
[2]*Indonesian Agency for Agricultural Research and Development, Ministry of Agriculture of Indonesia*

**Corresponding author:* Email: RenyDebora.Tambunan@lincolnuni.ac.nz

Abstract

The potential epidemiological benefit of reducing the peri-parturient relaxation in immunity to gastro-intestinal nematode parasites through supplementing twin-bearing ewes during the first four weeks of lactation was evaluated in a replicated farmlet study. In two sequential years, ewes either grazed pasture alone or grazed pasture while supplemented with 0.5 kgDM/d of a high-protein pellet. Supplementation did not affect ewe live weight or body condition score or weight of lamb weaned per ewe ($P>0.05$). Ewe faecal egg counts (FEC) showed a time x supplementation interaction ($P<0.05$), being reduced by 50% from week six of lactation in both years, although this only resulted in transient and inconsistent reductions in pasture larval contamination. After weaning, there was no consistent parasitological benefit to lambs grazing areas where ewes had been supplemented that were reflected in either pasture larvae concentrations, lamb FEC, the requirement for anthelmintic treatment or lamb growth rate ($P>0.05$ for all). Despite supplementation of ewes during the first four weeks of lactation successfully reducing ewe faecal egg count by 50%, this was not sufficient to provide a measureable and consistent epidemiological benefit to the lambs.

Keywords: supplementation; sheep; peri-parturient; epidemiological; nematode

Introduction

Nematode parasites are a major animal health impediment for grazing ruminants around the world. Sheep farmers in New Zealand consider nematode parasites as their most significant animal health issue (Lawrence et al. 2007), and can cause significant losses in productivity and welfare if not prevented. Many control methods have been used to reduce the effect of parasite infection, including the use of anthelmintics. However, due to the development of anthelmintic resistance, alternative control options are needed.

A potential method of control is the nutritional supplementation of the ewe during the peri-parturient period (Beasley et al. 2012; Donaldson et al. 1998, 2001; Houdijk 2008; Kahn 2003). Donaldson et al. (1998) suggested that manipulation of nutrient supply to breeding ewes could potentially reduce larval contamination of pasture, especially for prolific flocks, as they are more vulnerable to breakdown of immunity. Moreover, because of lower milk intake their lambs will be forced to graze at a younger age and hence receive a greater larval challenge while experiencing a lower protein intake. However, most of these studies have only investigated the effects in either indoor studies or for a relatively short term. Consequently, the potential for an epidemiological benefit through reduced larval contamination for grazing lambs throughout the remainder of the season because of lower nematode egg excretion by the ewes has not yet been explored. Thus, this experiment aimed to evaluate the benefits of supplementing lactating ewes on pasture to reduce the peri-parturient relaxation in immunity (PPRI) to gastro-intestinal nematode parasitism and its provision of an epidemiological benefit to grazing lambs after weaning.

Materials and methods

Experimental design

The studies were conducted at summer-safe unit of LincolnSheep, Lincoln University, Canterbury, New Zealand over two sequential years (2015/2016 and 2016/2017) with the approval of the Lincoln University Animal Ethics Committee (approval #635 and 2016-25). Prior to lambing in 2015, twin-bearing crossbred ewes (n=140 in 2015) were randomly allocated to one of two farmlet treatments, *viz*, supplemented or not, and set-stocked in each replicate paddock. In 2015 each treatment was replicated on two paddocks, with a total of four paddocks (29-38 ewes/paddock, based on pasture availability). Ewes remained in their farmlets across both years and in 2016 (n=128) each treatment was replicated on four paddocks, with a total of eight paddocks (12-20 ewes/paddock, based on pasture availability).

Ewes in the supplemented treatment were given access to a commercially available high-protein sheep pellet (Farmlands stock feeds Ltd) through an Advantage Feeder (NGF800, Advantage Feeders Ltd, NZ). The pellet contained barley, wheat, soybean meal, peas, canola, wheat by-products, maize, oats and mollases with 25% crude protein and 12.8 MJ/kg dry matter (DM) of estimated metabolizable energy, as per product label. The feeder was initially restricted to supply 50 g/d/ewe three weeks prior to lambing and subsequently increased to 500 g/d/ewe during the first four weeks of lactation at which point the supplementation ceased. The amount of supplement consumed for each paddock was recorded and calculated as mean supplement intake per ewe. Amounts of the supplement were calculated to supply an additional 100 g of metabolizable protein (MP)/head/d assumming that unsupplemented ewes may consume 2 kg DM/day

with a total MP intake of 160 g/d (AFRC 1993). Thus, assuming no substitution, supplementation of 0.5 kgDM/d was calculated to increase total MP supply to about 260 g/d and a total DM intake of 3.5% of body weight. These supplementation rates were selected based on indoor studies which have indicated this MP supply may reduce worm burden in peri-parturient ewes by up to 50% (Donaldson et al. 2001). All ewes were allowed to graze on ryegrass/white clover pasture. Ewes remained in their respective paddocks until weaning at approximately 12 weeks after mean lambing date.

