

Strategic combination of nutritional approaches for gastrointestinal parasite control in sheep

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Dedication

To my brother Areb Kidane without whose assistance I would never have thought of completing my high school.

Declaration

I hereby declare that this thesis is of my own work and all assistance has been duly acknowledged. The results presented herein have not previously been submitted for any other degree or qualification.

09/06/10

Alemayehu Kidane Sagaye

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Summary

Gastrointestinal nematode parasitism is one of the major causes for reduced performance, compromised health and welfare of grazing ruminants. The extensive, and sometimes inappropriate, use of anthelmintics for gastrointestinal nematode parasite control has led to anthelmintic resistance. The latter has urged the development of alternative approaches that would reduce reliance on anthelmintics (***Chapter One***). This thesis reports effects of combining such alternative approaches on sheep productivity and parasitism, investigated under both pen (***Chapters Two*** and ***Three***) and grazing (***Chapters Four, Five*** and ***Six***) conditions.

The first two experiments report the effects of periparturient metabolizable protein nutrition of twin-rearing ewes, on the degree of resistance to an abomasal nematode parasite, *Teladorsagia circumcincta,* and performance under varying degree of experimental parasite infection (***Chapter Two***) and with two breeds that differ in their production potential (***Chapter Three***). The results suggest that maternal protein supplementation improves litter performance independently of infection pressure and production potential, and reduces nematode egg excretion by ~60% in the more productive breed. However, protein scarcity had only limited effects on resistance to parasites in the less productive breed used.

The last three experimental chapters describe grazing studies where maternal protein supplementation was combined with grazing chicory relative to grass/clover from around weaning (***Chapter Four***) and early lactation (***Chapters Five*** and ***Six***) to lamb finishing. Maternal protein supplementation and grazing on chicory consistently improved ewe and lamb performance and reduced lamb parasitism in an additive manner. On an initially parasite infested pasture (***Chapter Six***), maternal protein supplementation and grazing on chicory reduced lamb drench requirement by 31 and 40%, respectively. The grazing experiments strongly suggest that the effects of chicory could be through (1) improved nutrient supply, boosting lamb resilience and/or resistance, (2) reduced larvae intake and (3) direct anthelmintic-like effects. The absence of significant interactive effects between maternal protein nutrition and grazing on chicory suggests that under the grazing conditions used, achieved level of ewe protein intake from the combination was, at least, not more than adequate or the anthelmintic-like property of chicory was subtle. Exploitation of the effects of combination

from the above alternative approaches should lead to sustainable sheep production by minimizing reliance on anthelmintics.

Chapter Seven briefly discusses the results obtained in the above experiments in relation to the current knowledge on the use of alternative approaches and puts recommendations for future work in the area of parasite control in sheep with minimal reliance on anthelmintics.

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List of acronyms

ADF	*Acid detergent fibre*
ADG	*Average daily gain*
AFRC	*Agricultural and Food Research Council*
AHEE	*Acid hydrolysable ether extract*
ANOVA	*Analysis of variance*
BCS	*Body condition score*
BF	*Scottish Blackface breed*
BW	*Body weight*
Ca	*Calcium*
CI	*Confidence interval*
COWP	*Copper oxide wire particles*
CP	*Crude protein (expressed as Nitrogen *6.25)*
Cu	*Copper*
DM	*Dry matter*
DMd	*Apparent dry matter digestibility*
epg	*Eggs per gram of fresh faeces*
FAO	*Food and Agricultural Organization of the United Nations*
FEC	*Faecal egg count*
g	*Gravity*
g/d	*Gram per day*
HCl	*Hydrochloric acid*
HP	*High protein (1.3 times estimated metabolizable protein requirement)*
K	*Potassium*
L_3	*Third stage infective larvae*
LP	*Low protein (0.8 times estimated metabolizable protein requirement)*
MAFF	*Ministry of Agriculture, Fisheries and Food*
ME	*Metabolizable energy*
MEr	*Metabolizable energy requirement*
Mn	*Manganese*
Mo	*Molybdenum*
MP	*Metabolizable protein*
MPr	*Metabolizable protein requirement*
MU	*Mule breed (Scottish Blackface X Bluefaced Leicester)*
N	*Nitrogen*
Na	*Sodium*
NCGD	*Neutral cellulase and gammanase digestibility*
NDF	*Neutral detergent fibre*
P	*Phosphorus*
PPRI	*Periparturient relaxation of immunity*
REML	*Restricted maximum likelihood*
SED	*Standard error of the difference between means*
SEM	*Standard error of the mean*

CHAPTER ONE

General Introduction

1.1 Introduction

Domestic ruminants exposed to natural grazing environments harbor a wide array of parasites even though few could dominate the rest. This has been reported in many field experiments where different investigators observed multiple infections of grazing animals (Bisset *et al.*, 1996; Doligalska *et al.*, 1997; Mandonnet *et al.*, 2006; Morris *et al.*, 2005; Stear *et al.*, 1998; Wales, 1940). These different species are associated with specific locations within the gastrointestinal tract of the host animal (Holmes, 1993) and with varying degree of pathogenicity (Abbott *et al.*, 2009; Parkins and Holmes, 1989).

Nematode parasites are a major cause for compromised health and welfare of grazing animals. As such, they pose one of the greatest disease problems (Waller, 2006) and cause big economic loss in ruminants worldwide. Most of the latter are associated with gastrointestinal infections (Parkins and Holmes, 1989) in young and periparturient sheep, goat and cattle (Sykes, 1994). They cause extensive gastrointestinal pathology, reduction in performance and under severe conditions even death (Sykes and Coop, 1976; Sykes and Coop, 1977). Such a clinical and chronic subclinical parasitism is characterized by impaired production (Sykes and Coop, 1976) which could be manifested as weight loss, reduced weight gain and reproductive inefficiency. Furthermore, reduction in wool growth in sheep (MacRae, 1993) and debilitation of host survival and fecundity/ reproductive performance (Regoes *et al.*, 2002) are also apparent in (sub-)clinical parasite infection.

The mechanisms by which parasites cause impaired productivity have become the subject of intense study (Holmes 1993). The main losses of

production are reported to arise from reduction in food intake and efficiency of food utilization (Coop and Angus, 1981), and also reduction in apparent nutrient digestibility (Parkins and Holmes, 1989). In a prolonged sub-clinical infection of lambs with the intestinal nematode *Trichostrongylus colubriformis*, Kimambo *et al.*, (1988) observed growth check which resulted from inappetence and disruption in nitrogen digestion and metabolism. Parkins *et al* (1973, as cited by Parkins and Holmes 1989) reported negative nitrogen balance, which was attributed to both reduced feed intake and an increased urinary nitrogen excretion in sheep infected with *Ostertagia (Teladorsagia) circumcincta. T. circumcincta* and *T. colubriformis* may ingest tissue exudates causing considerable damage to the mucosa of abomasum and intestine allowing plasma leakage to the alimentary tract (Coop and Angus, 1981). It is also described that there is substantial loss of exfoliated epithelial cells, mucus and erythrocytes all of which constitute protein loss, in addition to plasma protein, into the gastrointestinal tract (Holmes, 1993). Other parasites, which actively ingest blood, for instance *Haemonchus spp* and *Fasciola hepatica,* frequently cause anemia. These create marked differences in the synthetic rate of protein in the liver and gastrointestinal tissue (Holmes, 1987) with increase cost for protein turnover.

The extent of damage induced by parasites depends on parasites involved and their feeding habit (Coop and Angus, 1981), size of larval challenge (Coop *et al.*, 1981; Regoes *et al.*, 2002), host immunological status (Sandberg *et al.*, 2007), host nutrition (Abbott *et al.*, 1985; Gbakima, 1993; van Houtert *et al.*, 1995) and host reproductive status (Houdijk *et al.*, 2001b). However, given other things remain the same, the level of resistance that hosts develop to a parasite following continuous exposure will affect parasite establishment and subsequent ill effects of parasitism (Coop and Kyriazakis, 2001) providing an

opportunity to make use of differences between breeds/lines or even class of animals in these characteristics for the benefit of parasite control strategies.

1.1.1 Sheep production and nematode parasitism

At the global level, small ruminant production constitutes a large share of the agricultural sector with the latter varying widely between regions (see recent report by FAO (2007)) due to multitude of reasons (Zygoyiannis, 2006). Sheep, in turn, constitute a large part of the small ruminant population and recent reported estimates indicate that the global sheep population, which is 1024 million, is 1.33 times that of goats (Zygoyiannis, 2006).

Despite the level of contribution to the agricultural sector, sheep industry is affected by several factors. Gastrointestinal nematode parasitism is one of these factors. A monetary value for losses from parasites is difficult to ascertain, but it can be very substantial (Correa *et al.*, 1998). *T. circumcncta* is one of the most prevalent and economically important nematode parasites of small ruminants in temperate climates (Abbott *et al.*, 2004; Abbott *et al.*, 2009; Stear *et al.*, 1998).

1.1.2 Nematode parasite life cycle

Nematode parasites display a wide variety of life cycles and life histories and, as such, parasitize almost all groups of plants and animals, and occur in virtually every marine, terrestrial and freshwater habitats (Blouin *et al.*, 1999).

However, most of the pathogenic nematode parasites of domestic livestock undergo a direct life cycle (for a typical nematode parasite, see **Figure 1.1)**. A female adult worm residing in a predilection site lays eggs which are later defecated through faeces into the pasture. Once in the pasture, the eggs hatch into a free-living larvae which successively molt into an infective 3rd stage larvae. During grazing, animals consume these infective larvae which lose their protective sheath and invade the mucosa of the abomasum, small intestine, or large intestine depending on which nematode is involved. Whilst in the mucosa, larvae develop to the fourth larval stage and then return to the surface of the gut mucosa where they become adult worms and eventually patent (Miller and Horohov, 2006). For *T. circumcincta* this cycle takes only 16-21 days.

Every developmental stage requires certain conditions to progress into the successive stage. Once hatched, for example, the development from a free-living into infective larvae has specific requirement for environmental conditions. In this regard, temperature and moisture are the dominant influences on the free-living stages with the effects of pasture conditions playing a significant modulating role (O'Connor *et al.*, 2006). Only when the conditions are met at an adequate level that parasites survive and develop (Olsen, 1974). These conditions are parasite species specific to some extent. As such, early in the free-living phase, the developmental success of the gastrointestinal nematode parasites is limited by susceptibility to cold temperatures (O'Connor *et al.*, 2006). For instance, *T. circumcincta* and *Ostertagia ostertagi* could resist cold/winter conditions whereas *H. contortus* and *Oesophagostomum columbianum* are reported not to resist the winter conditions (O'Connor *et al.*, 2006; Wales, 1940). Under *in vitro* conditions

Ostertagia ostertagi required a minimum temperature of 4^{0}C for hatching whereas it could hatch at the maximum of 35^{0}C (Pandey, 1972).

Some show a temporal plasticity by going through a phase of arrested development, called hypobiosis, under unfavorable environmental conditions. Hypobiosis usually occurs when there is insufficient moisture in the environment or temperatures are too cold for pasture larval development and survival (Miller and Horohov, 2006; Wales, 1940) and ensures enhanced survival of parasites during such conditions (van Dijk *et al.*, 2010). This occurs inside the definitive host (Blood *et al.*, 1979) but results from environmental signals received by free-living infective larvae. Once the environmental conditions appear to be conducive the normal cycle resumes. This phenomenon is observed in the major gastrointestinal nematode parasites of small ruminants (Herd *et al.*, 1984; Waller *et al.*, 2004) and cattle (Myers and Taylor, 1989) and as such, has an important significance in the epidemiology and control of parasites in grazing livestock.

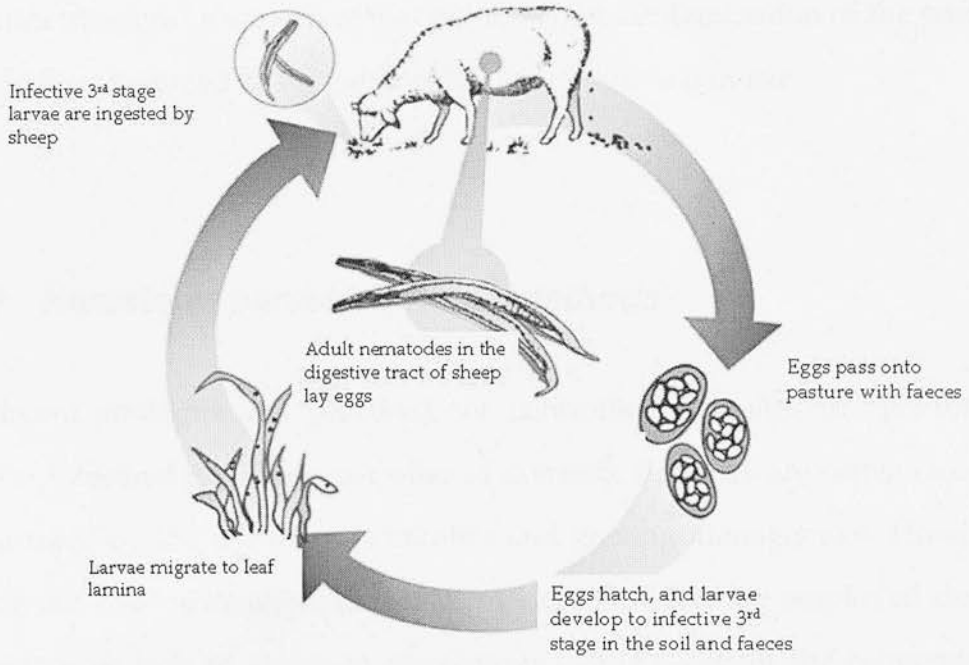


Figure 1.1 Typical gastrointestinal nematode parasite life cycle (Retrieved on 07/08/2009 from: ***http://www.pubs.ext.vt.edu/410/410-027/410-027.html)***

Under grazing conditions, animals get infected by ingesting infective larvae that reside on the leaf/stem of the grazed forage. The level of infection from the pasture depends mainly on the quantity of DM intake form an infested pasture and the distribution of infective larvae in the grazed area. The latter in turn depends on the faecal egg output from an infected patent host that grazed the area, environmental condition under which the eggs hatch, and success of hatching and development for individual species (Kao *et al.*, 2000). *T. circumcincta*, for example, has a high development success for eggs deposited from mid-spring through mid-autumn, and as such, its distribution in the field and the risk of infection for a susceptible host varies from season to season (van Dijk *et al.*, 2010). Appropriate and sustainable

control measures should target at points where contamination of the pasture could be minimized at any point of the life cycle of a parasite.

1.2 Nematode parasite control methods

Different strategies are practiced for controlling parasites in agriculture. Gastrointestinal nematode parasites of domestic livestock are conventionally controlled by the use of anthelmintics and grazing management. However, there are also other alternative control strategies that are employed due to presence or lack of access to conventional drugs, cost of the conventional drugs, regulation or restriction of the use of conventional drugs on the type of production system, and currently to delay the development of anthelmintic resistance for the existing classes of drugs.

1.2.1 Anthelmintics

Anthelmintics are drugs that are used to treat infections with parasitic worms. In the absence of vaccines for gastrointestinal nematodes, control of infections relies mainly on chemotherapy (Kaminsky *et al.*, 2008). As such, it has been suggested that without anthelmintic drugs the sheep industry could have not existed in its current form (Stear *et al.*, 2000).

Three classes of broad-spectrum anthelmintics are available for use in parasitized livestock; a class defined as a group of compounds that are related on the basis of common structure, common mechanism of action or

cross-resistance (Geary *et al.*, 1999; Waller, 2006). These are benzimidazoles - that act by inducing loss of cytoplasmic microtubules of the tegumental and intestinal cells of nematode parasites; macrocyclic lactones - that act by selectively paralysing the parasite with increased muscle chloride ion permeability (glutamate-gated chloride receptor potentiation) and produce paralysis of pharyngeal pumping; and the imidazothiazoles –that produce contraction and spastic paralysis of the parasite (Geary *et al.*, 1999; Kaminsky *et al.*, 2008; Martin, 1997).

The existing classes of anthelmintics had been used extensively and effectively until the use was threatened by prevalence of anthelmintic resistance (Waller, 1994). Anthelmintic resistance develops in such a way that during anthelmintic treatments, a small number of worms survive, these being the most resistant proportion of the population. These worms contaminate the pasture with a majority of resistant larvae for subsequent generations, leading gradually to the selection pressure of anthelmintic resistance. Any treatment strategy that contributes towards increased number of survivor worms will contribute to development of resistance.

However, nematodes will ultimately develop resistance to any new drug (Kaminsky *et al.*, 2008) and the need for alternatives in integrate parasite control strategy is always crucial. Therefore, due mainly to the prevalence of anthelmintic resistant strains of nematodes (Jackson and Coop, 2000), increasing public awareness of and concern about potential drug residues in consumable products (Coop and Kyriazakis, 2001) and hence, an increasing demand for reduced levels of drug residues in food and the environment (Stear and Murray, 1994) and the requirement for reduced anthelmintic input in organic systems of production there is a need for development of

sustainable alternatives to anthelmintics in small ruminant production systems.

1.2.2 Host nutrition

Host nutrition has long been considered to be an important factor influencing the host-parasite relationship and the impact of gastrointestinal nematode infections on host performance (van Houtert and Sykes, 1996). The influence of host nutrition on the development and consequences of parasitism is suggested to be through improved resilience, improved resistance, and/or direct effect on parasite through anti-parasitic effects (Coop and Kyriazakis, 2001; Houdijk and Athanasiadou, 2003). Defense against pathogens, by mounting an immune response, confers evident benefits but may also incur costs (Bonneaud *et al.*, 2003). Parasitised hosts, hence regulate worm fecundity and/or worm burden by mounting an immune response at the expense of (scarce) nutrients. In periparturient ewes parasitized with *T. circumcincta* it is estimated that approximately 5 per cent the maximum metabolizable protein requirement of the of the ewe could be required for expression of immunity (Houdijk *et al.* 2001) but this seems to be an underestimation. Under nutrient scarce conditions, this cost could be offset by compromising other bodily functions. Bonneaud *et al* (2003) assessed the cost of mounting an immune response in the house sparrow (*Passer domesticus*) exposed to an antigen (lipopolysaccharide) from *Escherchia coli* where they observed reduced activity and increased body mass loss of captive individuals and drop in feeding rate and reproductive success of breeding females. The sustained gastrointestinal mucosa damage which

results in the loss of nutrients and an associated reduced nutrient utilization efficiency, and increased nutrient demand for immune functions in the face parasite challenge could explain loss of productive functions in heavily parasitized animals.

Periparturient hosts experience higher worm burden and a rise in faecal egg counts (Taylor, 1935) due to relaxed immunity which contributes to pasture contamination (White and Cushnie, 1952). It is hypothesized that this has a nutritional basis as periparturient hosts would prioritize nutrient allocation to reproductive functions over expression of immunity at times of nutrient scarcity (Coop and Kyriazakis, 1999).

Improved protein nutrition to sheep parasitised with gastrointestinal nematodes has, therefore, resulted in reduced faecal egg counts and worm burden (Houdijk *et al.*, 2000; Houdijk *et al.*, 2001a; Louvandini *et al.*, 2006; van Houtert *et al.*, 1995) through its influence on host resistance (Coop and Kyriazakis, 2001; Houdijk *et al.*, 2006). In a mice model where 5 week old female mice were infected with *Trichinella spiralis*, and fed on two contrasting diets differing in their protein content (4% *vs* 26%) Gbakima (1993) showed that adult worm expulsion from the intestine of low protein group was slower and the mice had retarded immune response when compared to the high protein group indicating that host dietary protein is critical in immunocompetence and resistance to infection. It is suggested that the immune effector arms are primarily derived from protein, and metabolizable protein (MP) supplementation at times of increased demand or reduced supply can, thus, influence the different effector mechanisms of the immune response (Coop and Kyriazakis, 2001). Nutrition and specific effector responses have been widely reviewed (Athanasiadou *et al.*, 2008). It is also

speculated that the effects of supplementation could be greater with higher parasite load (White and Cushnie, 1952) as the expected response could be greater when the metabolic drain on the host is expected to be higher.

1.2.3 Use of genetic differences

The use of genetically resistant hosts should permit a reduced frequency of anthelmintic treatment, and hence reduce the rate of development of anthelmintic resistance in the parasite. Variation within sheep breeds in resistance to nematode infection is as great as that between breeds with moderate heritability (Barger, 1989). This existence of genetic variability for resistance within and between breeds, in small ruminants, to internal nematode parasitism has been clearly demonstrated by various authors. Woolaston (1992) and Woolaston *et al.*, (1990) observed increased resistance, as indicated by reduced FEC during periparturient period, to *H. contortus* infections in Merino sheep selectively bred for resistance vs. control randomly selected ewes. Romjali *et al.*, (1997) observed higher periparturient rise in egg counts in Sumatra ewes when compared to the Barbados Blackbelly X Sumatra cross (F1) for *H. contortus* infections. Good *et al.*, (2006) observed the influence of breed on resistance to naturally acquired trichostrongyle infection in which Suffolk had significantly higher FEC and worm burden when compared to Texel lambs following natural challenge. Merino lambs from a resistant selection line showed increased resistance to *H. contortus* infection, (at 97 days of age), in which FEC was 72% lower than their random-bred counterparts (Kahn *et al.*, 2003a).

Quantitative genetic analyses indicate the presence of substantial genetic variation among lambs in faecal egg counts and worm length but analyses provide no evidence for variation in worm burden (Stear *et al.*, 1999a). Furthermore, decreased worm length has been strongly associated with decreased worm fecundity.

It is possible that genetic differences in resistance to nematode infections may be associated with changes in protein metabolism with gut and gut-based immune effectors (Kahn *et al.*, 2003a), providing higher priority for use of absorbed amino acids at the expense of other tissues. These authors provide data in support of this with reduction in: wool growth (15%), annual clean fleece weight (8%) and lamb birth weight (5%) in resistant line Merino ewes relative to random bred counterparts. If an animal already has a genetic level of resistance to parasite challenge it is unlikely that it will benefit further by an increase in nutrient supply unless under low plane of nutrition (Coop and Kyriazakis, 1999). However, it is not well understood if breeds and /or lines selected for increased resistance to nematode parasites could be equally responsive to nutritional supplementation under the challenge of parasites.

1.2.4 Grazing management

Grazing management strategy which avoids the build-up of infective larvae in the grazing field is one of the approaches to reduce parasite load for a susceptible host. As such, evasive strategies that aim at avoiding disease producing infections of a contaminated area by moving to a clean or safe pasture are recommended as relevant for ruminants (Schoenian, 2009; Thamsborg *et al.*, 1999).

Sequential and/or mixed grazing of different species of domestic livestock, for instance cattle and sheep, is also recommended for parasite control and improved performance (Marley *et al.*, 2006b). Therefore, a pasture grazed by cattle and/or horses is considered safe, since sheep/goats and cattle/horses do not share the same parasites (Schoenian, 2009). Rotational grazing of small ruminants with adequate rest period between rotations (65 days or more; (Schoenian, 2009; Schoenian, 2010)) may also provide a reduction in parasite load in the grazed pasture.

Grazing on bioactive forages [forages rich in plant secondary metabolites such as sainfoin (*Onobrychis viciifolia),* chicory (*Cichorium intybus),* sulla (*Hedysarum coronarium*)] has been found to reduce worm egg count and worm burden. Feeding with sulla reduced worm egg count and worm burden in sheep infected with *Teladorsagia circumcincta* (Niezen *et al.*, 1998; Niezen *et al.*, 2002). Plant secondary metabolites such as condensed tannins may have direct antiparasitic properties (Athanasiadou *et al.*, 2000a). Paolini *et al.*, (2003) reported that feeding condensed tannins (quabracho extracts at 5% of the diet DM) reduced worm fecundity and hence nematode egg excretion of *H. contortus* infected goats without significant effect on worm burden. Furthermore, in a grazing experiment with goats infected naturally with both intestinal and abaoamasal parasites and fed 7 days indoors with either sainfoin (a tanniferous plant) or rygrass , Paolini *et al.*, (2005) reported reduced worm fecundity and faecal egg count on goats from the sainfoin group. In a field experiment using chicory (a plant containing a different plant secondary metabolite: lactones) vs grass/clover, lambs grazing on chicory harbored less worm burden than those grazing on grass/clover mixture (Tzamaloukas *et al.*, 2005). Investigating the potential benefits of grazing lactating ewes and their lambs on chicory, Athanasiadou *et al.,(2007)*

observed reduced faecal counts and higher body weight gains from lambs grazing on chicory when compared to those fed on grass/clover pasture. Direct anthelmintic effect of condensed tannins have been reported in sheep parasitised with *Trichostrongylus colubriformis* and drenched Quebracho condensed tannins (Athanasiadou *et al.*, 2000b). Heckendorn *et al.*, (2006) observed a nematocidal effect of feeding sainfoin hay and silage on an adult *H. contortus* in lambs. It is also suggested that condensed tannins form complexes with dietary protein which dissociates in the lower part of the alimentary canal increasing the flow and availability of amino acids, and thus conferring indirect route of protein nutrition (Coop and Kyriazakis, 2001) or increased nutrient availability (Waghorn *et al.*, 1987).

1.2.5 Other methods

Control of nematode parasites by larvae-trapping fungi, for instance *Duddingtonia flagrans*, is another alternative approach. Fungal spores from *D. flagrans* fed to sheep orally, showed promising results in reducing the *Trichostrongylus colubriformis* larval numbers in faeces culture (Faedo *et al.*, 1997). In Merino sheep given chlamydospores by intra-ruminal infusion and faeces collected from these sheep were deposited on pasture, Faedo *et al.* (1998) showed that numbers of larvae recovered from faeces and pasture were both lower on plots from sheep dosed with fungus than on plots from control sheep. Three positive attributes of these fungi were stated in the review of Waller (2006) as the ability to withstand the gut environment of the livestock, the propensity to grow rapidly in freshly deposited dung and the voracious nematophagus capacity. However, there are limitations of this

approach that during PPRI the increased number of nematode eggs which results in higher larvae population may not all be fed by the fungi and also the requirement for daily dosing of the fungi (S.M. Thamsborg, personal communication). Overall, control of nematodes by larvae-trapping fungi looks promising for ruminants and certain monogastric animals but delivery systems and practical dosing regimes integrated with grazing management have to be developed (Thamsborg *et al.*, 1999).

Copper oxide wire particles (COWP) have also been used in nematode parasite control in sheep and goats. The use of COWP has shown reduction in faecal egg counts of sheep and goats infected with *H. contortus* (Burke *et al.*, 2004; Burke *et al.*, 2007a; Burke *et al.*, 2007b). The limitation of the use of COWP is that it is targeted against a specific nematode species, *H. contortus*, whereas it has no effect against other gastrointestinal nematodes (Athanasiadou *et al.*, 2008).

In general, there are several management schemes that can reduce the severity of infection but they are insufficient on their own to control infection (Stear *et al.*, 2007). Furthermore, majority of the works done so far as alternatives to chemical control of nematode parasites have mainly focused on independent attempts of the alternative approaches and strategic combinations of these approaches for parasite control need investigation for any accrued effects (Athanasiadou *et al.*, 2008; Hoste *et al.*, 2005; Thamsborg *et al.*, 1999).

1.3 Why chicory?

Forage is an important part of livestock production systems in Europe (Hogh-Jensen *et al.*, 2006). Chicory is blue-flowered perennial plant of the family Asteraceae, which is characterized by tap root system with a rosette growth habit (Rumball, 1986) and currently used as a promising forage for parasite control in sheep. The family of Asteraceae is reported to contain plant secondary metabolites (Adekenov, 1995; Sessa *et al.*, 2000). Furthermore, chicory cv. Puna II is bred to contain even higher levels of these plant secondary metabolites specially sesquiterpene lactones, lactucin and lactucopicrin (Rumball *et al.*, 2003). These plant secondary metabolites are suggested to have antimicrobial/antiparasitic properties (Barrera *et al.*, 2008; Molan *et al.*, 2003; Picman, 1986; Picman *et al.*, 1982).

The beneficial effects of chicory in terms of animal performance and/or parasite control have been demonstrated in different areas and different class of animals. Min *et al* (1997) reported grazing red and hybrid deer on chicory compared to perennial ryegrass/white clover pasture improved mean carcass weight, which was due to increased live-weight gain and higher dressing-out percentage at slaughter. Athanasiadou *et al* (2007) and Komolong *et al* (1992) reported improved performance of lambs when chicory was compared to other conventionally used forage species. Based either on its nutritional value and yield over the experimental years (Labreveux *et al.*, 2004; Labreveux *et al.*, 2006) or on its mineral composition (Sanderson *et al.*, 2003) chicory was recommended as a promising crop in northeastern USA. Currently, in the United Kingdom the cultivation of chicory in sheep production system is getting attention in order to make use of the above qualities.

1.4 Objectives of this thesis

In this PhD project, the combined effects of three alternative parasite control approaches were investigated; namely maternal protein supplementation, grazing on chicory and exploiting between-line variation in perceived resistance to parasites under a condition where animals were exposed to the most important abomasal parasite of the temperate regions (Miller and Horohov, 2006; Stear *et al.*, 1998) which is *T. circumcincta*. Firstly, the effects of periparturient MP supplementation to ewes infected at different levels of infection pressure were investigated (***Chapter Two***). Secondly, these effects of MP supplementation on the periparturient performance and parasitism were investigated in two breeds of ewes with purported difference in resistance to nematode parasites and performance (***Chapter Three***). Thirdly, the beneficial effects of maternal MP nutrition and grazing chicory relative to grass/clover were compared in three separate experiments (***Chapters Four, Five and Six***) where the two alternative approaches were combined at different ages of lambs and under different degree of pasture initial infectivity. Briefly, lambs from two different maternal MP feeding backgrounds were weaned on initially parasite clean chicory or grass/clover (***Chapter Four***); periparturient ewes infected with *T. circumcincta* and given MP supplementation or not were turned out onto initially clean chicory or grass/clover from 32 days post-parturition (***Chapter Five***) and lastly periparturient ewes infected with *T. circumcincta* and given MP supplementation or not were turned out onto initially parasite contaminated chicory or grass/clover from 32 days post-parturition (***Chapter Six***).

In brief, the beneficial and combined effects of these alternative parasite control methods were assessed through strategic combination targeted at periparturient ewe and subsequent lamb performance with minimal reliance on chemical drugs. The positive epidemiological implications of this project are assessed through a combination of different responses like; (*i*) lower ewe parasitism around lambing, improved ewe body weight and better body condition of ewes at weaning, (*ii*) lower pasture contamination around turnout, (*iii*) improved lamb performance and (*iv*) finally reduced input of anthelmintics in the system.

The major objective of this thesis was to evaluate the combination of non-pharmaceutical strategies to control gastrointestinal parasitism in sheep with minimal anthelmintic input.

The Specific objectives of this PhD project were to investigate:

1. The consequences of periparturient protein supplementation in ewes challenged to varying levels of abomasal nematode parasite (***Chapter Two***).

2. The effects of protein supplementation on the degree of periparturient relaxation of immunity in two breeds of ewes with different production level and varying degrees of resistance to nematode parasites (***Chapter Three***).

3. The effects of periparturient maternal protein nutrition and subsequent lamb grazing on parasite clean chicory compared to grass/clover around weaning on lamb parasitism and performance (***Chapter Four***).

4. The combined effects of maternal protein nutrition and ewe and lamb grazing on parasite clean chicory compared to grass/clover from early lactation to lamb finishing on lamb parasitism and performance (***Chapter Five***), and finally

5. The combined effects of maternal protein nutrition and ewe and lamb grazing on a parasite contaminated chicory compared to grass/clover from early lactation to lamb finishing on performance, parasitism and lamb drench requirement (***Chapter Six***)

The findings are reported and discussed under each experimental chapter and summarized in the general discussion chapter.

CHAPTER TWO

Consequences of infection pressure and protein nutrition on periparturient resistance to *Teladorsagia circumcincta* and performance in ewes

Abstract

The consequences of protein nutrition on the degree of periparturient relaxation of immunity to nematode parasites in sheep may be more pronounced at higher levels of infection pressure. Here, the interactive effects of metabolizable protein (MP) nutrition and infection pressure on resistance and lactational performance of ewes were assessed. Twin-rearing ewes were trickle infected with either 1000, 5000 or 10,000 infective Teladorsagia circumcincta larvae and fed either at 0.8 (low protein, LP) or 1.3 (high protein, HP) times their estimated MP requirement. Expected interactions between feeding treatment and infection pressure were not observed. Periparturient relaxation of immunity, as indicated by variation in faecal egg counts, was higher in LP ewes than in HP ewes and FEC showed an inverse relationship with infection pressure indicating possible density dependency effects on worm fecundity. Plasma pepsinogen concentration linearly increased with infection pressure. Daily total nematode egg excretion, assessed at week three of lactation, was not significantly affected by infection pressure but was reduced by 65% in HP ewes compared to LP ewes. MP supplementation improved lamb performance but had little effect on ewe body weight and plasma protein concentrations, whilst lactational performance, as judged from lamb performance, tended to be reduced with increased infection pressure. The results suggest periparturient MP supplementation to ewes reduces nematode egg excretion independent of infection pressure and improves lactational performance of parasitized ewes even in the presence of moderate MP scarcity.

Keywords: *Teladorsagia circumcincta, metabolizable protein, infection pressure, faecal egg count*

2.1 Introduction

The periparturient relaxation of immunity (PPRI) to nematode parasites plays an important role in the epidemiology of gastrointestinal nematode infections in grazing sheep. It is characterized by an increase in nematode egg excretion from periparturient ewes onto the pasture, which can be a main source of infection for the immunologically naïve lambs (Heath and Michel, 1969). Therefore, controlling worm egg excretion from periparturient ewes should be included in any strategy aimed at a reduction of parasitism in young grazing lambs.

It has been postulated that the extent of PPRI has a nutritional basis (Coop and Kyriazakis, 1999), arising from a prioritized scarce metabolizable protein (MP) allocation to reproductive rather than to immune functions at times when MP demand for progressing gestation and subsequent lactation are increased. The extent of PPRI to gastrointestinal nematode parasites can be characterized by increased faecal egg count (FEC) and/or worm burden in periparturient ewes relative to non-reproducing counterparts (Barger, 1993), and can indeed be reduced by reducing MP scarcity, e.g. through increased MP supplementation or reduced MP demand through a reduction in the reproductive effort; see Sykes and Kyriazakis (2007) and Houdijk (2008) for recent reviews.

So far, single levels of trickle infections have been used to investigate the effects of MP supplementation on gastrointestinal parasitism in periparturient ewes (Donaldson *et al.*, 2001; Houdijk *et al.*, 2001a; Houdijk *et al.*, 2003a; Houdijk *et al.*, 2006). Effects of MP supply on PPRI may depend on the level of MP demand (Houdijk, 2008), and the latter may be sensitive to

infection pressure, arising from the expected negative effects of nematode infection pressure on host performance (Sandberg *et al.*, 2007). For instance, lambs trickle infected with increasing doses of *Trichostrongylus colubriformis* (Steel *et al.*, 1980) and *Teladorsagia circumcincta* (Sykes and Coop, 1977; Symons *et al.*, 1981) showed progressively reduced performance. Such effects on host performance may be seen at least to some extent as the outcome of an increased nutrient (MP) demand arising from an increased infection pressure and this could also be applicable to periparturient ewes.

The objective of this experiment was to investigate interactive effects of infection pressure and MP nutrition in periparturient ewes on their lactational performance and extent of PPRI. It was hypothesized that increased infection pressure would reduce periparturient performance most pronouncedly at times of MP scarcity, and that the beneficial effects of MP supplementation on PPRI would be most clearly observed in ewes trickle infected with the highest dose of infective larvae. The latter would be consistent with the view that effects of MP supply on PPRI are more pronounced at higher levels of nutrient (MP) demand (Houdijk *et al.*, 2001b).

2.2 Materials and Methods

2.2.1 Animals and housing

Seventy-two, 4-5 year old Scottish Blackface x Bluefaced Leicester ewes (Mules), all expected to lamb at the span 2 weeks and scanned for twin pregnancy, were used in this experiment. The ewes were recruited from the same flock, had similar genetic background and grazing history. The experiment lasted for approximately 11 weeks, i.e. from day$_{-56}$ to day$_{24}$ (day$_0$ was mean parturition day). Ewes were group housed from day$_{-56}$ until day$_{-24}$, and then individually housed until the end of the experiment, in pens sized 1.48 x 1.88 m. The shed received natural illumination, and was well ventilated. Fresh saw dust was used as bedding and added daily. Fresh water was supplied *ad libitum* and water troughs were checked daily and cleaned as needed. The experimental details described below were approved by the Animal Experiment Committee of Scottish Agricultural College (ED AE 03/2007) and carried out under Home Office regulations (PPL 60/3782).

Feeding. Two feeding treatments (n=36 each) were designed to supply 0.8 (LP) and 1.3 (HP) times the estimated MP requirement of the ewes during the periparturient period (see below). These were achieved by providing restricted access to two iso-energetic feeds offered to supply metabolizable energy (ME) at 0.9 times the estimated ME requirement of periparturient ewes. For the estimation of nutrient requirements during pregnancy, a litter birth weight of 10.3 kg, no maternal bodyweight gain (Houdijk *et al.*, 2005) and 20.4g MP per day for wool growth (AFRC, 1993) were assumed.

Furthermore, similar requirements for wool growth during lactation, maternal body weight (BW) loss of 100 g per day and milk yields of 3.3, 3.6 and 3.9 kg per day in the 1st, 2nd and 3rd week of lactation, respectively (Houdijk *et al.*, 2003a) were assumed. All MP and ME requirement estimates were based on the Agricultural and Food Research Council (1993) recommendations.

The experiment consisted of 3 periods: mid-pregnancy (day_{-56} to day_{-21}), late pregnancy (day_{-21} to day_{0}) and early lactation (day_{0} to day_{24}). The latter two periods were collectively termed the periparturient period (day_{-21} to day_{24}). The mid-pregnancy period was an adaptation period to reduce body reserves and to introduce the different levels of infection pressure (see below). The LP and HP feeding treatments were applied during the periparturient period. The daily allowance consisted of a mixture of medium quality hay and concentrates. During mid-pregnancy, all ewes were given ad libitum hay and 250g of a low protein pregnancy feed. In the periparturient period, ewes were fed a restricted amount the feeds based on individually estimated ME requirements. **Table 2.1** presents the ingredients and chemical composition of all feeds. The high MP diet was achieved through substitution of part of the LP diet with soypass, which is rich in rumen undegradable digestible protein. Since the estimated ME content of soypass was similar to that of the LP feed, substitution on weight for weight basis was not expected to affect ME content. The quantity of mineral premix in the feeds was adjusted to achieve similar mineral concentrations in the HP and LP feeds.

Table 2.1 Experimental diets and ingredients used during periparturient period

Ingredients[1] (g/kg fresh)	Late pregnancy		Early lactation	
	LP	HP	LP	HP
Barley	361	281	333	233
Oat feed	70.0	54.6	70.0	49.0
Soybean meal	-	-	66.0	46.2
Soypass[2]	-	146	-	210
Molasses	50.0	39.0	50.0	35.0
Beet pulp molassed	100	78.0	100	70.0
Urea	12.0	9.40	10.0	7.00
DiCal[3]	11.7	9.10	10.9	7.60
Salt	3.90	3.00	3.90	2.70
CalMag[4]	5.20	4.10	5.00	3.50
50% Fat premix	30.0	23.4	30.0	21.0
Megalac[5]	20.0	15.6	20.0	14.0
Min Vit	1.80	1.80	1.80	1.80
Hay (medium quality)	335	335	300	300
Chemical composition (on as fed basis from wet chemistry)				
Dry matter (g/kg)	837	833	831	828
Crude protein (g/kg)[6]	114	157	134	190
NCGD[7] (%)	60.0	60.4	60.0	60.7
NDF[8] (g/kg)	276	281	267	274
ADF[9](g/kg)	149	143	143	135
ME (MJ/kg)	9.50	9.44	9.31	9.33
MP (g/kg) (estimated)	65.4	107	80.6	133

[1] Hay was offered chopped and other ingredients were given mixed as mash feed
[2] Xylose treated soybean meal
[3] Dicalcium phosphate, 18% P
[4] Calcined magenesite, 85 % MgO
[5] Calcium soap product
[6] Crude protein =N X 6.25
[7] Neutral cellulase and gamanase digestibility
[8] Neutral detergent fiber
[9] Acid detergent fiber

Experimental infection. Because the ewes were 4-5 years old, they were expected to have considerable prior exposure to gastrointestinal nematodes and were treated at housing on day$_{-56}$ with levamisole (Levacide, Norbrook, Newry, UK) and ivermectin (Oramec, Merial, Harlow, UK) at a rate of 7.5 and 0.2 mg/kg BW, respectively, to clear any resident worms. They were kept indoors throughout the experiment. Within each feeding treatment, ewes were then randomly allocated to one of three infectious pressure groups, with similar mean starting BW, body condition score (BCS), expected lambing date and initial faecal egg count. Ewes were trickle infected from day$_{-42}$ until day$_{24}$ with 1,000 (n =12), 5,000 (n=12) or 10,000 (n=48) infective larvae (L3) of *T. circumcincta* per infection day. Each infective dose was suspended in water and administered using 10ml syringes, three days a week (Monday, Wednesday and Friday) during the morning hours. The number of ewes per level of infection pressure differed because ewes exposed to the highest level of infection pressure were destined to a subsequent experiment, which needed a substantial number of replicates.

2.2.2 Measurements

Animal performance. The ewes were weighed at day$_{-56}$ and then weekly from day$_{-42}$ onwards, as well as within 12h of parturition. The lambs were weighed within 12h after birth and weekly afterwards. The body condition of the ewes was scored regularly, by lumbar palpation on a 0 to 5 scale, and to an accuracy of a quarter, as described in Russel (1984).

The experimental diets were sampled whilst preparing the daily allowance; the samples were bulked and analyzed for dry matter, crude protein, neutral detergent fibre, acid detergent fibre and in vitro digestibility (neutral cellulose and gamanase digestibility). Resultant values were used to estimate dietary ME contents. The ewes were fed restrictedly but the sometimes occurring small refusals were collected twice a week and analysed for dry matter content. Average achieved dry matter feed intake was calculated as the difference between dry matter offered and dry matter refused.

Male lambs were castrated using elastic bands within one week after birth.

Faecal egg count (FEC). An FEC, expressed as eggs per gram of fresh faeces (epg), was carried out upon housing (initial FEC) and then twice a week from day$_{-27}$ onwards. Post-lambing FEC was also assessed on samples collected within 12 h after lambing. The FEC was measured according to a modified floatation method (Christie and Jackson, 1982) using polyallomer centrifuge tubes to collect the nematode eggs from the meniscus.

Daily nematode egg excretion. In addition to FEC, daily nematode egg excretion was assessed to account for possible effects of experimental treatments on faeces production, and thus effects of faecal dilution on FEC (Houdijk, 2008; Vagenas *et al.*, 2007). Daily nematode egg excretion (eggs/day) is assessed by multiplying FEC (epg) with fresh faeces production (g/day). To this effect, feed samples were collected every day during the experiment while weighing allowances and were pooled before analyses. Faeces were collected directly from the rectum of all ewes for 3 consecutive days (day$_{19}$ to day$_{21}$) and were pooled per individual ewe before analyses.

Pooled feed and faecal samples were analyzed for dry matter (DM) and acid insoluble ash (AIA) using the 2N HCL procedure of van Keulen and Young (1977). AIA is used as an internal marker to determine DM digestibility (van Keulen and Young, 1977). Observed DM intake, faeces DM contents and DM digestibility then allow to calculate faeces production under the assumption that daily intake of AIA and its excretion is the same (Houdijk *et al.*, 2003a). The amount of faeces produced per day was multiplied with FEC of the corresponding date to estimate the daily nematode egg excretion per ewe between day_{19} and day_{21}.