Live weight (LW), body condition score (BCS) and faecal egg counts (FEC) of all ewes were monitored at set stocking, tailing (approximately four weeks after lambing) and fortnightly thereafter until weaning. Faecal eggs were counted using the modified McMaster method (MAFF 1986) with a sensitivity of 100 eggs/gram (epg). Pasture grab samples were collected using a W-shape pattern from each paddock for the measurement of pasture parasite larval concentration fortnightly from set-stocking and infective third-stage (L3) larvae were recovered from the pasture using a modified Baermann technique (MAFF 1986). L3 present were then counted and morphologically differentiated from free-living larvae under a microscope. Two readings were performed for each sample and expressed as L_3/kg DM.

At weaning all lambs were drenched to remove residual parasite contamination and then exposed to a targeted selective treatment regime while grazing the areas in which ewes had been or not supplemented to determine if any epidemiological benefit of supplementation existed. To account for any potential carry-over effect, lambs (60 lambs/replicate in 2015 and 35 lambs/replicate in 2016) originating from each treatment were stratified across the treatment area, with each replicate (n=2, for 2015 and n=3, for 2016) consisting of 50% of lambs originating from a supplemented ewes and 50% of lambs originating from unsupplemented ewes. For each lamb replicate the potential for growth was assessed using sentinel lambs (n=10 in 2015 and n=6 in 2016) that were treated with a long-acting anthelmintic (1 ml/20 kg LW; Cydectin, Pfizer Animal Health, Auckland, NZ). Selection of these was based on placement when ranked hierarchically by LW. The remaining lambs in each replicate were subjected to a targeted selective treatment (TST) regime where the need for anthelmintic was based on animals achieving acceptable growth rates. In 2015, treatment thresholds were determined using Happy Factor Model (Greer et al. 2009) with the treatment threshold set to an efficiency of 0.74 (Greer et al. 2010). In 2016, treatment thresholds were set at 80% of the mean growth rate of sentinel lambs. Within each treatment and replicate lambs and ewes were rotationally grazed for the remainder of the grazing season with ewes following the lambs. The ewes were moved into a paddock on the day the lambs were moved out. To simulate on-farm conditions where lambs may be sent to slaughter, lambs were removed from the study once their body weight exceeded 40 kg. Lambs were weighed fortnightly with the use of a swing-gate autodrafter (Prattely Industries Ltd) fitted with a tag reader. Any individual failing to reach their minimum target liveweight gain (LWG) was automatically drafted to one side, treated with anthelmintic and returned to graze with the remainder of the group. Faecal samples per rectum from the sentinal lambs and six TST lambs from each replicate were collected fortnightly.

After the completion of the first year, the paddocks were then grazed by the ewes for one more rotation before they were removed from the pastures and grazed on winter crops until being set-stocked for the 2016 study.

Statistical analysis

The LW and BCS of ewes, FEC and pasture larval counts data were analysed using the Restricted Maximum Likelihood (REML) using GENSTAT statistical package (16th Edition ver.16.1.10916, VSN International Ltd, UK). Other parameters were analysed using one-way analysis of variance (ANOVA) by Minitab statistical package (16Th Ed.). FEC and pasture larval counts were log transformed (log10 (count + 1)) before analysis to obtain a normal distribution, and presented as back-transformed means. Where the F-test for treatment was significant ($P<0.05$), treatments were compared with a least significant differences test with a significance value of 5%. Due to the weight of conceptus at the start of lambing, change in ewe LW from four weeks after lambing only was assessed. The LWG, number of drench and LWG/drench of sentinel and TST lambs after weaning were analysed using one-way analysis of variance (ANOVA) by Minitab statistical package (16th Ed.).