Plasma samples. Blood samples were taken from the jugular vein of the ewes into heparinised vacutainers before infection (day_{-49}), prior to start of feeding the experimental diets (day_{-25}), the last week of pregnancy (day_{-4}) and at the end of the experiment (day_{24}). The last three sample days were effectively at the end of the three experimental periods described earlier, and were selected because any treatment effects (feeding treatment and infection pressure) were expected to be most pronounced by then. The plasma was separated by centrifuging for 15 min at 1340 X *g* and stored at –20°C pending analyses. The plasma samples were analyzed for plasma urea (*m*mol/*L*), plasma pepsinogen (expressed in iu*1000 per liter as mu/*L*), plasma albumin (g/*L*) and plasma total protein (g/*L*) concentration. In addition to this, ewes were also monitored for risk of twin lamb disease during late pregnancy with weekly blood sampling to assess beta-hyroxybutyrate (BHBA). Ewes with BHBA level above 2 mmol/*L* were given propylene glycol as a life-aid until the BHBA level was regarded safe.

2.2.3 Statistical analyses

Data collected over the experimental period for individual ewes were re-arranged from calendar date to day relative to parturition (day_0). This was done to account for the influence of small differences in parturition dates on the data obtained during the periparturient period.

Results obtained during the periparturient period were analysed separately for late pregnancy and early lactation. These periods were considered to be very distinct with regards to the quantity and composition of feed offered, as well as ewe physiological state and were, therefore, treated separately for analysis of variance. During each of these periods, ewe BW, BCS and FEC were analyzed using repeated measures analysis of variance (ANOVA) in split-plot design with a ewe treated as a block and time points within ewe as subplots. The ANOVA was a 2 x 3 factorial design, assessing the effects of feeding treatment (LP vs HP), infection pressure (1000, 5000 or 10,000 L3 per day as a class variable) and their interaction. When interactions with time were observed, the ANOVA was carried out on each time point to locate these effects. Post-lambing BW of ewes and litter birth weight were compared using ANOVA with ewe initial BW taken as a covariate whereas litter average daily weight gain (ADG, in g per day) and litter final BW were compared with litter birth weight introduced as a covariate in the model. For all the BW measurements least squares means with standard error of the difference between means (SED) are reported unless otherwise stated. FEC and total daily nematode egg excretion was transformed via log(x+1) prior to statistical analysis. These are reported as backtransformed least-square means, accompanied by a lower and upper 95% confidence interval (CI), calculated from backtransforming least-square mean of transformed data (μ),

μ - s.e. and μ + s.e., respectively. The ANOVA for FEC and plasma constituents during adaptation and periparturient period included initial FEC and pre-infection plasma levels as covariates, respectively. For most plasma constituent measurements, but not for FEC, the covariates contributed significantly to the respective model in which they were incorporated. Full probabilities of effects are given in the tables, whilst in the text only $P<0.15$ are reported in full with $P<0.05$ considered to be significant. Statistical analyses were performed using SAS 9.1 and GenStat 11 (VSN International Ltd., 2008).

2.3 Results

2.3.1 Dry matter intake and digestibility

Table 2.2 shows the observed least squares mean daily intake of DM, ME and MP over the different phases of the experiment. Small amount of feed refusals were observed, which averaged (±SD) 35±12.8 g per ewe per day. Estimated ME contents were similar between LP and HP foods, within each of the late pregnancy and early lactation. Mean achieved DM and ME intake, during both late pregnancy and early lactation were not affected by infection pressure, feeding treatment or their interaction (see **Table 2.2**). However, achieved MP intake was, as planned, significantly higher for the HP feeding treatment throughout the experiment but was not affected by infection pressure or the feeding treatment x infection pressure interaction.

There was no significant interaction between infection pressure and feeding treatment on apparent DM digestibility of the feed offered during early lactation (**Table 2.4**). Mean apparent DM digestibility was higher for those in the low infection pressure group (75.8%) compared to the high infection pressure group (71.5%), with an intermediate value for the medium infection pressure group (73.0%; SED 1.70%; *P=0.036*). Apparent DM digestibility was higher for HP ewes than for LP ewes (74.3 vs 70.6%; SED 1.20%; *P=0.003*).

Table 2.2 Least squares means for daily intake of dry matter (DM), metabolizable energy (ME) and metabolizable protein (MP) of twin bearing and -rearing Mule ewes, trickle infected at different doses with abomasal nematode *Teladorsagia circumcincta* and fed at either 0.8 (LP) or 1.3 (HP) times their assumed MP requirement during late pregnancy and early lactation

Treatment			Observed daily intake					
			DM (g)		ME (MJ)		MP (g)	
MP[1]	Infec.[2]	*n*	Preg.	Lact.	Preg.	Lact.	Preg.	Lact.
LP	1000	6	1382	2439	15.7	27.3	108	237
	5000	6	1372	2305	15.6	25.8	107	224
	10000	24	1374	2379	15.6	26.6	107	231
HP	1000	6	1420	2426	16.0	27.3	182	390
	5000	6	1320	2402	14.9	27.0	169	386
	10000	24	1374	2404	15.5	27.0	176	387
		SED	*54.2*	*69.7*	*0.613*	*0.780*	*5.62*	*7.08*
P-value		MP	*0.91*	*0.30*	*0.63*	*0.178*	*<0.001*	*<0.001*
		Infec.	*0.36*	*0.25*	*0.36*	*0.26*	*0.24*	*0.22*
		MPxInfec	*0.50*	*0.49*	*0.51*	*0.49*	*0.33*	*0.59*

Pre. and Lact. stand for late pregnancy period (last three weeks of pregnancy) and early lactation period (three weeks into lactation) respectively.

[1] Feeding treatment (either LP or HP)

[2] Infec. is level of larval dose which was administered as 1000, 5000 or 10,000 L_3/day of *T. circumcincta* per infection day three days a week; SED was based on minimum replication size (n) for feeding treatment X infection interaction.

2.3.2 Animal performance

Table 2.3 shows the least squares mean BW of ewes at the end of late pregnancy, immediately post-lambing and at the end of early lactation. During late pregnancy, the three-way interaction between time, feeding treatment and infection pressure, the two-way interaction between feeding treatment and infection pressure and the effect of infection pressure per se on ewe BW were not significant (*P>0.15*). However, whilst ewes in all treatments gained weight during late pregnancy (*P<0.001*), the interaction between time and feeding treatment (*P<0.001*) indicated that HP ewes grew faster than LP ewes. This resulted in heavier HP ewes on day$_{-4}$ than LP ewes (81.5 vs 78.6; SED 0.711kg; *P<0.001*).

Immediately post lambing, HP ewes were heavier than LP ewes (67.9 vs 65.4; SED 0.728 kg; *P<0.001*) but post lambing ewe BW was not affected by infection pressure or interaction between feeding treatment and infection pressure (**Table 2.3**). The effects of feeding treatment on ewe BW were maintained throughout lactation, with HP and LP ewes weighing 67.1 and 64.6 kg, respectively, by day$_{24}$ (SED 0.876 kg; *P=0.007*). During lactation, there was no interaction between time and feeding treatment (*P>0.15*), and although increasing infection pressure tended to reduce mean ewe BW (*P=0.092*), infection pressure did not affect final ewe BW (**Table 2.3**). The three way interaction between time, feeding treatment and infection pressure on ewe BW was not significant (*P>0.15*).

Figure 2.1 shows periparturient ewe BCS. During late pregnancy, feeding treatment and infection pressure interacted for BCS (*P=0.020*), whilst ewe

BCS increased over time (*P<0.001*). As such, ewes at low infection pressure offered HP had a higher BCS compared to their LP counterparts during the last week of pregnancy. However, this effect was not seen in the medium or high infection pressure group.

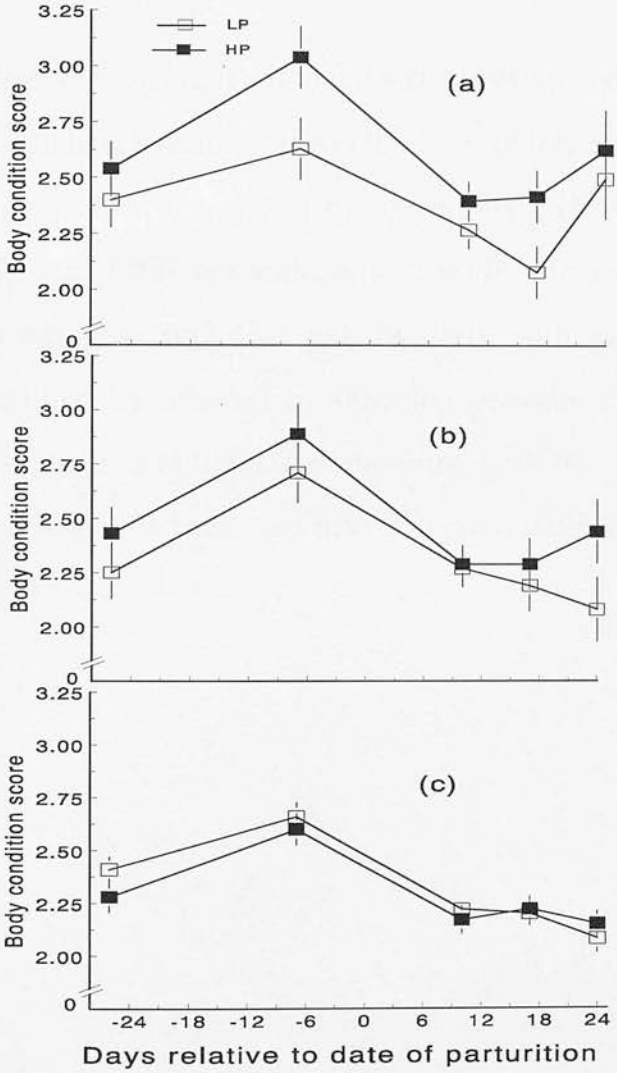


Figure 2.1 Body condition score of ewes trickle infected with (a) 1000, (b) 5000 and (c) 10000 L3 of *T. circumcincta* per day three times a week from day$_{-42}$ onwards and fed either 0.8 (LP) [open box] or 1.3 times (HP) [solid box] their periparturient MP requirement *[vertical bars give SEM]*

Time, feeding treatment and infection pressure interacted for condition score during lactation (*P=0.007*). Body condition improved over time only for HP ewes from the 2nd week of lactation onwards in the low and medium infection pressure groups. At the end of early lactation, ewes in the low, medium and high infection pressure group had a BCS of 2.50, 2.20 and 2.13 (SED=0.07; *P<0.001*), respectively.

Litter birth weight averaged 9.80±0.141 kg and was not significantly affected by feeding treatment (*P=0.146*), level of infection or their interaction (*P>0.15*). However, at the end of the experiment, HP litters had significantly higher BW than LP litters, indicating that HP litters grew faster than LP litters (708 vs 651 g/d; SED 15.5 g/d; *P<0.001*). Although litter growth rate was not significantly affected by infection pressure or interaction between feeding treatment and infection pressure (*P>0.15*), final litter weight tended to decrease with increased infection pressure (See **Table 2.3**).

Table 2.3 Least squares mean body weight of twin bearing and -rearing Mule ewes and their lambs (kg) and average daily weight gain (ADG) of litter at day_{24} (g/day) when the ewes were trickle infected at three different doses with abomasal nematode *T. circumcincta* and fed at either 0.8 (LP) or 1.3 (HP) times their assumed MP requirement during late pregnancy and early lactation period

Treatment		Animal performance					
		Ewes (kg)[1]			Litter[2]		
MP[3]	Infec[4].	Day_{-3}	Post-lambing (day_0)	Day_{24}	Litter birth wt (kg)	Day_{24} (kg)	ADG (g/day)
LP	1000	82.2	65.7	66.3	9.9	26.7	678
	5000	78.6	65.4	66.4	9.7	25.1	611
	10,000	78.5	65.3	63.8	9.5	26.2	652
HP	1000	81.8	68.7	68.3	9.3	28.6	746
	5000	81.4	66.9	66.2	10.2	27.6	716
	10,000	81.5	67.9	67.0	10.1	27.1	692
	SED[5]	1.74	1.78	2.15	0.708	0.999	40.0
ANOVA (P-value)	MP	*<0.001*	*0.001*	*0.007*	*0.14*	*<0.001*	*<0.001*
	Infec.	*0.52*	*0.67*	*0.25*	*0.78*	*0.11*	*0.18*
	MPxInfec	*0.68*	*0.79*	*0.36*	*0.28*	*0.30*	*0.36*

[1] for replication with ewes see table 2 above

[2] for litter measurements (n=11 for 1000 and 5000, and 46 for 10,000)

[3] Feeding treatment (either LP or HP)

[4] Infec. is level of larval dose which was administered as 1000, 5000 or 10,000 L_3/day of *T. circumcincta* per infection day three days a week

[5] SED was based on minimum replication size (n) for the feeding treatment X infection interaction.

2.3.3 Faecal egg count and daily egg excretion

Figure 2.2 shows the back-transformed mean FEC (with 95% CI) during the periparturient period. No first or second order interactions with time were significant for FEC during late pregnancy. However, mean FEC decreased with increasing infection pressure (*P=0.021*) and increased over time (*P=0.032*). Although mean FEC was higher for LP ewes than for HP ewes during late pregnancy, this effect was not statistically significant (*P=0.12*). In agreement with these effects, FEC immediately post parturition was not affected by feeding treatment or by the interaction effect of feeding treatment and infection pressure (*P>0.15*) but decreased with increasing infection pressure (*P=0.035*).

FEC during early lactation was not affected by interaction between time, feeding treatment and infection pressure, by interaction between feeding treatment and infection pressure, and between time and infection pressure (*P>0.15*). However, HP ewes had lower FEC than LP ewes during early lactation (*P=0.027*), whilst FEC was not affected by infection pressure (*P>0.15*). In addition, time tended to interact with feeding treatment (*P=0.069*), suggesting that the effects of feeding treatment on FEC increased over time.

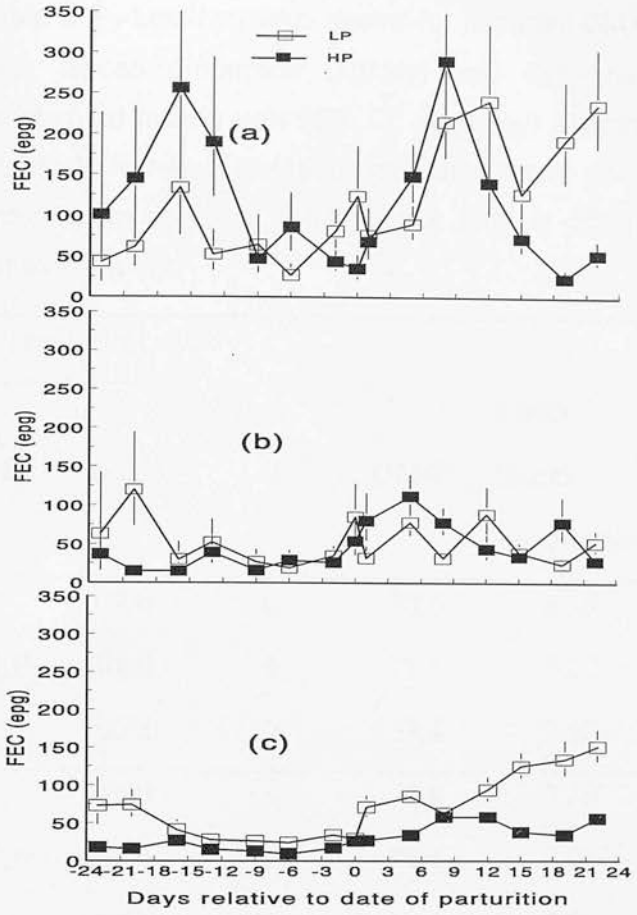


Figure 2.2 Backtransformed mean faecal egg count of ewes trickle infected with (a) 1000, (b) 5000 and (c) 10000 L3 of *T. circumcincta* per day three times a week from day$_{-42}$ onwards and fed either 0.8 (LP) [open box] or 1.3 times (HP) [solid box] their periparturient MP requirement *[vertical bars give 95% confidence intervals, which were too small to be visible for some of the results]*

For estimated daily faeces production and egg excretion in the third week of lactation see **Table 2.4**. There was no effect of infection pressure or interaction between feeding treatment and infection pressure on faeces production and nematode egg excretion (*P>0.15*). However, HP ewes produced significantly less faeces (*P=0.028*) and excreted fewer nematode eggs (*P=0.009*) than LP ewes.

Table 2.4 . Least squares means for apparent DM digestibility (%), estimated daily fresh faeces production (kg/day) and daily nematode egg excretion (back transformed means with 95% CI; eggs/day) until the 3rd week of lactation of twin rearing Mule ewes, trickle infected at different doses with abomasal nematode *T. circumcincta* and fed at either 0.8 (LP) or 1.3 (HP) times their assumed MP requirement

Treatment					
MP[1]	Infec.[2]	*n*	DMd[4]	Fresh faeces production	Egg excretion (x10[3]) per dayduring lactation [5]
LP	1000	6	73.8	3.19	636 (333 – 1215)
	5000	4	71.6	3.22	198 (90 - 438)
	10000	23	69.6	3.45	459 (330 - 638)
HP	1000	5	77.8	2.55	193 (95 - 392)
	5000	6	74.4	3.03	200 (105 - 381)
	10000	23	73.4	3.08	142 (102 - 197)
		SED[3]	*2.48*	*0.373*	
ANOVA		MP	*0.003*	*0.028*	*0.009*
		Infec.	*0.036*	*0.260*	*0.751*
		MPxInfec.	*0.953*	*0.750*	*0.753*

[1, 2 and 3] are as described in Table 2.2 above.

[4] apparent dry matter digestibility

[5] back transformed mean with 95% confidence interval

2.3.4 Plasma constituents

Table 2.5 presents effects of feeding treatment and infection pressure on plasma constituents. Feeding treatment and infection pressure did not

interact for any of the plasma constituents measured (*P>0.15*) and hence **Table 2.5** presents main effects only.

Pre-infection plasma pepsinogen concentration averaged 543±29.6 mu/*L*. At the end of mid-pregnancy phase, plasma pepsinogen concentration increased with increased infection pressure (*P=0.013*). Although averaged plasma pepsinogen concentration tended to increase with increasing level of infection throughout the periparturient period, this effect as well as the effect of feeding treatment were not significant (*P>0.15*).

Pre-infection plasma urea averaged 1.97±0.063 *m*mol/*L*. Plasma urea was not affected by infection pressure on day$_{-25}$ (*P>0.15*) and averaged 3.1±0.3 *m*mol/L. HP ewes had higher plasma urea than LP ewes on day$_{-4}$ and day$_{24}$ (*P<0.001*). The effect of infection pressure on plasma urea concentration tended to be significant (*P=0.052*) on day$_{-4}$ but this effect disappeared on day$_{24}$ (*P>0.15*).

Pre-infection plasma total protein and albumin concentrations averaged 71.5±0.640 and 35.4±0.270 g/L, respectively. The low infection pressure group had higher total protein (*P=0.054*) and plasma albumin (*P=0.020*) concentrations than the medium and high infection pressure group on day$_{-25}$ but plasma total protein and albumin concentrations on day$_{-4}$ or day$_{24}$ were not affected by feeding treatment or infection pressure (*P>0.15*).

Table 2.5 Least square means for plasma albumin (g/L), plasma total protein (g/L), plasma urea (*m*mol/*L*) and plasma pepsinogen (*m*U/L) of ewes trickle infected with either 1000, 5000 or 10000L_3 of *T. circumcincta* three times a week and fed either 0.8 (LP) or 1.3 (HP) times their MP requirement

Plasma constituents (mean ±SEM) at different time points		Treatments					ANOVA (P-value)		
		Infection			MP				
		1000	5000	10,000	LP	HP	Infection	MP	Infection*MP
Albumin	Day-25	32.8±1.29[a]	27.6±1.29[b]	29.9±0.64[b]			*0.020*	*-na-*	*-na-*
	Day-4	29.6±2.03	30.5±2.03	30.6±1.01	29.4±1.43	31.0±1.44	*0.900*	*0.438*	*0.808*
	Day24	32.5±1.98	31.5±1.98	30.3±0.99	29.9±1.40	32.8±1.40	*0.591*	*0.157*	*0.586*
Total protein	Day-25	59.9±2.55	50.9±2.56	55.6±1.28			*0.054*	*-na-*	*-na-*
	Day-4	49.2±3.80	51.9±3.8	51.8±1.90	50.0±2.73	52.0±2.74	*0.824*	*0.620*	*0.822*
	Day24	56.8±3.81	55.8±3.81	54.0±1.90	53.9±2.75	57.2±2.74	*0.770*	*0.404*	*0.890*
Urea	Day-25	3.35±0.26	3.07±0.26	3.01±0.13			*0.508*		
	Day-4	5.40±0.43	4.67±0.43	5.87±0.21	4.58±0.30	6.04±0.30	*0.052*	*0.001*	*0.412*
	Day24	10.7±0.56	10.9±0.56	10.0±0.28	8.26±0.39	12.8±0.39	*0.306*	*<0.001*	*0.924*
Pepsinogen	Day-25	640±164[a]	924±165[ab]	1177±82.3[b]			*0.013*	*-na-*	*-na-*
	Day-4	944±197	986±197	1265±98.5	1117±140	1013±139	*0.220*	*0.600*	*0.537*
	Day24	776±165	884±165	1104±83	973±117	870±117	*0.155*	*0.534*	*0.611*

[a,b,c] Means in a row with different superscripts are significantly different from each other at P=0.05; MP is feeding treatment (LP or HP); *-na-* feeding treatments were not in place at this time point and the effects of feeding treatment were not tested

2.4 Discussion

The objective of this experiment was to investigate possible interactions between MP nutrition and parasite infection pressure in periparturient ewes on lactational performance and extent of PPRI. It was hypothesized that the effects of MP supplementation on lactational performance and the extent of PPRI would be more pronounced in ewes trickle infected with the highest dose of infection pressure compared to the medium and low level of infection pressure. However, this expected interaction was only demonstrated in a limited number of traits assessed in the experiment.

Firstly, lactational performance data only tend to support the hypothesis that ewe and litter BW (gain) would be penalized with an increasing infection pressure. Based on previous evidence, it was expected that with increasing level of infection there would be increased level of plasma leakage leading to increased plasma protein turnover, and substantial quantity of endogenous nitrogen loss into the abomasum along with reduced utilization efficiency of absorbed nutrients (Coop and Angus, 1981; Symons *et al.*, 1981). Furthermore, a shift in protein synthesis from peripheral tissues to the maintenance of the integrity of alimentary tract was expected (Sykes and Greer, 2003). Taken together, these were expected to lead to impaired periparturient performance. However, the data did not clearly show that periparturient performance was sensitive to parasite infection pressure. Similar conclusions were reached in twin-bearing ewes, trickle infected with 5000, 10000 or 20000 L3 *T. circumcincta* per day (Donaldson *et al.*, 1998) and in lactating rats re-infected with 400, 800 or 1600 L3 *Nippostrongylus brasiliensis* (Houdijk *et al.*, 2003b). However, the increased infection pressure did show a

reduction in albumin concentration during mid-pregnancy, with similar trends for plasma total protein. At the same time point increased plasma pepsinogen concentration in the high infection group compared to the low infection group was observed. However, these effects on plasma parameters disappeared as time went on suggesting that the penalty of a parasite infection, in the range of infection pressure included here and elsewhere, on the performance of restrictedly fed immune hosts may be limited.

Evidence from parasite-naïve lambs under different infection pressure from *T. circumcincta* suggests that a minimum level of daily larvae intake may be required to observe significant effects on host physiological and metabolic functions (Symons *et al.*, 1981). It is true that comparison of findings done under different environments may not hold true as the class of animal, type of parasite and level of feeding involved, among others, may affect the outcome. It should be noted that lambs in the aforementioned studies were fed *ad libitum* allowing them to display anorexia, which is the main contributor to reduced productivity under infection (Kyriazakis *et al.*, 1998). Since trickle infection with 10,000 *T. circumcincta* of *ad libitum* fed periparturient ewes resulted in anorexia and reduced lactational performance (Zaralis *et al.*, 2009), it might be argued that the restricted feeding used in this experiment and by Donaldson *et al.* (1998) did not allow the expression of differences in lactational performance arising from variation in infection pressure.

The additional MP supplied to the HP ewes was expected to offset the physiological and metabolic costs associated with parasitism in the LP ewes. It was also expected higher albumin concentrations for HP ewes than for LP ewes because the additional MP could be used for albumin synthesis to

replenish plasma proteins lost into the abomasum during abomasal infections (Amarante *et al.*, 1999; Coop and Angus, 1981; Steel *et al.*, 1982; Symons *et al.*, 1981; Wallace *et al.*, 1996). However, the lack of significant difference in BW development and plasma protein concentrations between the different feeding treatments within each infection level suggests that the achieved intake of MP in LP ewes might not have been sufficiently limiting to observe such effects, although HP ewes had consistently higher concentrations of plasma total protein and albumin than LP ewes. Symons *et al.* (1981) observed significant depression in plasma albumin concentration at doses of 12,000 *T. circumcincta* per week or higher. However, the latter study was with parasite-naïve lambs rather than (partially) immune ewes, and therefore under the currently achieved level of MP nutrition (see below), it can be argued that infection pressure should be considerably higher to observe effects on plasma albumin.

Achieved differences between plasma albumin and urea in LP and HP ewes were considerably smaller than those observed in previous experiments, where similar LP feeding treatments and experimental conditions were used (Houdijk *et al.*, 2005). Furthermore, the estimation of MP requirements assumed that ewes would lose BW (100 g/day) during lactation and have >20 g MP per day for wool (AFRC, 1993). This assumed BW loss was not seen even under the pressure of sub-clinical parasitism, and the requirement for wool growth may have been an over estimation, as AFRC (1993) assumes similar MP requirements for wool in growing lambs and adult ewes. Taken all together, it is highly likely that MP supply from the current LP ewes was not as scarce as observed in other previous studies (Houdijk *et al.*, 2005; Houdijk *et al.*, 2006). However, during lactation the faster litter growth rate from the HP group compared to the LP group was an indication that MP

scarcity may have been more pronounced during lactation. Therefore, it can not be excluded that had the experiment lasted longer, expected effects of feeding treatment on ewe BW gain and plasma albumin concentrations during lactation might have been observed.

Litter birth weight was not significantly penalized by LP feeding even though post-lambing BW was lower for LP ewes compared with HP ewes. The observed effects of feeding treatment on BCS indicate that this difference in post parturition BW may arise to some extent from differences in body reserves. As such, the absence of significant effect of feeding treatment on litter birth weight supports the view that scarce nutrient allocation would be prioritized to reproductive effort over maintaining body reserves, as postulated by Coop and Kyriazakis (1999).

The effect of MP supplementation on FEC was stronger during lactation than during late pregnancy, which is in support of earlier reports (Houdijk *et al.*, 2005; Kahn *et al.*, 2003b; Zaralis *et al.*, 2009). Although, the overall observed FEC from this experiment was lower than previously reported results with reproducing ewes trickle infected with *T. circumcincta* (Houdijk *et al.*, 2005; Houdijk *et al.*, 2006), observed effect of MP scarcity on FEC during early lactation were consistent with the nutrient partitioning framework (Coop and Kyriazakis, 1999) that postulates a penalty on immunity to parasites at times of MP scarcity. In addition, the reduced FEC following MP supplementation was associated with a 65% reduction of nematode egg excretion. Similar or higher magnitude of effects have been observed previously (Houdijk *et al.*, 2003a), and demonstrate the possibility of exploiting improved nutrition to reduce pasture contamination for the immunologically naïve lambs at the early stage of their life.

Increased plasma pepsinogen concentrations, together with reduced acid secretion and increased plasma gastrin concentrations, are associated with abomasal parasitism in ruminants (Murrell *et al.*, 1989; Nogareda *et al.*, 2006; Simpson, 2000). Pepsinogen is a precursor for the proteolytic enzyme pepsin secreted by chief cells which leaks into the circulatory system through epithelial cell tight junctions that are disrupted by mast cell proteases, which are released through Th2-cytokine action during parasitism (Patel *et al.*, 2009). Hence, the observed rise in plasma pepsinogen concentration following infection was expected. It has been suggested that this is mainly because of the presence of adult worms in the abomasal lumen (Scott *et al.*, 2000; Simpson, 2000). As such, the increased plasma pepsinogen at day$_{-25}$, day$_{-4}$ and day$_{24}$ at the highest infection pressure compared to the medium and low infection pressure may indicate that the degree of abomasal mucosa damage and/or abomasal permeability (Brundson, 1972; Houdijk *et al.*, 2000; Smith *et al.*, 1983b) is subject to level of infection, and that the increased level of infection resulted in an increased worm burden.

The inverse relationship between FEC and level of infection during the experimental period was unexpected but agrees with similar findings in parasitized rats (Paterson and Viney, 2002) and in sheep (Bishop and Stear, 2000; Callinan and Arundel, 1982; Hong *et al.*, 1987; Jackson and Christie, 1979; Symons *et al.*, 1981). For example, Jackson and Christie (1979) observed similar FEC between lambs daily infected with either 100, 320 or 4000 L_3 of *T. circumcincta*. Similarly, Hong *et al.* (1987) reported similar FEC between lambs infected daily with either 250, 500 or 1000 *T. circumcincta*, although worm burdens were positively related to the levels of infection. Symons *et al.* (1981) observed two-fold higher FEC in lambs following infection with 1200, 3750 and 12000 L_3 *T. circumcincta* per week compared to those receiving

37,500 and 120,000 L_3 per week between 4 to 8 weeks since the start of trickle infection. Level of secondary infection with *Nippostrongylus brasiliensis* increased worm burdens in lactating rats but did not affect their nematode colon egg counts (Houdijk *et al.*, 2003b). Furthermore, level of *T. circumcincta* infection only tended to increase FEC and worm burden at parturition in twin-bearing ewes (Donaldson *et al.*, 1998). These and the result from this experiment suggest that using faecal egg count as a phenotypic trait to estimate variation in infection pressure might not necessarily be reliable especially for partially immune animals. In animals that have previous exposure to nematode parasites, this may be related to the fact that any of the worm life history traits that control FEC, including rate of establishment, larval development, fecundity and expulsion (Smith *et al.*, 1983a; Stear *et al.*, 1999b) could be affected by host immune responses and density dependency effects. Paterson and Viney (2002) illustrated this in rats infected with *Strongyloides ratti*, where they observed dose related negative density dependent effects between worm burden and worm fecundity. Bleay *et al.*, (2007) reported that with increasing density of *S. ratti* infection in rats, Th2-type response increased accordingly. They also reported that parasite fecundity was significantly negatively related to the concentration of IgA. Since in the current experiment worm burdens and immune responses were not measured, further work would be required to establish which of the above mechanisms dominated in the current observed inverse relationship between FEC and infection pressure.

This experiment showed that FEC and worm egg excretion may be inversely related to infection pressure in lactating ewes. This implies that deliberately grazing ewes under low infection pressure for *T. circumcincta* may not necessarily assist control strategies aimed to reduce pasture infectivity

throughout and across seasons, but that the latter will have to arise from other (non-chemical) parasite control strategies. Rather, independent of infection pressure, the results support a large body of evidence that increased protein supplementation, especially during lactation, reduces worm egg output in periparturient ewes. The observed 65% reduction in worm egg output following protein supplementation would under grazing conditions contribute to reduced pasture infectivity to the lambs and hence reduced need for the use of chemical drugs for nematode control strategies. Over time, a continuing presence of such interventions would result in slowed evolution of drug resistance among nematode parasites for the existing chemical drugs.

CHAPTER THREE

Nutritional sensitivity of periparturient resistance to nematode parasites in two breeds of sheep with different nutrient demands

Abstract

The periparturient relaxation of immunity (PPRI) to parasites in mammals is sensitive both to metabolizable protein (MP) supply and animal genotype (different reproductive outputs). It was hypothesised that the sensitivity of PRRI to MP scarcity would not differ between different levels of reproductive output when nutrient intake is adjusted for associated differences in MP demand; this hypothesis assumes that PRRI has a nutritional basis only. Scottish Blackface (BF) and the more productive Mule (MU) ewes were infected with the abomasal parasite Teladorsagia circumcincta, and from day$_{-21}$ to day$_{32}$ (day$_0$ is parturition) restrictedly fed at either 0.8 (LP) or 1.3 (HP) times their breed-specific estimated MP requirement (n=18 for each breed-feeding treatment combination). During late pregnancy LP feeding reduced ewe body weight gain in both breeds, tended to increase faecal egg count (FEC), but did not affect plasma pepsinogen. During lactation, LP feeding reduced litter growth rate, ewe plasma urea and plasma albumin concentration compared to HP feeding in both breeds. However, breed and feeding treatment interacted for ewe FEC, worm egg excretion and plasma pepsinogen, which were higher for LP-MU ewes compared to HP-MU and BF ewes. The lower degree of PPRI of BF ewes during lactation compared to MU ewes at similar degree of MP scarcity suggests that effect of reproductive output on nutritional sensitivity of PPRI can not be explained by associated differences in nutrient demand only.

Key words: *breed; metabolizable protein; faecal egg count; T. circumcincta*

3.1 Introduction

During the periparturient period, resistance to nematode parasites often breaks down, resulting in increased worm burdens and nematode egg excretion in animals that are immune to parasites when non-reproducing (Barger, 1993). The degree of this periparturient relaxation of immunity (PPRI) to parasites may have a nutritional basis and be sensitive to metabolizable protein (MP) supply (Coop and Kyriazakis, 1999). Periparturient MP supply is often limited due to feeding on low quality roughage-based feeds or as part of the management system, whilst at the same time MP demand is known to increase up to six fold or more relative to maintenance (AFRC, 1993; Bell *et al.*, 2000; Jessop, 1997). At times of MP scarcity, expression of acquired immunity to nematode parasites may be penalized because of a prioritized allocation of scarce MP to reproductive functions rather than immune functions (Coop and Kyriazakis, 1999). Consequently, an increased MP supply would be expected to reduce the degree of PPRI. It has indeed been repeatedly shown that MP supplementation reduces worm burdens and/or nematode egg excretion in periparturient sheep (Donaldson *et al.*, 1998; Houdijk *et al.*, 2000; Kahn *et al.*, 2003b; Kidane *et al.*, 2009) although increasing MP supply without overcoming MP scarcity may increase milk yield without reducing worm burdens (Houdijk *et al.*, 2003a).

The degree of PPRI also varies considerably between small ruminant genotypes with different reproductive outputs (e.g. litter growth), and thus nutrient demand (Houdijk, 2008). For example, under *ad libitum* feeding on low quality diets, Scottish Blackface ewes had a lower degree of PPRI than

the more productive Mule ewes (Zaralis *et al.*, 2009). Likewise, under natural grazing conditions, Horro ewes showed a higher degree of PPRI than less productive Menz ewes (Mukasa-Mugerwa *et al.*, 2000; Tembely *et al.*, 1998). The design of these experiments does not allow to distinguish whether such between breed differences are due to differences in nutrient scarcity arising from differences in nutrient demand (Houdijk, 2008), or whether they are associated with genetic resistance *per se*. A lower degree of nutritional demand arising from rearing a smaller number of offspring consistently reduces the degree of PPRI in mammals (Baker *et al.*, 1998; Donaldson *et al.*, 1998; Houdijk *et al.*, 2001b; Houdijk *et al.*, 2006; Normanton *et al.*, 2007), and this may account for the above between-breed differences. This experiment was designed to test the hypothesis that if the magnitude of PPRI has a nutritional basis only, then the sensitivity of PPRI to MP scarcity would not differ between different levels of reproductive output when feeding is adjusted for associated differences in MP demand. The hypothesis was tested in an experiment using two breeds of sheep with different reproductive outputs and hence nutrient demands, and its outcome could inform on genotype-specific feeding strategies to minimise PPRI.

3.2 Materials and Methods

3.2.1 Animals and housing

Thirty-six twin-bearing Scottish Blackface ewes (BF) and 36 Mule ewes (MU), all 4-5 year old, were recruited from a group of pregnant ewes at ~56 days before expected mean parturition date (day_0). Their BW (mean ± SEM) was 57.6±0.70 and 69.1±0.73 kg, respectively and all had a similar body condition score (BCS) of 2.65±0.10 on day_{-56}. BF ewes were mated with BF rams, whilst MU ewes were mated with Suffolk rams. From day_{-56} to day_{-25}, the ewes were group housed, and received *ad libitum* medium quality hay and 200 g/day/head of commercial ewe nut (12.5 MJ ME/kg DM and 208 g CP/kg DM). From day_{-24} until the end of the experiment (day_{32}), the ewes were housed individually, in pens sized ~1.5 m x ~2.0 m. The shed was naturally ventilated and illuminated, with additional low-level lighting at night times during lambing. Fresh wood shavings were used as bedding and added daily and fresh water was supplied *ad libitum*.

Male lambs were castrated within one week after birth using elastrator band.

The experiment was approved by SAC's Ethical Review Committee (ED AE 25/2007) and carried under Home Office authorization (PPL 60/3782).

3.2.2 Feeding treatments

At day_{-23} BF and MU ewes were allocated to one of two feeding treatment groups (n=18), balanced for initial faecal egg count (FEC), body weight (BW)

and BCS. Feeding treatments were calculated to supply 0.8 (LP) or 1.3 (HP) times their estimated MP requirements (MPr) and 0.9 times metabolizable energy requirements (MEr) through restricted feeding. For the estimation of MPr and MEr during pregnancy, a litter birth weight of 6.6 kg for BF ewes and 10.3 kg for MU ewes, as observed in previous studies, no maternal BW gain and 10.2 g MP per day for wool growth (Houdijk *et al.*, 2005; Zaralis *et al.*, 2009) were assumed. Expected milk yields were estimated on the basis of previously used or reported values (Houdijk *et al.*, 2005; Peart, 1970; Peart *et al.*, 1979), as 2.4, 2.6, 2.8 and 2.8 kg per day for BF ewes, and 3.0, 3.3, 3.6 and 3.6 kg per day for MU ewes in week 1, 2, 3 and 4 of lactation, respectively. Furthermore, a 10.2 g MP per day for wool growth for both breeds and a BW loss of 80 and 100 g per day was assumed during lactation for BF and MU ewes, respectively (Houdijk *et al.*, 2001a; Houdijk *et al.*, 2001b; Houdijk *et al.*, 2006). Body weight loss was reduced proportionally to the initial BF to MU BW ratio to obtain expected BW loss for BF ewes. All MPr and MEr were based on the Agricultural and Food Research Council (1993) recommendations. MPr and MEr averaged 85 g/day and 13 MJ/day for BF ewes and 115 g/day and 16 MJ/day for MU ewes, respectively, during the last three weeks of pregnancy. During the first four weeks of lactation, MPr and MEr averaged 237 g/day and 27 MJ/day for BF ewes and 287 g/day and 32 MJ/day for MU ewes, respectively.

Daily allowances consisted of ~1/3 of medium quality hay and ~2/3 concentrates, on as fed basis, during both pregnancy and lactation. The daily feed allowance was gradually increased after parturition over three days until the planned lactational allowance was reached. Differences in dietary MP supply were achieved mainly through including xylose-treated soybean meal at the expense of partially alkaline treated straw in the concentrate,

whilst diets were maintained iso-energetic through varying the barley to fat ratio (for details see **Table 3.1**).

Table 3.1. Ingredient and chemical composition of the diets used during late pregnancy and early lactation

	Pregnancy		Lactation	
Ingredients* (g/kg fresh)	LP	HP	LP	HP
Barley	260	279	212	243
Soybean Meal	-	-	67.8	67.8
SoyPass	-	95.0	-	185
Molasses	31.0	31.0	33.4	33.4
Straw partially treated	286	191	308	123
Urea	12.4	9.30	11.0	5.10
DiCal	3.80	-	7.30	-
Limestone	-	4.76	0.72	9.45
Salt	0.30	3.23	1.50	7.20
CalMag	1.10	0.48	1.10	-
Soya oil	20.0	11.4	19.5	5.10
50% Fat Premix	31.0	31.0	33.4	33.4
Megalac	19.1	9.50	20.5	5.10
Sulphur	0.48	-	0.92	-
Mineral and vitamins	1.67	1.67	1.80	1.80
Hay (medium quality)	333	333	281	281
Chemical composition on as fed basis (in g/kg; unless mentioned)				
Dry matter	832	833	840	833
Crude protein	97.4	130	129	187
Ash	57.0	53.0	66.0	63.0
Calcium	5.68	4.31	7.98	7.23
Phosphorus	2.61	2.51	3.91	3.69
Sulphur	1.94	1.68	2.78	2.46
NCGD (%)	50.3	53.8	53.3	57.8
AHEE	47.2	50.3	53.1	34.6
Neutral detergent fiber	403	368	362	308
Acid detergent fiber	249	211	232	165
ME (MJ/kg)†	8.20	8.30	8.80	9.00
MP (estimated)‡	52.9	81.1	68.5	122.4

LP, low protein; HP, high protein; Soypass, Xylose treated soybean meal; DiCal, Dicalcium phosphate, 18% P; CalMAg, calcined magenesite (85 % MgO); Megalac, calcium soap product; NCGD, neutral cellulase and gammanase digestibility; AHEE, Acid-hydrolysable ether extract

* Hay was offered chopped, whilst other ingredients were mixed in a mash form

† metabolizable energy calculated based on NCGD and AHEE values (Thomas *et al.*, 1988)

‡ metabolizable protein predicted from feed tables

3.2.3 Experimental infection

Because the ewes were 4 to 5 years old and previously grazed on natural pastures, they were expected to have substantial prior exposure to a variety of gastrointestinal nematode parasites through grazing. They were treated at housing on day$_{-56}$ with levamisole (Levacide, Norbrook, Newry, UK) and ivermectin (Oramec, Merial, Harlow, UK) at the rate of 7.5 and 0.2 mg/kg BW, respectively, to clear any resident worms. Ewes were trickle infected with 10,000 infective larvae (L_3) on each infection day with a parasite *Teladorsagia circumcincta*, an abomasal nematode that is of particular concern in the temperate regions (Miller and Horohov, 2006). The *T. circumcincta* strain used was the Moredun Ovine Susceptible Isolate that has been maintained in the laboratory for several years. The infective dose was administered three days a week (Monday, Wednesday and Friday) from day$_{-41}$ until day$_{32}$ suspended in water using 10ml syringes during the morning hours, and this infection model has repeatedly been shown to induce sub-clinical parasitism in periparturient ewes (Houdijk *et al.*, 2001a; Houdijk *et al.*, 2006; Zaralis *et al.*, 2009) .

3.2.4 Measurements

The ewes were weighed on day$_{-56}$ and then weekly from day$_{-42}$ onwards, as well as within 12 hours of parturition. The lambs were weighed within 12 hours after birth and weekly afterwards. Ewe BCS was taken regularly, by

lumbar palpation on a zero to five point scale, and to an accuracy of a quarter, as described by Russel (1984).

Since the ewes were fed restrictedly, refusals were not expected. However, whenever refusals did occur they were collected and weighed twice a week and dried. Average achieved daily dry matter (DM) intakes, and associated ME and MP intakes were calculated from the difference between dry matter offered and refused, and the ME and MP yields per kg DM. Furthermore, feed samples were collected every day during the experiment while weighing daily allowances and were pooled at a treatment group level before chemical analyses.

FEC and daily nematode egg excretion. FEC, expressed as eggs per gram of fresh faeces (epg), was taken at housing and then monitored twice weekly from day$_{-23}$ to day$_{32}$ using a modified floatation method (Christie and Jackson, 1982). Daily nematode egg excretion was assessed during lactation to account for possible effects of breed and feeding treatment on faeces production and thus the potential diluting effect of the latter on FEC (Houdijk, 2008; Vagenas *et al.*, 2007). To this effect, faeces production was estimated through using acid insoluble ash (AIA) as an internal, indigestible marker. Feed samples were collected every day during lactation whilst weighing allowances and were pooled for AIA and DM analyses. Faeces were collected directly from the rectum of all ewes for four consecutive days (day$_{23}$ to day$_{26}$) and were pooled per individual ewe and kept frozen at -20^{0}C before analysis of AIA and DM. AIA was analyzed using the 2N HCl procedure as described by van Keulen and Young (1977), and used to calculate total tract DM digestibility (DMd; %). Daily fresh faeces production was calculated using achieved DM intake, faeces DM contents and DMd.