Results

Ewes and lambs performance throughout lactation

The performance of ewes and lambs during lactation period of 2015 and 2016 is given in Table 1. Supplementation had no effect ($P>0.05$) on ewe BCS in both years and only on ewe mean LW in 2016 where unsupplemented ewes

Table 1 Effect of supplementation on ewe live weight, BCS, number of lambs weaned per ewe and weight of lamb weaned per ewe throughout lactation. Data represents mean ± SEM.

Parameter	Supplemented	Unsupplemented	P value
Mean live weight (kg)			
2015	60.30±0.59	62.20±0.77	0.18
2016	63.83±1.72	67.92±1.72	0.02
BCS			
2015	2.73±0.06	2.83±0.07	0.41
2016	3.06±0.08	3.11±0.08	0.56
Number of lambs weaned per ewe			
2015	1.69±0.14	1.56±0.05	0.47
2016	1.96±0.13	1.84±0.09	0.48
Weight of lamb weaned per ewe (kg)			
2015	34.88±3.98	31.77±0.89	0.53
2016	48.01±1.97	50.02±1.14	0.41

Table 2 The effect of supplementing ewes on pasture on the performance of their offspring after weaning. Data represents mean ± SEM.

Parameter	Sentinel lambs			TST lambs		
	Supplemented	Unsupplemented	P value	Supplemented	Unsupplemented	P value
LWG (g/d)						
2015	139.50±13.50	180.77±4.46	0.10	146.76±2.04	165.30±0.94	0.01
2016	142.39±3.66	135.78±5.86	0.39	150.74±4.08	147.71±4.27	0.64
Number of drench						
2015	-	-	-	3.03±0.01	3.20±0.04	0.04
2016	-	-	-	2.22±0.06	2.35±0.12	0.40
LWG/drench (g/d)						
2015	-	-	-	48.44±0.83	51.61±0.88	0.12
2016	-	-	-	81.97±6.08	77.78±4.00	0.59

Figure 1 Log 10-back-transformed means of faecal egg count (FEC) for ewes (closed symbols) that were supplemented (solid line) or not (dashed line) during the first four weeks of lactation and for lambs (open symbols) that were suppresively drench (squares) or exposed to a targeted selective treatment anthelmintic regime (circles) that subsequently grazed areas where ewes had been supplemented (solid line) or unsupplemented (dashed line) in: a) 2015; b) 2016.

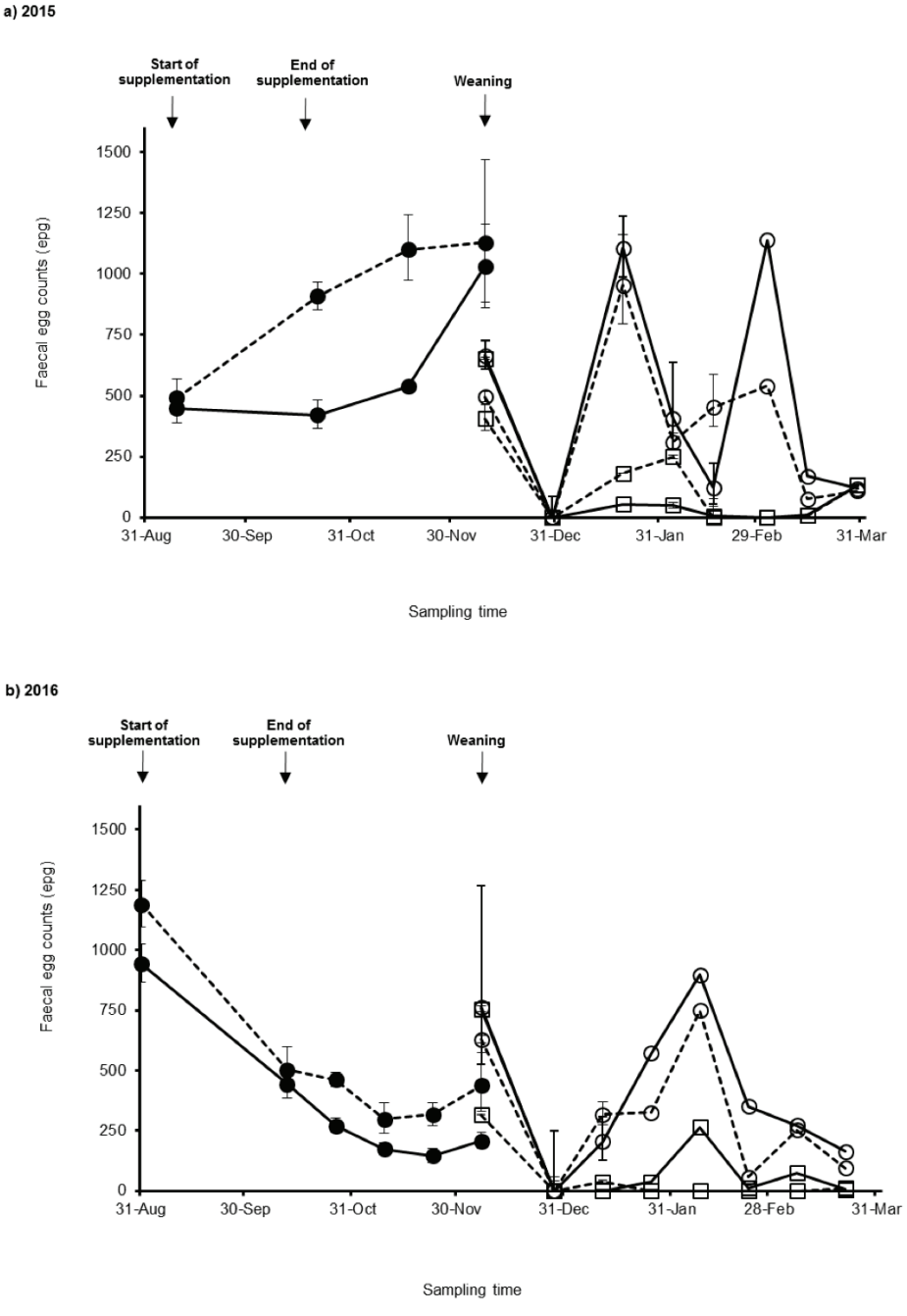
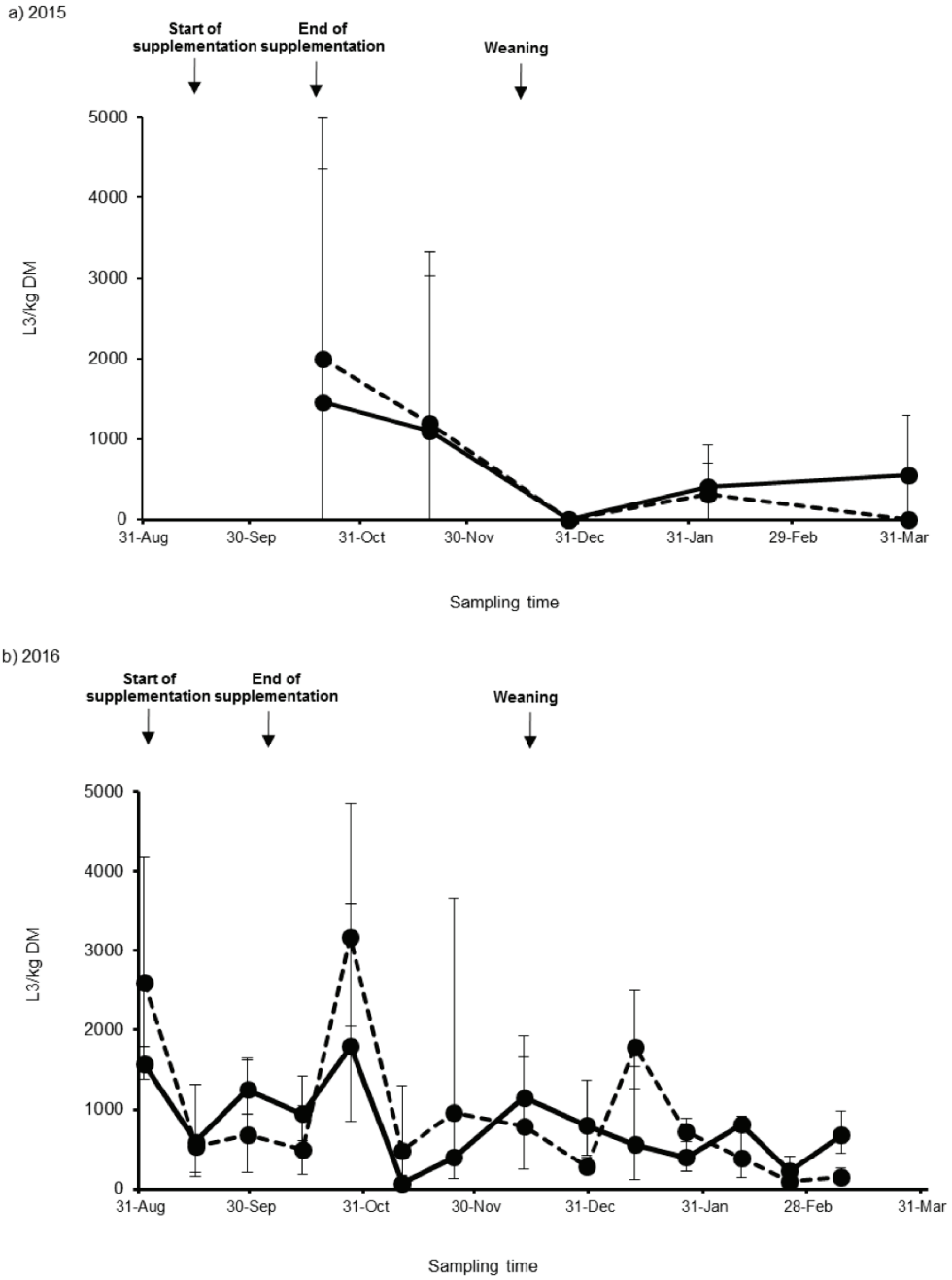