Daily nematode egg excretion throughout lactation (eggs/d) was estimated by multiplying FEC (eggs/g) with the calculated fresh faeces production (g/day) for each day FEC was analyzed, under the assumption that DMd measured from day_{23} to day_{26} can be extrapolated over the lactation period since ewes were fed the same diet throughout lactation.

Plasma samples. Blood samples were taken from the jugular vein of the ewes into heparinised vacutainers immediately before infection (day_{-41}) and at regular intervals afterwards throughout the experiment. The plasma was separated by centrifuging for 15 min at 1340 x *g* at 4°C and stored at –20°C pending analyses. The plasma samples were analyzed for plasma urea (*m*mol/L), plasma pepsinogen (expressed in *IU**1000 per liter as *m*U/L) and plasma albumin (g/L) concentration. In addition, ewes were also monitored for risk of twin lamb disease during late pregnancy with weekly blood sampling to assess beta-hyroxybutyrate (BHBA). Ewes with BHBA level above 2 *m*mol/L were considered at risk for twin lamb disease and were given propylene glycol as an additional energy source until the BHBA level was regarded safe.

3.2.5 Statistical analyses

One ewe lost her twins immediately after lambing and three ewes reared singles because siblings did not survive long after birth. These ewes were excluded from analyses of the lactation part of the experiment.

FEC and nematode egg excretion were log-transformed for statistical analyses, and reported as backtransformed means (with 95% confidence

intervals, CI). Data collected during late pregnancy and during lactation were analyzed separately because of the distinctness in the quality and quantity of feed offered and the difference in ewe physiological state. During lactation, average daily gain of ewe and litter (ADG; in g/d) were estimated by linear regression, as visual inspection of the graphs suggested linear trends with time. Ewe and litter BW, FEC and nematode egg excretion were analyzed using Restricted Maximum Likelihood (REML) in repeated measures analysis of variance (ANOVA) with power model for correlation within subject across time which assumes a tailing-off correlation between repeated measurements as time points between the measurements get wider. Ewe BW taken at the end of pregnancy and immediately post-lambing, litter birth weight, and ewe and litter ADG were analyzed using a 2 x 2 factorial ANOVA with scaled ewe initial BW used as a covariate to account for the *a priori* differences between BF and MU ewes. Total tract DM digestibility was analyzed using a 2 x 2 factorial ANOVA. Plasma constituents taken during late pregnancy (day_{-11} and day_{-3}) and lactation (day_{7} and day_{25}) were analyzed using repeated measures ANOVA with pre-infection values as covariates. All statistical analyses were performed using GenStat 11 (VSN International Ltd., 2008).

3.3 Results

During the experiment all ewes remained healthy with no signs of clinical parasitism. During the last week of pregnancy, only one ewe had mean plasma BHBA above 2 *m*mol/L and was treated with propylene glycol.

3.3.1 Dry matter, ME and MP intake and estimated faeces production

Achieved DM intake, and estimated ME and MP intake during the experiment are presented in **Figure 3.1** and **Table 3.2**. During both pregnancy and lactation, feeding treatment and the interaction between breed and feeding treatment did not affect achieved DM intake and estimated ME intake (P>0.05). Achieved MP intake differed between LP and HP ewes, whilst the interaction with breed illustrated that this difference was larger for MU ewes than for BF ewes.

Faeces DM content was higher for BF ewes than MU ewes (299 *vs.* 283 g/kg; SED 6.10 g/kg; *P=0.01*). However, the effect of feeding treatment on faeces DM content was not significant (P=0.714). DMd was higher for the BF ewes than for the MU ewes (67.1 *vs.* 64.1%; SED 0.82%; *P<0.001*). In addition, HP-ewes had a higher DMd than LP-ewes (72.0 *vs.* 59.0%; SED 0.82%; *P<0.001*). Estimated fresh faeces production was significantly higher for MU ewes than BF ewes (2797 *vs.* 2024 g/d; SED 96 g/d; *P<0.001*) and for the LP ewes than HP ewes (3010 *vs.* 2051 g/d; SED 96 g/d; *P < 0.001*). The breed x feeding treatment interaction was not significant for faeces DM contents (*P=0.30*), DMd (*P=0.48*) and daily fresh faeces production (*P=0.18*).

Table 3.2. Daily intake of dry matter (DM), metabolizable energy (ME) and metabolizable protein (MP), and total tract DM digestibility (DMd) of twin bearing and -rearing Blackface (BF) and Mule (MU) ewes, trickle infected with *T. circumcincta* and fed at either 0.8 (LP) or 1.3 (HP) times their assumed MP requirement during late pregnancy and early lactation

Treatments		Observed daily intake						DMd (%)
		DM (g)		ME (MJ)		MP (g)		
Breed	Feeding	Preg	Lact	Preg	Lact	Preg	Lact	
BF	LP	1041	1882	10.2	20.0	66[a]	157[a]	60.3
	HP	1037	1897	10.4	20.5	101[c]	280[c]	73.9
MU	LP	1446	2385	14.3	25.0	92[b]	196[b]	57.9
	HP	1455	2381	14.5	25.6	142[d]	350[d]	70.3
	SED	*14.3*	*20.4*	*0.13*	*0.13*	*1.21*	*1.63*	*1.20*
ANOVA	*Breed*	***	***	***	***	***	***	***
	Feeding	*ns*	*ns*	*ns*	***	***	***	***
	Breed x Feed	*ns*	*ns*	*ns*	*ns*	***	***	*ns*

Preg: late pregnancy (day$_{-23}$ to day$_0$); Lact: lactation (day$_1$ to day$_{32}$); Feed: feeding treatment

[a,b,c,d] means in a column with different superscripts are significantly different at P<0.05

[†] SED of the interaction term from ANOVA

****P<0.001; ns, not significant at P=0.05*

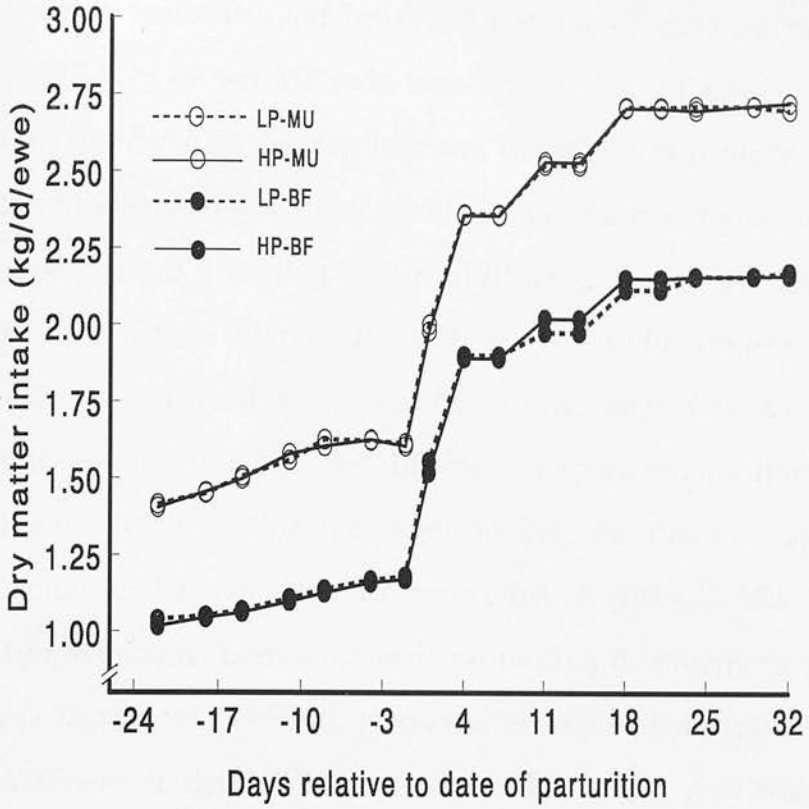


Figure 3.1 Mean daily dry matter intake of twin bearing and -rearing Blackface (BF) and Mule (MU) ewes, trickle infected with *T. circumcincta* and fed at either 0.8 (LP) or 1.3 (HP) times their assumed MP requirements during late pregnancy and lactation *[SEM is very small and can not be seen on the graph]*

3.3.2 Animal performance

Mean BW of ewes and their litter is presented in **Figure 3.2.** Ewe BW increased over time for both breeds (*P<0.001*) and this increase was higher for HP ewes than LP ewes (*P=0.015*). As a result, HP ewes were heavier than the LP ewes (*P=0.001*) at the end of pregnancy and this increment tended to be higher for MU ewes than BF ewes (breed x feeding treatment interaction; *P=0.070*).

Feeding treatment and breed did not interact for post parturition ewe BW (*P=0.27*). However, HP ewes were heavier than LP ewes (58.7 *vs.* 57.1 kg; SED 0.68 kg; *P=0.025*). During lactation the effects of feeding treatment (*P=0.78*), breed x feeding treatment (*P=0.53*), time and feeding treatment (P=0.83), and time x breed x feeding treatment (*P=0.65*) on ewe BW were not significant. However, time interacted with breed (*P < 0.001*) on ewe BW development. This was reflected in the rate of ewe BW change, (-174 *vs.* -111 g/d; SED 18.6 g/d; *P=0.001*; for MU and BF ewes, respectively). Although time did not interact with feeding treatment on BW, the rate of ewe BW change was higher for HP ewes than LP ewes (-165 *vs.* -121 g/d; SED 18.6 g/d; *P=0.023*). The interaction between breed and feeding treatment on ewe daily BW was not significant (*P=0.692*). However, expressed per kg post-lambing BW, the difference in daily BW loss between MU and BF ewes was reduced and was no longer significant (2.64 *vs.* 2.19 g/kg/d; SED 0.313 g/kg/d; *P= 0.154*), although the difference between HP and LP ewes was still significant (2.74 *vs.* 2.09 g/kg/d; SED 0.310 g/kg/d; *P=0.042*).

Ewes of both breeds had similar BCS on day$_{-28}$ (2.43±0.07). However, on day$_{-14}$ condition scores tended to be lower for the LP ewes than HP ewes (2.35 *vs.* 2.46; SED 0.07; *P=0.09*) and was higher for MU than for BF ewes (2.48 *vs.* 2.3l; SED 0.064; *P=0.024*). Breed and feeding treatment did not interact on ewe BCS. During lactation, BCS decreased with time (P<0.001) and was lower for LP ewes than for HP ewes (2.08 *vs.* 2.21; SED 0.047; *P=0.008*). Furthermore, MU ewes tended to have higher condition scores than BF ewes (mean 2.18 *vs.* 2.10; SED 0.048; *P=0.09*). The interaction effects of breed and feeding treatment (*P=0.52*), time and breed (*P=0.79*), time and feeding treatment (*P=0.70*) and the three-way interaction of time, breed and feeding treatment (*P=0.18*) on ewe condition score were not significant.

Feeding treatment and breed interacted for litter birth weight (*P=0.008*). Litters from HP ewes were significantly heavier at birth than litter from LP ewes in MU only (mean 9.60 *vs.* 8.20 kg ; SED 0.266 kg; *P<0.001*). The corresponding values for the BF litter were 6.90 *vs.* 6.51 kg (SED 0.215 kg; *P=0.09*). The interaction effects of breed x feeding treatment (P=0.84), and time x breed x feeding treatment (*P=0.94*) on litter BW during lactation were not significant. However, time and breed (*P<0.001*), and time and feeding treatment (*P<0.001*) interacted on litter BW. This was reflected in differences in daily litter weight gain; MU litters grew faster than BF litters (615 *vs.* 444 g/d; SED 15.1 g/d; *P<0.001*), and HP litters grew faster than LP litters (616 *vs.* 453 g/d; SED 15.1 g/d; *P < 0.001*). However, the interaction effect of breed and feeding treatment on litter ADG was not significant (*P=0.93*).

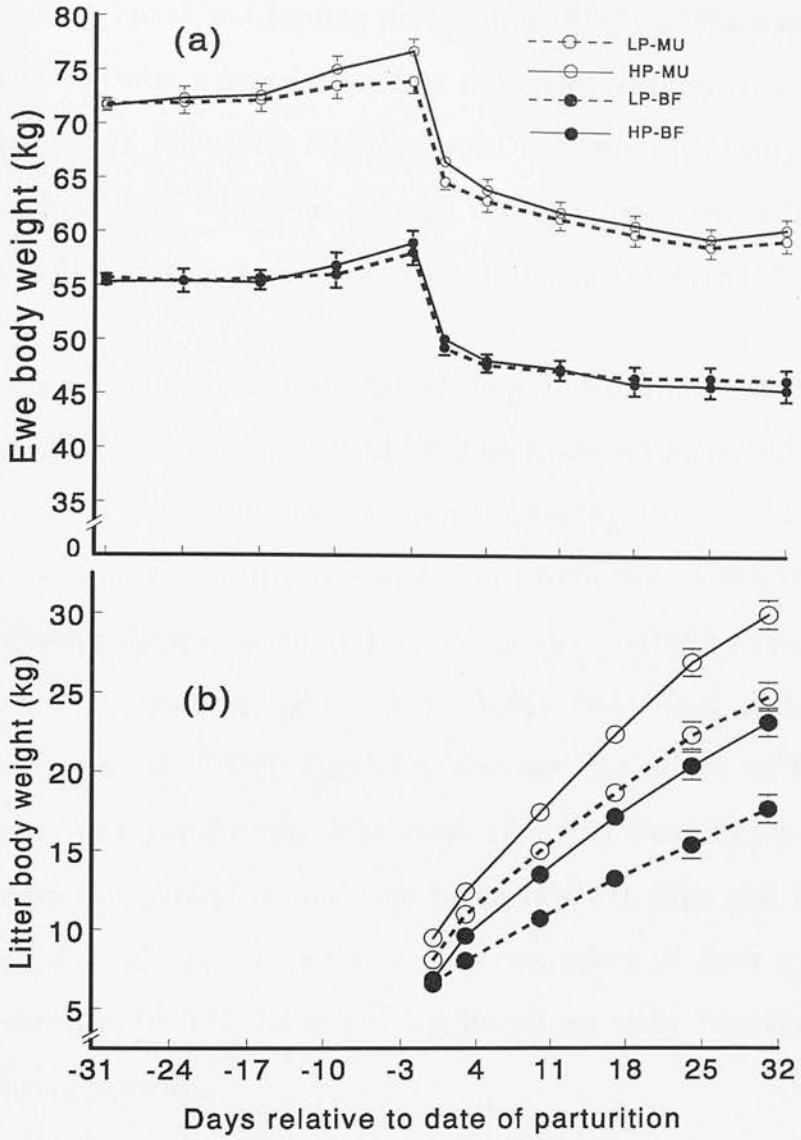


Figure 3.2 Ewe **(a)** and litter (**b**) body weight of twin bearing and -rearing Blackface (BF) and Mule (MU) ewes, trickle infected with *T. circumcincta* and fed at either 0.8 (LP) or 1.3 (HP) times their assumed MP requirement during late pregnancy and lactation

3.3.3 FEC and nematode egg excretion

Figure 3.3 shows backtransformed FEC (with 95% CI). During late pregnancy, the interaction effect of time and breed (*P=0.36*), time and feeding treatment

(*P=0.34*), breed and feeding treatment (*P=0.98*) and the three-way interaction effect of time x breed x feeding treatment (*P=0.28*) on ewe FEC were not significant. However, FEC increased over time (*P<0.001*) for all treatment combinations, whilst on average BF ewes had lower FEC than MU ewes (*P=0.018*) and LP ewes tended to have higher FEC than HP ewes (*P=0.08*).

During lactation, breed and feeding treatment interactions significantly affected FEC (*P=0.05*). The LP feeding treatment increased FEC in MU ewes (*P<0.001*) but not in BF ewes (*P=0.58*). In combination with the aforementioned difference in faeces production, estimated nematode egg excretion during lactation (**Figure 3.4**) was affected by time (*P=0.015*), breed (*P<0.001*), feeding treatment (P<0.001) and breed x feeding treatment interaction (*P=0.042*). The latter showed that effects of feeding treatment were stronger for the MU ewes (*P<0.001*) than BF ewes (*P=0.18*). The interaction effects of time and breed (*P=0.87*), time and feeding treatment (*P=0.40*) and the three-way interaction effect of time x breed x feeding treatment (*P=0.12*) were not significant for daily nematode egg excretion during lactation.

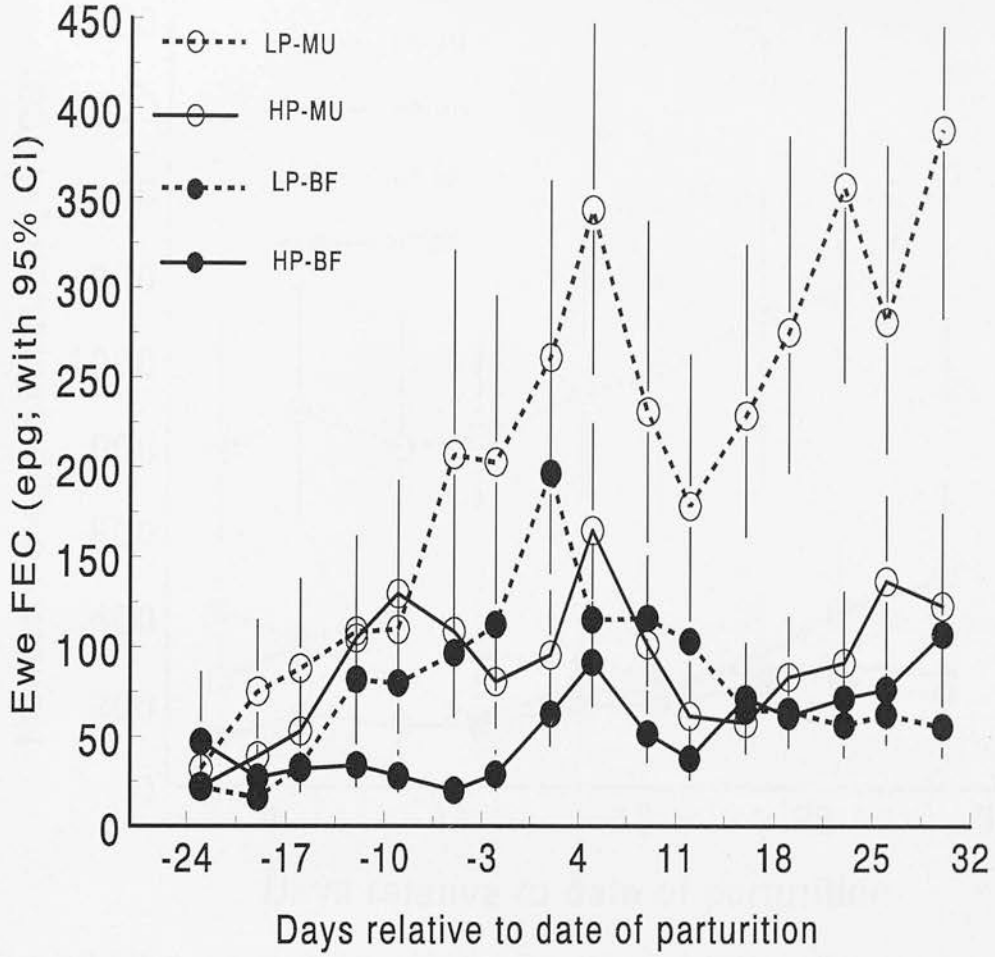


Figure 3.3 Backtransformed faecal egg count [FEC, in eggs per gram (epg) faeces] with 95% confidence interval of twin bearing and -rearing Blackface (BF) and Mule (MU) ewes, trickle infected with *T. circumcincta* and fed at either 0.8 (LP) or 1.3 (HP) times their assumed MP requirement during late pregnancy and lactation

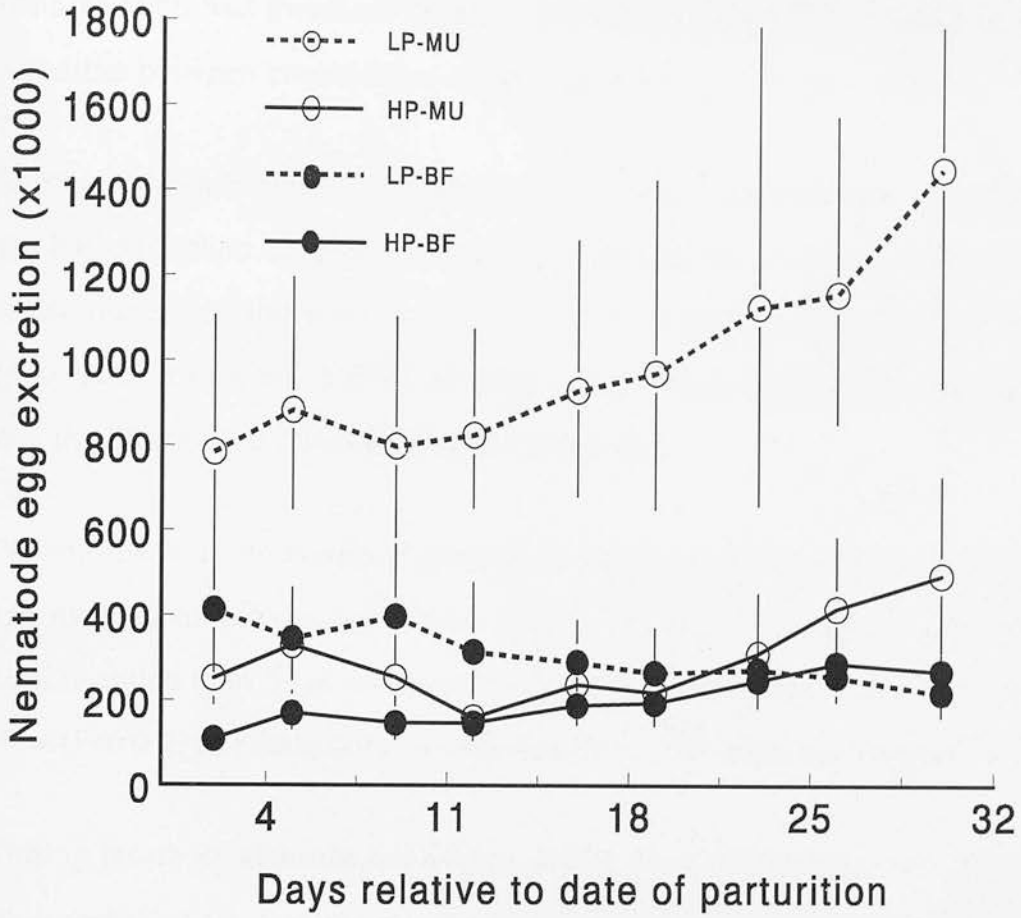


Figure 3.4 Daily nematode egg excretion (eggs/day with 95% confidence interval) of twin-rearing Blackface (BF) and Mule (MU) ewes, trickle infected with *T. circumcincta* and fed at either 0.8 (LP) or 1.3 (HP) times their assumed MP requirement during late pregnancy and lactation

3.3.4 Plasma constituents

Plasma albumin, urea and pepsinogen concentrations are presented in **Figure 3.5.** Pre-infection urea concentration was significantly higher (*P=0.014*) for BF ewes than MU ewes, whilst pre-infection albumin concentration was significantly higher for MU ewes than BF ewes (*P=0.029*). Following two weeks of trickle infection and immediately before supplementation albumin concentration had reduced (*P<0.001*) and urea

concentration had increased by day-26 ($P<0.001$), whilst these parameters did not differ between breeds when adjusted for the pre-infection values.