Figure 2 Log 10-back-transformed means of number of L3 larvae of strongyle nematodes per kgDM in paddocks grazed by the ewes and the lambs where ewes had been supplemented (solid line) or remained supplemented (dashed line) during the first four weeks of lactation in: a) 2015; b) 2016.



were heavier (P=0.02) than their counterparts. Additionally, supplementation had no effect on either the number of lambs weaned per ewe or the weight of lamb weaned per ewe during both years (P>0.05 for all).

Lamb performance after weaning

The performance of lambs after weaning that grazed areas where ewes had been supplemented or remained unsupplemented in 2015 and 2016, is given in Table 2. Overall, there were no differences (P>0.05) between the two treatment groups in all years, except for a greater LWG and a greater number of drenches administered per lamb of targeted selective treatment (TST) lambs in areas where ewes had not been supplemented in 2015.

Parasitological measurements: FEC and pasture larvae (L3)

There were no differences in parasitic load between farmlets and animals at the beginning of the study. Mean faecal egg counts of ewes and lambs in both years are given in Figure 1. For ewes in 2015, there was a time x supplementation interaction (P=0.04), reflecting similar FEC at the start of lactation, that increased in unsupplemented, but not supplemented ewes in weeks six and eight (P=0.03) although were not different at weaning (week 12). In 2016 similar reductions in ewe FEC were observed, however the reduction continued until weaning. For lambs there were no effects of treatment (P>0.05) in FEC after weaning in both years.

The number of L3 larvae of strongyle nematodes *(Nematodirus spp* and other strongyles) present on pasture grazed by ewes and lambs before and after weaning are presented in Figure 2. Overall, there were no differences (P>0.05) in numbers of L3 larvae/kgDM on pastures grazed by the ewes and the lambs before and after weaning in both years.

Discussion

The primary objective was to evaluate the benefits of supplementing lactating ewes on pasture and its provision of an epidemiological parasite benefit to grazing lambs after weaning. The short period of targeted supplementation was chosen in an attempt to provide a cost-effective supplementation regime which provided additional nutrient supply to the ewes at the time of greatest need, such as until peak lactation. Overall, supplementation of lactating ewes in both years was successful in reducing ewe FEC, being a 50% reduction during periods of lactation. Although MP supply was not measured in this study, the observation of a 50% reduction in FEC is comparable with the 50% reduction in worm burden observed in parasitised lactating ewes relative to MP supply shown by Donaldson et al. (2001). However, in 2015 this effect was transient, as FEC of supplemented ewes increased and was not different at week 12 of lactation. In part, this increase in FEC by weaning was not expected as the demand for nutrients for lactation may be expected to be relatively low. However, climatic conditions were not favourable for pasture growth during this year, resulting in low pasture availability, which may have resulted in unintentional nutritional stress, with mean pasture mass declining to less than 700 kg DM/ha in all paddocks, below recommended levels (Corner-Thomas et al. 2015). In 2016, the reduction in ewe FEC in supplemented animals was more consistent throughout lactation indicating a longer-term benefit to the ewe as this extended beyond the pre-patent period of any larvae that would have been ingested post-supplementation. Nevertheless, while supplementation appeared to offer some benefit in reducing the peri-parturient relaxation in immunity to nematode parasites, the FEC in neither treatment in either year was reduced to low levels by weaning, possibly indicating that the nutritional stress caused by lactation extends well beyond peak lactation.