During the last two weeks of pregnancy, albumin concentration was higher for HP ewes than LP ewes ($P<0.001$), and this difference diverged over the measurement period with significant time by feeding treatment interaction ($P=0.040$). During this period, all other effects including that of breed were not significant ($P>0.11$) on albumin concentration..

During the last two weeks of pregnancy, urea concentration was higher for HP ewes than LP ewes ($P<0.001$). MU ewes tended to have higher urea concentration than BF ewes ($P=0.079$). The urea concentration decreased over time ($P<0.001$) but none of the interaction effects with time were significant.

During lactation, albumin and plasma urea concentrations were not affected by breed ($P>0.27$), and breed x feeding treatment interaction ($P>0.10$). Over time, urea concentration significantly increased ($P<0.001$), whereas albumin concentration did not change ($P=0.59$). However, feeding treatment significantly affected both albumin and plasma urea concentrations ($P<0.001$). Compared to their LP counterparts, HP-ewes had higher albumin concentration (34.1 *vs.* 30.4 g/L; SED 0.50 g/L) and urea concentration (13.4 *vs.* 8.8 mmol/L; SED 0.32 mmol/L).

Mean pre-infection pepsinogen concentration was higher for the MU ewes than the BF ewes (383 *vs.* 319; SED 29.4 *mU/L*; $P=0.033$). After two weeks of trickle infection (on day-26), pepsinogen concentration increased for both breeds to an overall mean (± SEM) of 1133 ± 116 *mU/L* ($P<0.001$). In the last two weeks of pregnancy, however, pepsinogen concentration was higher for the BF ewes than MU ewes (1466 *vs.* 1140 *mU/L*; SED 166 *mU/L*; $P=0.023$).

During this time, the effects of feeding treatment (*P=0.31*), and its interaction effect with breed (*P=0.56*) on pepsinogen concentration were not significant.

During lactation, breed and feeding treatment interacted for pepsinogen concentration (*P=0.025*). The LP feeding treatment resulted in a higher pepsinogen concentration compared to HP feeding in MU ewes only, whilst BF ewes fed LP and HP diet had similar pepsinogen concentrations to MU-HP ewes. None of the interaction effects of time were significant for pepsinogen concentration (*P>0.40*).

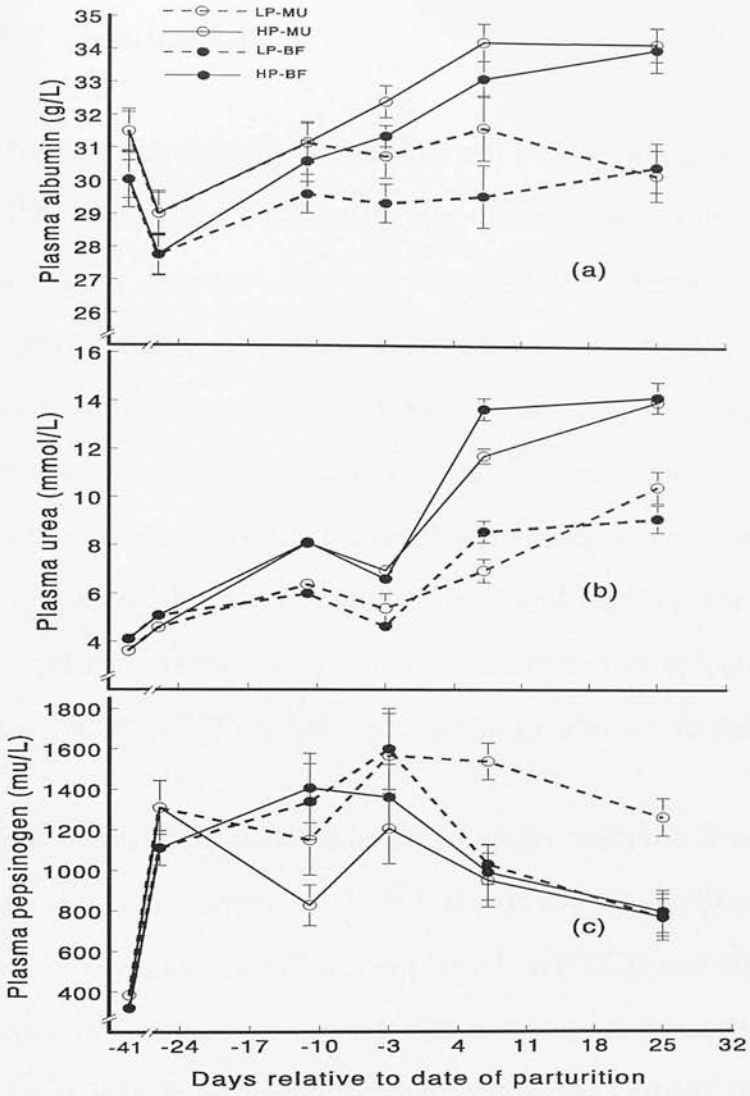


Figure 3.5 Plasma albumin (a), urea (b) and pepsinogen (c) concentration of twin bearing and rearing Blackface (BF) and Mule (MU) ewes, trickle infected with *T. circumcincta* and fed at either 0.8 (LP) or 1.3 (HP) times their assumed MP requirement during late pregnancy and lactation

3.4 Discussion

This experiment was designed to test the hypothesis that if the magnitude in PPRI has a nutritional basis only, then its sensitivity to MP scarcity would not differ between ewes of breeds with different levels of reproductive output when their feeding is adjusted for differences in MP demand. There were no interactions between breed and feeding treatment on the reproductive performance of the *T. circumcincta* infected ewes during lactation. However, the parasitological outcomes rejected the null hypothesis, as indicated by the significant breed and feeding treatment interactions for worm egg output and plasma pepsinogen during lactation, suggesting that sensitivity of PPRI to MP scarcity was greater for the more productive ewes.

This experiment was designed to attain, within a breed, different levels of MP intake at similar levels of DM and ME intake. The latter was confirmed by the estimates of ME content based on NCGD and ether extract values that were determined in pooled diet samples, daily collected during lactation (**Table 3.1**). In addition, the marker assisted DMd of the LP diet agrees well with its estimated ME content but there was a discrepancy for the HP diet. Its higher observed DMd would be expected to result in ~20% more ME from the HP diet than from the LP diet, which contrasts sharply with estimated ME contents. However, the observed higher rate of BW change (loss) of HP ewes compared to LP ewes would not be consistent with a considerably higher ME supply from the HP diet compared to the LP diet (see also discussion below). This suggests that DMd of the HP diet was likely overestimated, and as a result, faeces production during lactation of the HP ewes was probably slightly underestimated. However, in view of the very large effects of feeding treatment on FEC and daily egg excretion in MU ewes

(**Figure 3.3** and **Figure 3.4**), it is unlikely that this would have affected the outcome of the experiment.

The experiment was also designed to attain a similar degree of MP scarcity for both levels of reproductive output in the two breeds through restricted feeding relative to their respective nutrient demands. The lower litter weight gain in both LP-MU and LP-BF ewes relative to their HP counterparts indicates that the LP feeding treatment resulted, as intended, in a higher degree of MP scarcity compared to the HP feeding treatment. The absence of a feeding treatment x breed interaction effect on litter weight gain and ewe daily BW loss, along with similar feeding treatment effects on plasma urea and albumin in both MU and BF ewes support the view that the degree of MP scarcity achieved for both breeds during lactation was similar. Moreover, MU and BF ewes had similar rates of relative BW loss during lactation, whilst for both groups of ewes, the higher rate of BW loss was observed on the HP diet. The latter has also been observed in an earlier study (Houdijk *et al.*, 2000), and may have arisen from a higher degree of body (fat) reserve mobilization to sustain a higher level of milk production in HP than LP ewes at the restricted levels of ME intake. Mean litter BW gain of LP-BF and LP-MU ewes in the current experiment was 78% and 75%, respectively, of litter BW gain from *ad libitum* fed, protein supplemented, non-infected BF and MU ewes kept under comparable conditions (Zaralis *et al.*, 2009). This further supports the view that similar degrees of MP scarcity were achieved for the current BF and MU ewes fed LP diets during lactation.

The strongly elevated FEC, daily nematode egg excretion and pepsinogen levels for the LP-MU ewes during lactation compared to the HP-MU ewes is consistent with a large body of evidence supporting the view that the degree

of PPRI is sensitive to MP scarcity, as reviewed recently (Kyriazakis and Houdijk, 2006; Sykes and Kyriazakis, 2007). FEC is sensitive to dilution by the volume of faeces produced (Houdijk, 2008; Vagenas *et al.*, 2007), which in turn depends on variation in daily DM intake, faeces DM content and total tract DM digestibility. Expressing the results in terms of daily nematode egg excretion can account for the effects feeding treatments had on these parameters. Consequently, although faeces production of the HP ewes may have been slightly underestimated, the effect of MP scarcity on worm egg excretion in MU ewes was more pronounced than those on FEC (**Figure 3.4**).

The concentration of plasma pepsinogen generally increases at times of expression of immunity to infective *T. circumcincta* larvae (Yakoob *et al.*, 1983; Zaralis *et al.*, 2009), and this was also observed here following the start of the trickle infection in both MU and BF ewes. The rise in plasma pepsinogen concentration is mediated through an increased mucosal permeability, resulting from proteolytic action of mast cell protease on the tight junctions between the abomasal mucosa cells (Huntley *et al.*, 1987). Since it has been suggested that abomasal permeability may also correlate with worm burdens (Simpson, 2000), the rise of plasma pepsinogen during pregnancy in both BF and MU ewes suggests a build up of infection in both group of ewes. In addition, the further increased levels of plasma pepsinogen during lactation in the LP-MU ewes may indicate a larger worm burden compared to their HP counterparts, whilst their MP scarcity may have led to a reduced ability to restore mucosal integrity (Houdijk *et al.*, 2003a). The reason for the BF ewes to express an elevated level of plasma pepsinogen with low FEC during the last week of pregnancy, whilst the MU ewes showed a temporal change (see **Figure 3.5**) after the feeding treatments were introduced is unclear, but

may be related to stronger response and hypersensitivity to incoming larvae (Huntley *et al.*, 1987; Smith *et al.*, 1983a).

The results from this experiment suggest that MP scarcity may not reduce resistance to parasites in a breed with lower production output. These findings are consistent with Wallace *et al.* (1995; 1996), who observed reduced parasite resistance at times of MP scarcity in Hampshire lambs but not in the slower growing Blackface lambs, and Houdijk *et al.* (2001b) who noted lack of response to MP scarcity on PPRI in single rearing ewes but not in twin-rearing ewes. Similarly, under grazing condition, without any additional supplementation, the less productive Manx Leoghtan and Shetland breeds had very low FEC compared to heavier and faster growing Southdown breed (Golding and Small, 2009).

The aforementioned pronounced effects of MP scarcity during lactation on plasma pepsinogen, FEC and daily nematode egg excretion for MU ewes were virtually absent for the BF ewes. Zaralis *et al.* (2009) observed similar effects when assessing the effects of protein supplementation on parasitism in *ad libitum* fed MU and BF ewes. However, because both breeds in that study displayed a similar intake of a low protein basal food, the degree of MP scarcity in those BF ewes was calculated to be smaller than in those MU ewes (Zaralis *et al.*, 2009). The latter could have explained at least to some extent the lower degree of PPRI observed in those BF ewes (Coop and Kyriazakis, 1999; Houdijk *et al.*, 2003a). In the current experiment, such a confounding effect of breed and MP scarcity was removed through restricted feeding relative to breed-dependent MP requirements. Since this resulted in the same degree of MP scarcity between the BF and MU ewes, the lower level of FEC, daily nematode egg excretion and plasma pepsinogen concentration

during lactation in the LP-BF compared to the LP-MU ewes suggest that resistance to parasites is less sensitive to MP scarcity in the more resistant breed. The aforementioned results from Wallace *et al.* (1995; 1996) are consistent with this view, whilst it has also been observed that MP scarcity did not increase periparturient FEC in ewes genetically selected for low FEC in contrast to their randomly-bred counterparts (Kahn *et al.*, 2003a).

BF ewes had lower levels of DM intake than MU ewes, because feeding level was adjusted for the level of productivity and BW. During lactation, BF ewes also had faeces with higher DM content, and higher total tract DM digestibility than MU ewes even though the latter may have been slightly overestimated for the HP ewes of both breeds. With everything else being equal, these factors were expected to put a certain degree of magnification to the FEC of LP-BF compared to LP-MU, because of its sensitivity to faecal dilution, as described earlier. The opposite effects observed further suggest that the elevated degree of PPRI in the LP-MU ewes is unlikely to be entirely due to the higher level of productivity of the MU ewes *per se.* Their observed lower level of resistance could be partly explained by a higher emphasis of allocation of scarce resources to reproductive functions, as a correlated response of selection for improved productivity (Rauw *et al.*, 1998; Rauw *et al.*, 1999). Several levels of scarce MP supply, instead of the single level used here, would be needed to explore this hypothesis further (Houdijk *et al.*, 2003a). However, it can not be excluded that the differences observed arise to some extent from genetic differences in immune response *per se,* as recently demonstrated through micro-array studies on several sheep breeds (MacKinnon *et al.*, 2009a; 2009b).

The results from this experiment support the view that differences in periparturient resistance to parasites between the breeds compared here with different reproductive outputs may not be completely explained by differences in nutrient demand. Protein scarcity reduced periparturient resistance to parasites only in the more productive and probably more susceptible breed used. This suggests that using protein supplementation in parasite control strategies that aim to reduce reliance on chemoprophylaxis would be especially relevant for the more productive, and as such, responsive genotypes.

CHAPTER FOUR

Effects of maternal protein nutrition and subsquent grazing on chicory (*Cichorium intybus*) on parasitism and animal performance

Abstract

Forty-eight, four to five years old, Blackface X Bluefaced Leicester (Mule) ewes and their 24 day-old twin lambs were used to assess the combined effects of maternal protein nutrition and subsequent grazing on initially parasite clean Cichorium intybus (chicory) compared to Lolium perenne : Trifolium repens mix (ryegrass/clover) on sheep performance and parasitism. Prior to turn out onto a parasitologically safe pasture, as well as during the first 35 days of grazing on this safe pasture, all ewes were experimentally infected with Teladorsagia circumcincta, and were supplemented with protein (HP) or not (LP). After an additional 42 days grazing on this pasture, all LP and HP ewes and their lambs were moved onto newly established pastures sown with either chicory or grass/clover. Ewes were removed after grazing for 20 days on the experimental pasture. On safe pasture, HP ewes had lower faecal egg counts (FEC) than LP ewes, whilst HP lambs had temporarily lower FEC, consistently lower plasma pepsinogen levels and grew faster than LP lambs. On the experimental pastures, lambs grazing on chicory had consistently lower FEC and grew faster than lambs grazing on grass/clover but had higher levels of pepsinogen. Pasture larvae counts were lower for the chicory than for the grass/clover plots. There were no interactions between maternal protein nutrition and subsequent grazed forage type on either performance or parasitological measurements. The results indicate that increased maternal protein nutrition and subsequent grazing on chicory independently improve lamb performance and reduce lamb parasitism.

Keywords: *chicory; T. circumcincta; FEC; metabolizable protein; lamb performance*

4.1 Introduction

The heavy reliance on anthelmintics for gastrointestinal parasite control has led to anthelmintic resistance (Kaminsky, 2003), especially in small ruminants and in some cases towards all current classes of anthelmintics (Jackson and Coop, 2000). This has urged the development of alternative approaches that would reduce reliance on anthelmintics (Ketzis *et al.*, 2006; Torres-Acosta and Hoste, 2008), which include maternal protein supplementation (Houdijk *et al.*, 2006) and grazing on bioactive forages (Athanasiadou *et al.*, 2005).

Any parasite control strategy should aim to reduce pasture infectivity (Torres-Acosta and Hoste, 2008), as it is a key factor in parasite-reduced performance in growing lambs (Coop *et al.*, 1982). It has been observed that ewe and lamb egg excretion can be reduced through respectively improved protein nutrition (Houdijk *et al.*, 2006) and grazing on the bioactive forage chicory (Athanasiadou *et al.*, 2007). Because both approaches are expected to reduce pasture infectivity, both would also be expected to improve lamb performance (Coop *et al.*, 1982; Houdijk *et al.*, 2000; Komolong *et al.*, 1992). To date, these approaches have been investigated independently, although increased benefits in parasite control would accrue if different strategies are combined (Hoste *et al.*, 2005). The present experiment was, therefore, aimed at assessing the interactive effects of maternal protein nutrition and subsequent grazing on chicory on sheep performance and parasitic status. It was hypothesized that increased maternal protein nutrition would reduce the extent of PPRI on the safe pasture. This would lead to reduced parasite exposure and increased weight gain for lambs reared by supplemented ewes relative to lambs from non-supplemented ewes. It was further hypothesized

that grazing lambs on chicory relative to grass/clover would reduce parasitism and improve performance but these effects would be more pronounced in the lambs reared by previously non-supplemented ewes.

4.2 Materials and Methods

The reported findings here focus mainly on lambs. Whilst the combined effects of forage type and maternal protein nutrition on lamb performance and parasitism are reported from the actual grazing experiment, ewes were experimentally infected and adapted to the protein nutrition treatment prior to turn out, as described in detail elsewhere (Chapter 2) and summarized below. As to the timetable of the experiment, see **Figure 4.1.**

The experimental details described below were approved by the Animal Experiment Committee of Scottish Agricultural College (ED AE 04/2007) and carried out under Home Office regulations (PPL 60/3731).

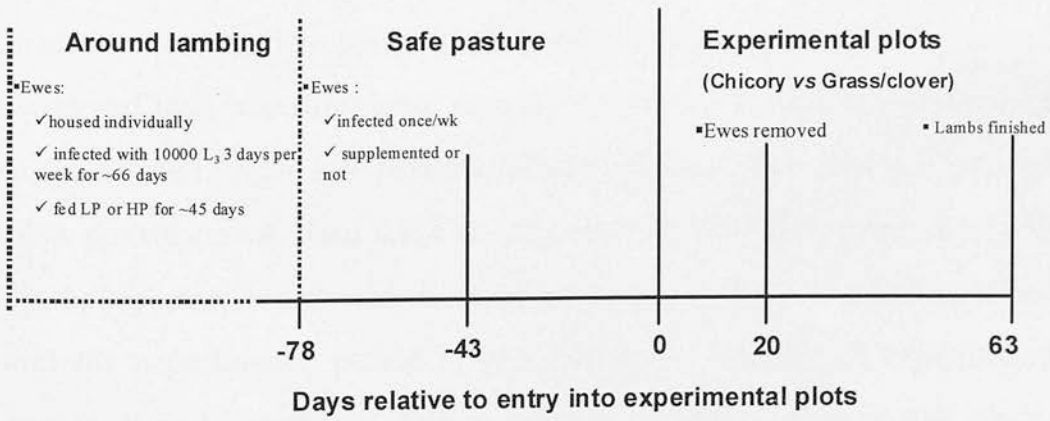


Figure 4.1 Timetable of the experiment

4.2.1 Animals

Forty-eight Mule (Blackface x Blueface Leicester) ewes mated to Suffolk rams with their twin lambs (aged 24 days at turnout) were used in the experiment.

Pre-experimental period. From 42 days before to around 24 days after lambing ewes were housed indoors and trickle infected with the abomasal nematode parasite dominant in temperate region (Stear *et al.*, 1998), *Teladorsagia circumcincta* at a dose of 10,000 infective larvae per infection day three days a week (on Mon-Wed-Fri basis). The isolate of *T. circumcincta* was the Moredun ovine susceptible one that has been maintained in the laboratory for several years (Houdijk *et al.*, 2006). Whilst housed, ewes were fed at either 0.8 (LP; n=24) or 1.3 (HP; n=24) times their estimated metabolizable protein (MP) requirement (AFRC, 1993). The restricted feed allowance consisted a 1/3 medium quality hay and 2/3 concentrates during the last three weeks of pregnancy and the first three weeks of lactation as detailed in Kidane *et al.*, (2009). Lambs were not supplemented and it was ensured that they did not consume any of the food offered to their mothers.

At approximately three weeks post-lambing, the ewes with their lambs were turned out onto a grass pasture, which had not been grazed by sheep for two years and thus was considered parasitologically clean (termed as of here as 'safe pasture'). In the safe pasture, LP and HP ewes were allocated into two plots per treatment, sized 0.372 ha (12 ewes with their twin lambs per plot). The safe pasture, which was used as a link between the periparturient period and the experimental period to give the newly established experimental pasture time to grow and sustain grazing, supplied metabolizable energy (ME) and MP at 10.5 MJ/kg DM and 142 g/kg DM, respectively. It had a

neutral detergent fibre (NDF) and acid detergent fibre (ADF) content of 465 and 246 g/kg DM, respectively. The neutral cellulose gamannase digestibility (NCGD) of the safe pasture was 69.2%.

In the safe pasture, ewes were trickle infected with 10,000 L_3 of *T. circumcincta* once a week for 35 days after which self-infection from the pasture was expected to have started for both the ewes and their lambs.

Whilst grazing on safe pasture, HP ewes continued to be supplemented with MP [in the form of 400 g xylose-treated soybean meal (Soypass) per ewe per day] for 35 days. It has been suggested that even the best managed grass/clover swards will not be able to meet the MP demands of parasitized twin-rearing ewes (Sykes and Kyriazakis, 2007). Therefore, ewes were expected to face MP scarcity, which was further reinforced through the relatively high stocking density used. The level of supplementation was based on an estimation that was used for housed ewes (Kidane *et al.*, 2009) where supplying such a quantity of Soypass above the basal diet increased the MP intake of twin-rearing ewes to 1.3 times the estimated MP requirement calculated according to AFRC (1993). Ewes and lambs remained on this pasture for 77 days before moving into the experimental plots, with day_0 indicating the last day on safe pasture.

Experimental period. In the experimental plots, animals grazed either plots sown with *Cichorium intybus* cv. Puna II (chicory) or sown with a 80:20 mix of *Lolium perenne* : *Trifolium repens* (grass/clover) of 0.24 ha each. There were 6 plots per pasture type that were each grazed by a group of four ewes and their lambs. LP and HP ewes were equally divided within each pasture type, and within maternal protein nutrition treatment, groups were balanced as

much as possible for lamb faecal egg count (FEC), lamb body weight (BW), ewe FEC and ewe BW (in order of reduced emphasis). Ewes were removed from the experimental plots on day$_{20}$ when lambs were weaned, whilst lambs continued grazing until day$_{62}$. Animals had *ad libitum* access to water.

4.2.2 Measurements

Animal measurements. Lamb and ewe BW was taken weekly in the safe pasture and fortnightly in the experimental plots, at the same time of a day on each occasion. The FEC, expressed in eggs per gram fresh faeces (epg), was assessed weekly in ewes and fortnightly in lambs whilst they were on the safe pasture and fortnightly in ewes and lambs in the experimental plots. A modified flotation methodology was used to perform the FEC, (Christie and Jackson, 1982) with the use of polyallomer centrifuge tubes to collect the nematode eggs from the meniscus.

Blood samples were taken from the jugular vein of the lambs into heparinised vacutainers on day$_{-36}$, day$_{-22}$, day$_{-8}$, day$_{6}$, day$_{20}$ and day$_{62}$. The plasma was separated by centrifuging for 15 min at 1340 x g at 4^{0}C and stored at –20^{0}C pending analyses. The plasma samples were later analyzed for plasma urea (mmol/L), plasma pepsinogen (expressed in IU*1000 per liter as mU/L) and plasma albumin (g/L) concentration. Plasma pepsinogen was used as a proxy to abomasal damage (Murrell *et al.*, 1989; Nogareda *et al.*, 2006) whereas plasma albumin and plasma urea were measured to show treatment effects on protein nutrition status.

Herbage measurements. Forage samples were collected from the experimental plots to characterize them in terms of chemical and botanical composition. Samples were collected on day$_0$ from 6 randomized areas of

each plot by walking in a 'W' transect and cutting at a height of ~4 cm above ground level to simulate grazing (Wright *et al.*, 2006). The material cut from each area of a plot was processed within 2 hours. Each of the 6 samples per plot was divided into two equal parts. The first half was pooled and sub-samples were taken for chemical analyses. The remaining 6 halves per plot were used to determine the botanical composition of the pastures to obtain an estimate of the proportion of sown species. To that end, samples were manually separated into 'sown species', 'seed bank species' and 'dead matter' (debris). They were then dried at 100°C to a constant weight, whereas samples for chemical analyses were dried at a temperature of 60°C for 48 hours. The later samples were ground to pass through 1mm mesh size.

At the end of the experiment fresh samples were collected from the experimental plots, as described above, for pasture larvae counts. Pasture larvae counts were made using the method described by Hutchings *et al.*, (2002) but without species identification. Sward height was measured every three weeks by using the Hill Farm Research Organization (HFRO) sward stick for monitoring feed availability (Murphy *et al.*, 1995).

The concentration of specific minerals of the forage sampled [macro minerals: magnesium (Mg), sodium (Na), potassium (K), phosphorus (P), sulphur (S) and micro minerals: zinc (Zn), boron (B), copper (Cu), manganese (Mn) and iron (Fe)] was determined by inductively coupled plasma–atomic emission spectrometry (ICP-AES) using Perkin Elmer OES Optima 4300DV. NDF and ADF were determined using the FOSS FaberCap System in accordance with Van Soest (1963). Feed total nitrogen (N) was determined by the Kjeldahl sulphuric acid digestion and steam distillation technique (AOAC, 1980) and per cent crude protein (CP) was calculated as N X 6.25.

AHEE was determined using MAFF (1993). In vivo digestibility was estimated by in vitro NCGD using the method of Dowman (1993). Metabolizable energy (ME; expressed in MJ/kg DM) content of the forages was estimated using the NCGD and AHEE values (Thomas *et al.*, 1988).

4.2.3 Statistical analyses

Data collected during the experimental period for individual ewes and lambs were re-arranged from calendar date to days relative to entry into experimental plots (day_0) prior to analyses. For sward measurements (sward height and botanical proportions) descriptive statistics was used.

Effects of pre-grazing treatments were accommodated in the statistical analysis by using scaled pre-grazing measurements (i.e. group mean deducted from individual measurement in each group which centre around zero) as covariates. The analysis of FEC and BW of ewes and lambs was performed over two periods: 'safe pasture' (day_{-78} to day_0) and experimental plot (day_0 to day_{62}). The periods were treated separately for analysis of variance. During each of these periods, plasma constituents, FEC and BW of lambs, and FEC and BW of ewes were analyzed using repeated measures analysis of variance (ANOVA). Lamb average daily weight gain (ADG, in g/d) was estimated by linear regression of BW on experimental day.

Pasture larvae count, final BW of ewes and ADG of lambs were analysed using general ANOVA. FEC and pasture larvae count were transformed via log(FEC+1) prior to statistical analysis and back-transformed means (with 95% confidence interval, CI) were reported. All statistical analyses were

performed using GenStat 11 (VSN International Ltd., 2008), using each plot as a block in the analyses.

4.3 Results

4.3.1 Sward measurements

Sward heights measured on day_0 were (mean ± SEM) 17.8 ± 3.6 cm and 22.3 ± 1.9 cm, for chicory and grass/clover plots respectively. Sward heights decreased sharply over the first 3 weeks of grazing to 6.5 ± 1.0 cm and 8.7 ± 1.1 cm and stabilized afterwards with end point (day_{63}) measurements averaging 4.2 ± 1.2 cm and 6.8 ± 1.2 cm, for the chicory and grass/clover plots, respectively.

Sward botanical composition (% on DM basis) from the experimental plots is presented in **Figure 4.2.** Seed bank species, (which consisted mainly of annual ryegrass and a very small proportion Chickweed and Thistle) constituted large portion of chicory plots. However, the proportion of chicory increased over time to ~50% on the chicory plots, whilst the proportion of grass/clover in the grass/clover plots was relatively stable at ~90%. In both chicory and grass/clover plots the proportion of dead matter showed a slight increase over time.

Terminal pasture larvae counts (with 95% CI) on the experimental plots averaged 5,817 /kg DM (3,970 to 8,142) for the chicory plots and 17,484 /kg DM (9,393 to 19,266) for the grass/clover plots (*P=0.073*). This was not affected by maternal protein nutrition or its interaction with forage type.

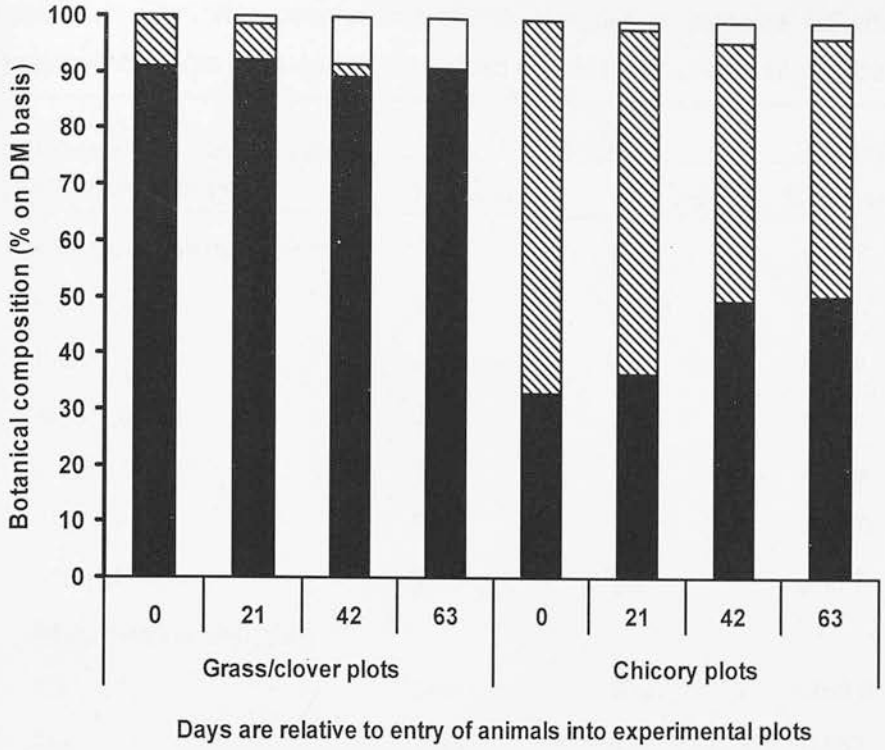


Figure 4.2 Sward botanical composition (% on DM basis) of sown species (■), seed bank species (▨) and dead matter (□) taken over 3 week intervals through out the experiment

Details on chemical composition of the chicory and grass/clover plots are presented in **Table 4.1**. Chicory has a lower DM content than grass/clover. However, the concentration of most of the nutrients, macro- and trace minerals analysed was higher in its DM content compared to grass/clover, whilst its NCGD and calculated level of ME were significantly lower.

Table 4.1. Chemical composition of the pre-grazing samples collected from the experimental plots (Values are expressed on DM basis unless otherwise mentioned)

Chemical composition (Wet chemistry)	Treatments[1]		Statistics	
	Grass/clover	Chicory	P-value	SED
DM (g/kg fresh matter)	179	120	*0.002*	*11.6*
CP (g/kg)	94.8	137.0	*< 0.001*	*8.23*
NDF (g/kg)	397	342	*0.042*	*23.7*
ADF (g/kg)	225	231	*0.596*	*12.4*
AHEE (g/kg)	38.1	38.5	*0.886*	*2.60*
NCGD (%)	81.9	73.8	*< 0.001*	*1.52*
ME (MJ/kg)	12.4	11.3	*0.001*	*0.209*
Macro-minerals, g/kg				
Ca	5.57	10.7	*< 0.001*	*0.506*
Na	4.43	6.22	*0.082*	*0.923*
Mg	1.72	3.43	*< 0.001*	*0.218*
K	15.6	27.3	*< 0.001*	*1.24*
P	3.33	5.65	*< 0.001*	*0.128*
S	2.08	2.87	*< 0.001*	*0.097*
Micro-minerals, mg/kg				
Zn	22.3	65.8	*< 0.001*	*4.47*
B	5.72	22.1	*< 0.001*	*1.28*
Cu	6.15	9.13	*< 0.001*	*0.416*
Mn	129	192	*0.002*	*14.6*
Fe	109	234	*0.028*	*48.8*

[1]Mean value for each treatment comes from a sample size of 6 and analyzed in duplicates

[2] AHEE=acid hydrolyzable ether extract

[3] NCGD=neutral detergent cellulase plus gamannase digestibility

[4] ME (MJ/kg DM) calculated as 0.014*NCGD (g/kg DM) + 0.025*AHEE (g/kg DM) (Thomas *et al.*, 1988)

4.3.2 Animal performance

Ewe body weight. The pattern of BW change of LP and HP ewes did not differ except the fact that the grazing groups started with different BW (see **Figure 4.3a)**. Ewes lost BW during the first 42 days after turnout (235 g/d; SEM=13.8 g/d; *P=0.66*) but gained weight during the next 35 days (mean=200 g/d; SEM=11.0 g/d; *P=0.40*). In the experimental plots, after adjusting for the starting BW, the effects of previous ewe protein nutrition (*P=0.173*), forage type (*P=0.28*) and the interaction effect between protein nutrition and forage type (*P=0.82*) were not significant on ewe final BW.

Lamb body weight. Error! Reference source not found.**b** shows that during the first 77 days of grazing in the safe pasture HP lambs grew faster than LP lambs (174 vs. 155 g/d; SED 9.0 g/d; *P=0.028*) whilst growth rate decreased between day$_{-50}$ and day$_{-22}$ in both HP and LP groups. Lambs grazing chicory grew faster than those grazing grass/clover (203 vs. 161 g/d; SED 13.0 g/d; *P=0.013*). During the experimental period, ADG of lambs was not affected by previous maternal protein nutrition (184 vs.181 g/d for HP vs. LP; SED 14.9 g/d; *P=0.75*) nor its interaction with forage type (*P=0.35*).

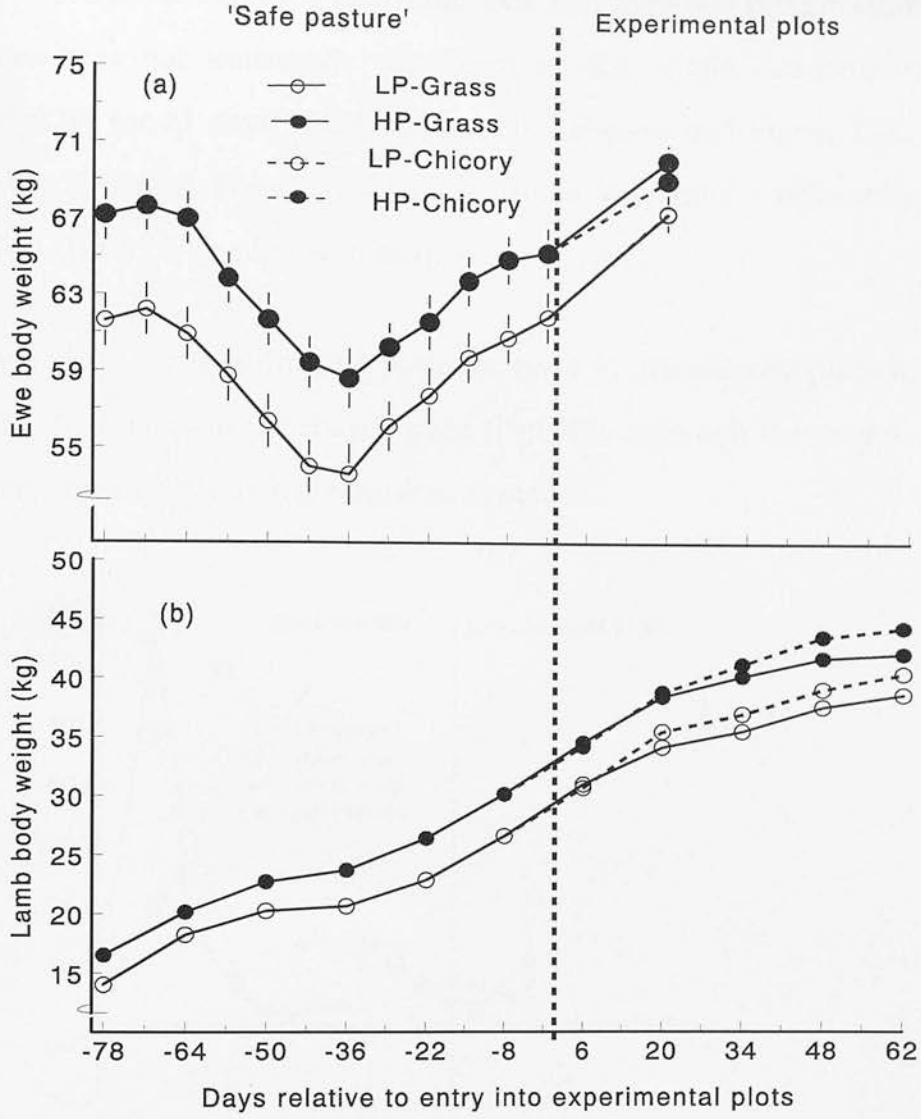


Figure 4.3 Body weight development over the experimental period: **(a)** ewes and **(b)** lambs *[SEM for lamb BW averaged 0.55 kg during safe pasture period and is not visible on the graph]*

4.3.3 FEC and plasma constituents

Ewe FEC. The FEC of ewes changed with time ($P<0.001$). It peaked on day$_{-78}$ for LP and day$_{-71}$ for HP ewes (**Figure 4.4**). The effect of maternal protein nutrition over the whole safe-pasture period tended to be significant

(*P=0.072*). Although the interaction between maternal protein nutrition and time was not statistically significant for the whole experimental period (*P=0.15*) for 21 days following turnout, LP ewes had higher FEC than HP ewes (*P=0.016*). From day-50 FEC remained low and the difference between the LP and HP groups disappeared.

Whilst on the experimental pastures, ewes in grass/clover plots had higher FEC than ewes in the chicory plots (*P=0.042*), although the overall FEC was very low over the two measurements made.

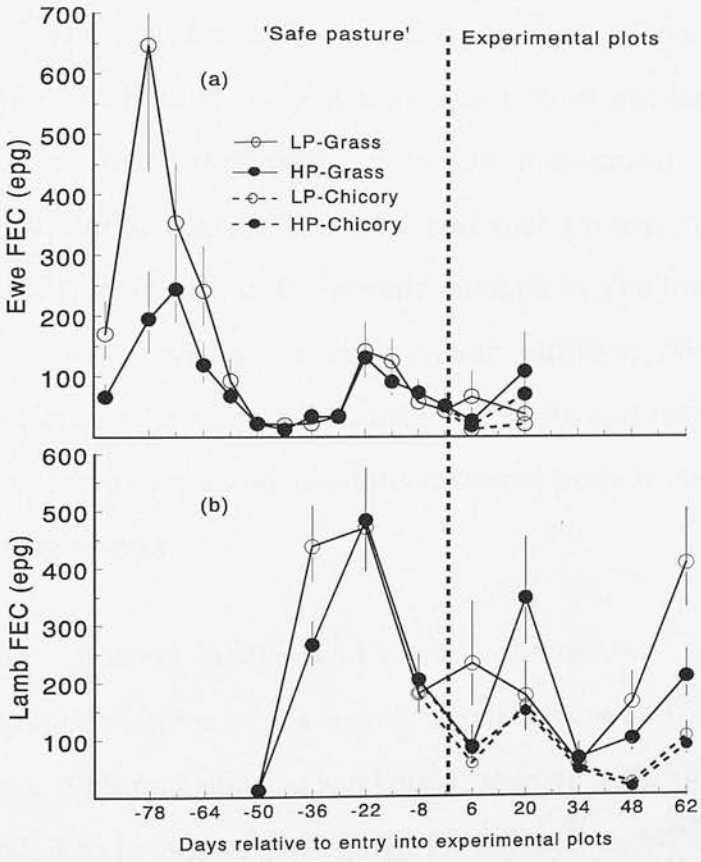


Figure 4.4 Back-transformed mean faecal egg count (epg with 95% CI) of: **(a)** ewes and **(b)** lambs

Lamb FEC. Lamb FEC both on safe pasture and experimental plots is shown **Figure 4.4b**. On safe pasture lamb FEC was the lowest on day$_{-50}$, averaging only 19 (range: 0 to 105) epg for both groups. FEC of lambs increased with time until day$_{-22}$ (*P<0.001*). Although, the interaction between maternal protein nutrition and time was not significant for the whole safe pasture period (*P=0.38*), on one occasion (day$_{-36}$), lambs of HP ewes had significantly lower FEC than lambs of LP ewes on (*P=0.011*). This difference disappeared in the subsequent two measurements.

There was a significant interaction between time and forage type on lamb FEC whilst on the experimental pastures (*P<0.001*). Relative to the chicory lambs, the FEC of lambs grazing grass/clover increased sharply towards the end of the experiment, and was numerically, although not always significantly, higher. Previous maternal protein nutrition did not affect (*P=0.22*) lamb FEC in the experimental plots. The interaction effects between time and previous maternal protein nutrition (*P=0.33*), forage type and previous maternal protein nutrition (*P=0.50*) and the three-way interaction of time, forage type and previous maternal protein nutrition (*P=0.13*) were all not significant.

Lamb plasma analysis. Plasma concentration of albumin, urea and pepsinogen in the lambs is presented in **Figure 4.5**. When grazing on the safe pasture plasma albumin level decreased with time (*P<0.001*) in all groups but tended to be higher throughout for lambs from HP ewes compared to those from LP ewes (28.7 vs. 28.0 g/l; SED 0.250 g/l; *P=0.057*). Whilst on the experimental pasture, plasma albumin increased with time (*P<0.001*), and a significant interaction between time and forage type (*P=0.002*) suggested that this increment was greater for chicory than for grass/clover lambs. Albumin

was not affected by previous maternal protein nutrition or any of its interactions. On safe pasture, time tended to interact with maternal protein nutrition for plasma urea (*P=0.063*). However, the effects of maternal protein nutrition on plasma urea were not significant (*P=0.11*).