Given the only measured difference between the ewes groups and their performance was the reduction in FEC, it seems reasonable to suggest this reflects diversion of nutrients into immune function rather than increases in ewe body weight gain or lactation performance. This interpretation would be in-line with the nutrient partitioning framework suggested by Coop and Kyriazakis (1999), as the additional nutrients supplied by supplementation appeared to be utilised by the ewes for maintenance of immunity. This possibly reflects the nutritional cost of the immune response in lactating ewes and is supported by a lack of effect of supplementation on change in ewe live weight, ewe BCS or lamb production. The lack of an effect on ewe LW or BCS or lamb weaning weight per ewe observed here is comparable to what has been reported in farm studies with interventions aimed at breaking the PPRI with long-acting drenches (Garland & Leathwick 2015; Miller et al. 2015). However, the design of the aforementioned studies was such that it did not allow an evaluation of the epidemiological benefit which may have accrued through interrupting the PPRI.

After weaning, supplementation of lactating ewes did not provide a clear benefit to lambs grazing the areas where ewes had been supplemented. Interpretation of the lamb LWG may be influenced by the potential differences between the pastures of each paddock, as such, comparisons between the TST and sentinel animals are preferred as these grazed the same areas. This aside, those lambs grazing areas on which ewes were supplemented did appear to have a lesser need for drench, although this only occurred in 2015. This indicates a small epidemiological advantage may have been conferred through the supplementation of ewes, although such effects were transient and were not great enough to result in a consistent difference in pasture larval contamination. Further, this apparent benefit is relatively low given the extent of the difference in FEC in the ewes for much of the lactation period. In part, this may reflect the design of the study, whereby the ewes followed the lambs, to mimic grazing practices on-farm. This may have contributed to the lack of effect due to the net removal of parasites by grazing non-lactating ewes (Leathwick et al. 2008). This may have been further exacerbated due to the low pasture availability in 2015 resulting in ewes grazing further down in the sward where a majority of the parasite population is believed to exist (Vlassoff 1982; Gazda et al. 2009). Alternatively, the lack of benefit may reflect relatively low transmission of disease from contamination supplied by lactating ewes. In the current study, pasture larval concentrations generally reduced during lactation despite the ewe FEC indicating a reasonable number of nematode eggs were being deposited. Nematode egg viability has been shown to be influenced by immune mechanisms of the host, with eggs from peri-parturient animals having a lower viability (Jørgensen et al. 1998), an effect which may be compounded by the relatively low egg development that has been reported during cooler periods of winter and early spring (Leathwick et al. 2011; Waghorn et al. 2011). Nevertheless, the results of the current study indicate that a 50% reduction in the FEC of lactating ewes is not sufficient under these conditions to result in an epidemiological advantage to the grazing lamb.

Conclusion

Supplementation of ewes during the first four weeks of lactation had no effect on ewe performance but was successful in temporarily reducing faecal egg by 50%, presumably reflecting better maintenance of immune function through greater nutrient supply. However, this reduction in parasite contamination was not sufficient to provide a measureable and consistent epidemiological

benefit to the grazing lambs that may assist with parasite control. It is suggested that for strategies to help break the parasite lifecycle through targeting the relaxation in immunity in the peri-parturient ewe, a reduction in ewe faecal egg count by more than 50% is required.

Acknowledgements

The authors acknowledge the technical support given by the staff of the Johnstone Memorial Laboratory (JML), Lincoln University. In addition, the authors thank Farmlands for financial support and supply of the high-protein pellet.

References

Agricultural and Food Research Council 1993. Energy and protein requirements of ruminants. An advisory manual prepared by the AFRC Technical Committee on Responses to Nutrients. CAB International, Wallingford.

Beasley AM, Kahn LP, Windon RG 2012. The influence of reproductive physiology and nutrient supply on the peri-parturient relaxation of immunity to the gastrointestinal nematode *Trichostrongylus colubriformis* in Merino ewes. Veterinary Parasitology 188: 306-324.

Coop RL, Kyriazakis I 1999. Nutrition–parasite interaction. Veterinary Parasitology 84: 187-204. doi 10.1016/ S0304-4017(99)00070-9.

Corner-Thomas RA, Hickson RE, Morris ST, Back PJ, Ridler AL, Stafford KJ, Kenyon PR 2015. Effects of body condition score and nutrition in lactation on twin-bearing ewe and lamb performance to weaning. New Zealand Journal of Agricultural Research 58(2): 156-169, doi: 10.1080/00288233.2014.987401.

Donaldson J, van Houtert MFJ, Sykes AR 1998. The effect of nutrition on the peri-parturient parasite status of mature ewes. Animal Science 67: 523-533.

Donaldson J, van Houtert MFJ, Sykes AR 2001. The effect of dietary fish-meal supplementation on parasite burdens of peri-parturient sheep. Animal Science 72: 149-158.

Garland CB, Leathwick DM 2015. A cost-benefit analysis of pre- and post-lambing drench treatments to twin-bearing ewes on commerical farms in the southern North Island of New Zealand. New Zealand Veterinary Journal 63(4): 220-226. doi 10.1080/ 00480169.2015.1012133.

Gazda TL, Piazzetta RG, Dittrich JR, Monteiro ALG, Thomas-Soccol V 2009. Distribution of nematode larvae of sheep in tropical pasture plants. Small Ruminant Research 82: 94-98. doi: 10.1016/j. smallrumres.2009.02.004.

Greer AW, Kenyon F, Bartley DJ, Jackson EB, Gordon Y, Donnan AA, McBean DW, Jackson F 2009. Development and field evaluation of a decision support model for anthelmintic treatments as part of a targeted selective treatment (TST) regime in lambs. Veterinary Parasitology 164(1): 12-20.

Greer AW, McAnulty RW, Logan CM, Hoskin SO 2010. Suitability of the Happy Factor decision support model as part of targeted selective anthelmintic treatment in Coopworth sheep. Proceedings of the New Zealand Society of Animal Production 70: 212-216.

Houdijk JGM 2008. Influence of peri-parturient nutritional demand on resistance to parasites in livestock. Parasite Immunology 30(2): 113-121.

Jørgensen LT, Leathwick DM, Charleston WAG, Godfrey PL, Vlassoff A, Sutherland IA 1998. Variation between hosts in the developmental success of the free-living stages of trichostrongyle infections of sheep. International Journal for Parasitology: 1347-1352.

Kahn L 2003. Regulation of the resistance and resilience of peri-parturient ewes to infection with gastrointestinal nematode parasites by dietary supplementation. Australian Journal of Experimental Agriculture 43(12): 1477-1485.

Lawrence KE, Leathwick DM, Rhodes AP, Jackson R, Heuer C, Pomroy WE, West DM, Waghorn TS, Moffat JR 2007. Management of gastrointestinal nematode parasites on sheep farms in New Zealand. New Zealand Veterinary Journal 55: 28-234.

Leathwick DM, Miller CM, Atkinson DS, Haack NA, Waghorn TS, Oliver AM 2008. Managing anthelmintic resistance: untreated adult ewes as a source of unselected parasites, and their role in reducing parasite populations. New Zealand Veterinary Journal 56: 184-195.

Leathwick DM, Miller CM, Waghorn TS 2011. Development and spatial distribution of the free-living stages of *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* on pasture: A pilot study. New Zealand Veterinary Journal 59: 272-278.

MAFF 1986. Manual of veterinary parasitological laboratory techniques (3rd ed.). London, UK: Her Majesty's Stationary Office (HMSO). Pg. 7-9.

Miller CM, Ganesh S, Garland CB, Leathwick DM 2015. Production benefits from pre- and post-lambing anthelmintic treatment of ewes on commercial farm in the southern North Island of New Zealand. New Zealand Veterinary Journal 63(4): 211-219. doi 10.1080/ 00480169.2015.1007108.

Vlassoff A 1982. Biology and population dynamics of the free living stages of gastrointestinal nematodes of sheep. In: Ross AD (ed). Control of Internal Parasites of Sheep- an Animal Industries Workshop. Lincoln College, Lincoln. Pg. 8-12.

Waghorn TS, Reynecke DP, Oliver A-MB, Miller CM, Vlassoff A, Koolaard JP, Leathwick DM 2011. Dynamics of the free-living stages of sheep intestinal parasites on pasture in the North Island of New Zealand. 1. Patterns of seasonal development. New Zealand Veterinary Journal 59: 279-286.