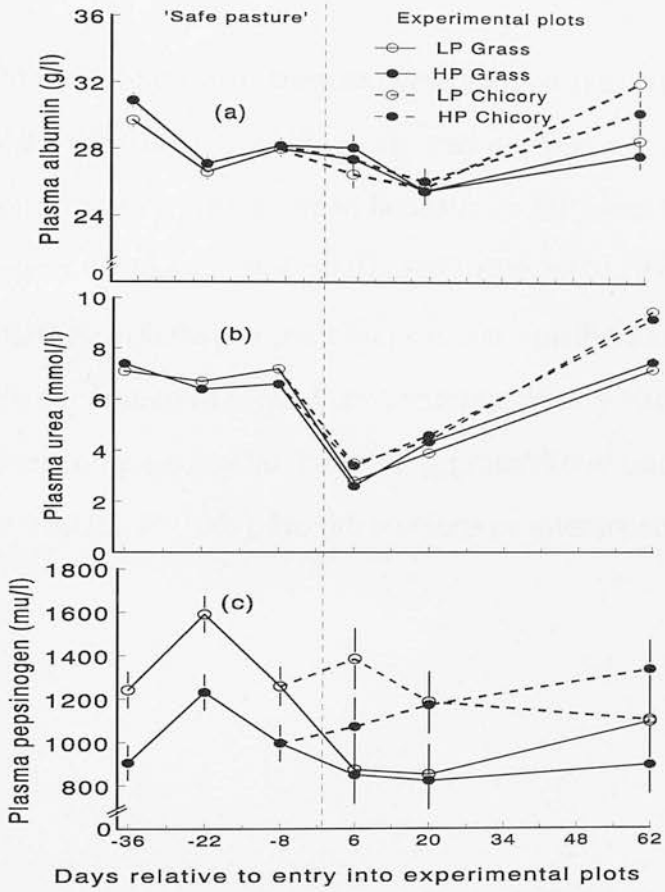


Figure 4.5 Mean plasma constituents on lambs coming from ewes fed LP (open circle) or HP (solid circle) and lambs grazing chicory (broken line) or grass/clover (solid line): **(a)** plasma albumin (g/*L*); **(b)** plasma urea (*m*mol/L) *[SEM for plasma urea averaged 0.182 and 0.243 mmol/L during safe pasture and experimental pasture periods respectively; and are not visible]*; **(c)** plasma pepsinogen (*mU*/L)

Whilst on the experimental plots, plasma urea concentration increased with time (*P<0.001*). There was, however, a significant time by forage type

interaction (*P<0.001*), such that, at the end of the experiment, lambs on chicory had higher plasma urea concentration than lambs on grass/clover (mean 9.35 *vs.* 7.33 *m*mol/L; SED 0.170 *m*mol/L; *P=0.001*). Neither maternal protein nutrition, nor any of its interactions affected plasma urea concentration on the experimental plots.

On the safe pasture, plasma pepsinogen concentration in lambs changed with time (*P<0.001*), peaking at day-22. Up to day0, plasma pepsinogen concentration was lower in lambs from HP ewes than in lambs from LP ewes (mean 1,045 *vs.* 1,364 *mU/L*; SED 61.9 *mU/L*; *P<0.001*), with no significant interaction between maternal protein nutrition and time (*P=0.75*). Whilst on the experimental plots, lambs grazing chicory had higher plasma pepsinogen level compared to lambs grazing grass/clover (mean 1,207 *vs.* 884 *mU/L*; SED 54.9 *mU/L*; *P<0.001*). No other effects or interactions were significant.

4.4 Discussion

The combined and possible interactive effects of maternal protein nutrition and subsequent grazing on chicory relative to grass/clover on sheep performance and parasitism were assessed. However, these strategies were not linked in time; the maternal protein nutrition treatment stopped 42 days before the start of grazing on chicory. Lambs derived from the two maternal feeding backgrounds then grazed either on the chicory or grass/clover plots. The results are discussed below.

4.4.1 Animal performance

Ewe performance. The loss of BW of the twin-rearing ewes in the five weeks after turnout occurred in both LP and HP treatments. The very wet weather of spring 2007 in the UK restricted grass growth which resulted in a sharp decrease in sward height during this period. These observations strongly suggest that the BW loss did not result from a protein limitation but that energy intake was insufficient to support both lactation demands and maintenance of body reserves. However, an initial depletion of body reserves later followed by repletion when lactation demands decrease, as observed here, is not uncommon in ewes (Lambe *et al.*, 2003).

Lamb performance. The faster growth rate of lambs from HP than from LP ewes on the safe pasture could suggest a beneficial effect of maternal protein supplementation on milk yield. The decline in growth rate of lambs reared

by both groups of ewes on the (initial) safe pasture, which started around day$_{-50}$, coincided with the expected reduction in milk production by ewes at this stage. The peak in FEC and plasma pepsinogen of lambs between day$_{-36}$ and day$_{-22}$ suggests that this also occurred at a time of increased larval intake by lambs as the reduced milk production would have necessitated an increased forage intake. Such an increased larval intake could have been responsible for the reduced performance observed (Coop *et al.*, 1982).

In the experimental plots, the live-weight gain of lambs grazing chicory was higher compared to lambs grazing grass/clover. Such effects were also reported in previous studies where chicory was compared to other conventionally used forage species (Athanasiadou *et al.*, 2007; Komolong *et al.*, 1992). Min *et al* (1997) also reported that grazing red and hybrid deer on chicory compared to other perennial ryegrass/white clover pasture improved mean carcass weight, which was due to increased live-weight gain and higher dressing-out percentage at slaughter. There are at least two possible explanations for the observed difference in performance of lambs grazing these different forages.

Firstly, this could be mainly a direct result of the differences in nutritional quality between the chicory and grass/clover forages. The data in **Table 4.1** show that per kg DM, the forage from the chicory plots supplied more CP (> 40 g per kg DM) and less NDF than forage from the grass/clover plots. Such nutritional benefits of chicory have been observed in many other studies (Belesky *et al.*, 2000; Collins and McCoy, 1997; Komolong *et al.*, 1992). The slightly higher plasma albumin and urea levels in the lambs grazing chicory are likely a reflection of the higher CP level in that forage compared to that on the grass/clover plots. Such a higher crude protein content yield usually

also results in higher DM intakes from forage in sheep (ARC, 1980; Blaxter, 1989). Although no evidence of mineral deficiencies was observed, chicory also provided more of most macro-minerals and micro-elements per kg DM than forage from the grass/clover plots. Such differences between forages in nutritional quality and its potential effects on DM intake alone could have led to the observed differences in lamb performance. Secondly, chicory grazing could have affected lamb performance through effects of the forage on the level of parasitism. In support of this view, Athanasiadou *et al.*, (2007) observed that the benefit of chicory over grass/clover on lamb performance was only apparent in the presence of parasitism. Such a benefit could have arisen from a reduced pasture infectivity as observed with consistently lower FEC in lambs grazing chicory compared to their grass/clover counterparts (Athanasiadou *et al.*, 2007).

Maternal protein supplementation and grazing on chicory instead of grass clover both affected lamb performance at some stage but the expected interactive effects of the two treatments were not observed. This might well be the result of the separation in time between the two treatments. The effects of maternal protein nutrition on lamb growth rate had disappeared during the last weeks of grazing on the safe pasture. This means that, apart from a remaining difference in average BW, the lambs reared by LP ewes were not different from lambs reared by HP ewes by the time they had access to the different pasture types.

4.4.2 Animal parasitism

Ewe FEC. The difference observed in ewe FEC during the early periods of the safe pasture supports previous works where protein supplementation reduced FEC of periparturient ewes (Houdijk *et al.*, 2000; Houdijk *et al.*, 2001b). However, the disappearance of this difference between the groups with advancing grazing period suggests the diminishing demand in MP of the twin rearing ewes as the later was expected to affect the degree of nematode egg excretion (Houdijk *et al.*, 2006; Houdijk, 2008). Furthermore, the relatively low FEC whilst grazing in the chicory vs. grass/clover plots in the later stage of the experiment supports the above argument.

Lamb parasitism. There were significant effects of maternal protein nutrition on lamb pepsinogen levels on the safe pasture, suggesting more nematode parasite damage to the abomasal wall (Berghen *et al.*, 1993) in lambs reared by LP ewes. This was also reflected by the higher FEC of lambs from LP ewes on one of the observation weeks in the safe pasture (**Figure 4.4b**). The observed difference in lamb FEC may reflect a difference in pasture infectivity arising from differences in ewe FEC. The trend of FEC of lambs during the safe pasture period, which followed that of ewes with a time lag of a few weeks, supports the idea that the ewes were the main source of infection for the lambs on the safe pasture. Furthermore, the very low FEC of lambs 28 days post turnout in the safe pasture (compared to ~500 epg on a similar date from a pasture previously grazed by sheep; data from lambs in the latter works (in ***Chapter Five and Six***) indeed suggest the background pasture infectivity on the safe pasture was either minimal or absent.

In the chicory and grass/clover plots, it was observed, however, that lambs grazing on chicory had consistently lower FEC compared to grass/clover. The effect of maternal protein nutrition on lamb FEC had disappeared during the last weeks of grazing on the safe pasture and it would, therefore, have been unlikely for maternal protein nutrition to affect lamb FEC on the experimental plots. Previous studies of lambs infected with *T. circumcincta* and grazing on chicory compared to other conventional forage species indicated reduced worm burdens (Marley *et al.*, 2003a; Tzamaloukas *et al.*, 2005) and FEC (Athanasiadou *et al.*, 2007). Mechanisms operating in worm control by chicory were suggested by Tzamaloukas *et al.*, (2005) to be direct anthelmintic-like effects and/or possible indirect effects through an enhanced immune response. The latter effect can be expected if more nutrients for mounting an immune response would be available to lambs grazing chicory compared to lambs grazing grass, as discussed above (Coop and Kyriazakis, 1999). The chicory used here was a cultivar that was bred to contain higher levels plant secondary metabolites (Rumball *et al.*, 2003) and specific component of the latter needs further study as the concentration of the condensed tannin of chicory is low and may not differ from that of grass/clover as reported by Athanasiadou *et al.*,(2007). In lambs trickle infected with *Trichostrongylus vitrinus* and fed diets differing in P content alone (1.88 gP/kg DM vs. 2.75 gP/kg DM), Coop and Field (1983) observed improved performance and reduced worm burden with lambs fed diet with the higher P content. Furthermore, in lambs experimentally infected with *Haemonchus contortus*, or *T. colubriformis* or a mix of both, it was reported that urea supplementation (3% of the basal diet of oaten chaff) reduced FEC for *H. contortus* and mixed infections (Knox and Steel, 1999). These could be a

basis for the differences observed in this experiment, given chicory's higher level of P and CP.

Athanasiadou *et al.*, (2007) have suggested that another cause of lower FEC in lambs grazing chicory could be that they are exposed to a lower level of infective larvae. Based on pot experiments, Marley *et al* (2006c; 2006a) reported that fewer larvae of *H. contortus* and *T. circumcincta* migrated onto chicory and legumes compared to ryegrass forages with out affecting survival of larvae. This was also noted in previous grazing experiments that found more infective nematode larvae on ryegrass swards than on chicory or lucerne swards when grazed by lambs experimentally infected with multiple parasite species (Scales *et al.*, 1995). Therefore, the consistently lower FEC in lambs grazing chicory, the lower pasture larvae counts on chicory compared to grass/clover plots at the end of this experiment and a possible reduced ability for infective larvae to migrate upwards, are consistent with the view that lambs grazing chicory may have been under a lower level of parasite challenge than lambs grazing grass/clover.

The divergence in plasma albumin and plasma urea, with time, between chicory and grass/clover groups could probably be due to the fact the experimental plots were clean (worm-free) at the beginning and contaminated progressively as time went on. Therefore, it was expected that with time the lambs could have picked up more infective larvae which would have exacerbated differences between grazing groups in which the grass/clover lambs were expected to show higher level of parasitism. Other studies on lambs infected with different doses of *T. circumcincta* (Symons *et al.*, 1981) and *T. colubrifomis* (Kyriazakis *et al.*, 1994) showed depressed levels of plasma albumin subject to level of infection or compared to non-infected

controls. Therefore, the lower level of plasma albumin in the grass/clover plots towards the end of the experiment could be a reflection of the higher level of parasitism in lambs on these plots.

The elevated level of plasma pepsinogen in the immunologically naïve LP lambs compared to their HP counterparts on the safe pasture could be attributed to an increased degree of parasite-induced abomasal mucosal permeability (Brundson, 1972; Houdijk *et al.*, 2000; Murrell *et al.*, 1989; Patel *et al.*, 2009; Smith *et al.*, 1983b; Symons *et al.*, 1981). In lambs infected with either 0, 250 or 1000 infective larvae of *T. circumcincta* per day and grazed on a minimally infected pasture plasma pepsinogen was reported to be the highest in the last group compared to the first two (Iposu *et al.*, 2008). In previous experiment (Kidane *et al.*, 2009) periparturient ewes infected at three different levels with *T. circumcincta,* plasma pepsinogen was also related to the level of infection. Under nutrient limiting conditions, lambs reared by LP ewes in this case, where worm burden could be higher as observed in weaned lambs compared to suckling lambs (Iposu *et al.*, 2008), elevated levels of plasma pepsinogen were expected. Increased nutrient intake through higher levels of milk supply could have partly offset the effect of parasites in HP lambs, and hence parasitism-associated abomasal damage might have been limited in the HP lambs.

Whilst in the experimental plots, lambs grazing on chicory had consistently higher plasma pepsinogen levels than lambs grazing on grass/clover. In light of earlier discussion, it was expected that plasma pepsinogen would have been higher for lambs grazing on grass/clover than chicory plots. It has been observed that plasma pepsinogen increases following exposure to infective larvae in immune sheep (Yakoob *et al.*, 1983). However, for the first few

weeks on the experimental pasture, larval exposure can be discounted for, as the chicory and grass/clover plots were initially clean, and the newly shed nematode eggs by ewes and lambs were expected to develop into an infective form after around two to three weeks only (Hoberg *et al.*, 2001). This would have resulted in a gradual increase in plasma pepsinogen over time, as indeed observed in lambs grazing the experimental grass/clover plots (**Figure 4.5**). Therefore, the immediate and sustained increased levels of plasma pepsinogen on the chicory plots during this period was unexpected and is most likely due to other reasons than parasitism-induced abomasal mucosal permeability. Gotteland *et al* (2001) observed increased gastric permeability for sucrose in human subjects given copper (10 mg Cu /L of distilled water as copper sulphate solution). It can not be discounted that in similar fashion, the higher levels of trace mineral intake, including copper, from the chicory plots could have resulted in the elevated level of plasma pepsinogen observed.

The observed higher lamb growth rate for HP ewes suggests that maternal protein supplementation has a positive effect on lamb performance. The results also show that lamb performance can be further improved by grazing chicory, irrespective of earlier maternal protein nutrition. This was observed at a level where chicory represented about 50% (on DM basis) in the sward. The hypothesized interaction effects between these two feeding treatments on lamb performance and FEC did, however, not occur. The reason for this may have been that the treatments were too separated in time to result in such an interaction. Furthermore, it has to be noted these effects were observed under this low level of representation of chicory in the sward, and the effects could have been bigger if the proportion of chicory had been higher. Thus, increased maternal protein nutrition and subsequent grazing

on chicory have the potential to finish lambs earlier in the season. The lower level of FEC and pasture larvae count from chicory plots suggest a reduced infection risk for lambs in the subsequent season. If such a combined use of alternative approaches could lead to reduced parasitism and improved performance over several seasons needs further study.

CHAPTER FIVE

Combined effects of maternal protein nutrition and grazing on initially clean chicory (C*ichorium intybus*) on sheep parasitism and performance

Abstract

The effects of maternal metabolizable protein nutrition and grazing on a bioactive forage (chicory) were investigated on ewe and lamb performance and parasitic status. Thirty-six Scottish Blackface ewes either protein supplemented (high protein=HP) or not (low protein=LP) with their twin lambs were turned out onto either chicory or grass/clover pasture in a 2 X 2 factorial design 32 days post-lambing. The ewes were trickle infected with an abomasal nematode parasite Teladorsagia circumcincta at a dose of 10,000 L_3 once a week until weaning. It was hypothesized that the effects of ewe protein supplementation were expected to improve ewe performance and reduce PPRI and improve lamb resilience through increased milk production. It was further hypothesized that grazing on chicory would improve animal performance and reduce parasitism but these effects would be more pronounced in the LP ewes and their lambs. Ewe performance was improved by HP feeding and chicory grazing in an additive manner. This improved body weight of ewes was accompanied by increased body condition score at weaning. Similarly, lamb body weight was improved by maternal HP feeding and ewe and lamb grazing on chicory relative to grass/clover independent of each other. Ewe FEC remained very low and was not affected by protein supplementation and forage type. Lamb FEC was reduced through out the experiment whilst grazing on chicory compared to grazing on grass/clover but not affected by maternal protein nutrition. The results support the view that the use of chicory can improve animal productivity and reduce parasitism in lambs. The reduced FEC whilst grazing on chicory at the early stage of turnout of parasite naïve lambs suggests that part of this effect could have been through some anti-parasitic effects. In conclusion, chicory can be included in a sustainable parasite control strategy, as part of an integrated approach, in small ruminant production systems.

Key words: *chicory; FEC; body weight; lamb performance*

5.1 Introduction

Currently nematode parasites show resistance towards all classes of anthelmintics (Jackson and Coop, 2000) and, hence, novel alternative approaches are needed to reduce the degree of reliance on anthelmintics and thereby slow the rate of evolution of anthelmintic resistance (Athanasiadou *et al.*, 2008; Gill and LeJambre, 1996; Kaplan, 2004).

Protein supplementation can reduce faecal egg count (FEC) in periparturient ewes, thus reducing the level of risk of infection for naïve lambs at the start of their first grazing season (Houdijk *et al.*, 2006). Similarly, grazing on chicory, compared to other forages, reduces FEC (Athanasiadou *et al.*, 2007) and worm burden (Tzamaloukas *et al.*, 2005), and improves lamb performance (Athanasiadou *et al.*, 2007; Komolong *et al.*, 1992). To date these approaches have been investigated independently. The strategic combination of such alternative approaches for synergism in parasite control and improved production has, however, not yet been explored in detail (Athanasiadou *et al.*, 2008). In earlier work of this project (Kidane *et al.*, 2008), periparturient maternal protein nutrition followed by grazing chicory post-weaning independently resulted in improved lamb performance and reduced parasitism, but no interaction was observed between the two effects. It was then suggested that the lack of interaction effects between the two alternative approaches could have been due to the departure in time between the approaches. The present experiment was, therefore, aimed at assessing the combined effects maternal protein nutrition and concurrent grazing of ewes and their lambs on initially parasite clean chicory relative to grass/clover on animal performance and parasitic status.

It was hypothesized that the beneficial effects of maternal protein nutrition on ewe PPRI would reduce pasture contamination and improve lamb performance. It was further hypothesised that grazing on chicory compared to grass/clover would improve performance and reduce parasitism in ewes and lambs. These effects were expected through the antiparasitic-like effects (Tzamaloukas *et al.*, 2005) and nutritional qualities (Komolong *et al.*, 1992) of chicory. Over all, the expected effects of grazing chicory would be higher for ewes and lambs from the protein non-supplemented group and this was anticipated to be observed by the interaction effect between the two alternative approaches.

5.2 Materials and Methods

The combined effects of forage type and maternal protein nutrition on sheep performance and parasitism are reported from the actual grazing experiment. However, ewes were experimentally infected and adapted to the maternal protein nutrition treatment prior to turn out as summarized below.

5.2.1 Animals and pre-grazing history

Thirty-six twin-rearing Scottish Blackface ewes, all 4-5 years old, were used in the experiment. Ewes and their lambs came from a pen experiment where they received treatments detailed in ***Chapter Three***. In brief, ewes were trickle infected with an abomasal nematode parasite, *T. circumcincta*, at a dose of 10,000 L_3 three times a week for 70 days. During the last 24 days of pregnancy and the first 32 days of lactation all ewes were fed 1/3 medium quality hay and 2/3 concentrate diet. During this time, ewes were fed at 0.9 times their assumed metabolizable energy requirement, and either 0.8 (LP; n=18) or 1.3 (HP; n=18) times their estimated metabolizable protein (MP) requirement (AFRC, 1993). For ingredients and chemical composition of the diets, see **Table 3.1.**

5.2.2 Infection

The ewes continued to be infected with *T. circumcincta* at a dose of 10,000 L_3 once a week for 28 days from turnout (day_0). The expectation was that by day_{28} post-turnout the nematode eggs seeded by ewes should have come

through as L_3 for self infection (which was indeed confirmed later by the FEC of initially parasite naïve lambs). The *T. circumcincta* strain used was Moredun Ovine Susceptible Isolate that has been maintained in the laboratory for several years (Houdijk *et al.*, 2006).

5.2.3 Grazing

At 32 days post-lambing, the ewes and their lambs were turned out onto experimental plots. In the experimental plots, animals grazed either chicory cv. Puna II (*Cichorium intybus* L.) or grass/clover (*Lolium perenne* : *Trifolium repnes;* commercial blend) sown with an 80:20 mix, 6 plots for each species (3 ewes with their twin lambs in a plot with an area of 0.24 ha), balanced for ewe FEC, lamb body weight (BW) and ewe BW with decreasing order of importance. Whilst grazing, HP ewes were offered ~350g soypass (for chemical composition, see **Table 5.1**) per ewe per day until day_{70}. In a previous pen experiment (Kidane *et al.*, 2009), this level of soypass supplementation on a basal diet had increased the MP intake of twin-rearing ewes from 0.8 to 1.3 times the estimated MP intake. LP ewes were not offered any additional supplement. Lambs were weaned on day_{70} (at a mean age of 102 days) and continued grazing on the experimental plots until day_{132}. Water was supplied *ad libitum* to the animals with an automatic trough in each plot.

5.2.4 Measurements

Animal measurements. Lamb and ewe BW was assessed once every week until weaning and then after fortnightly for lambs. The body condition score (BCS) of the ewes was assessed regularly, by lumbar palpation on a 0 to 5 scale, and to an accuracy of a quarter, as described in Russel (1984) as a subjective indicator of the degree of back-fat.

Faecal egg count [FEC expressed in eggs per gram fresh faeces (epg)] was assessed weekly in ewes (from day_0 until day_{42}) and fortnightly in lambs (from day_{28} until day_{132}). The FEC of ewes was not done after day_{42} as the overall FEC was extremely low regardless of treatment group. A modified flotation methodology was used to perform the FEC, (Christie and Jackson, 1982).

Blood samples were taken from the jugular vein of the lambs into heparinised vacutainers on day_{28} (lambs actively started grazing), day_{56} (mid-way to weaning), day_{70} (weaning point), day_{105} (mid-way between weaning and end of the experiment) and day_{132} (end point). The plasma was separated by centrifuging for 15 min at 1340 x *g* at 4°C and stored at –20°C pending analyses. The plasma samples were later analyzed for urea (*m*mol/L), pepsinogen (expressed in *IU**1000 per liter as *m*U/L) and albumin (g/L) concentration. Plasma pepsinogen would reflect abomasal damage/permeability (Murrell *et al.*, 1989; Nogareda *et al.*, 2006; Yakoob *et al.*, 1983) whereas albumin and urea would reflect level of protein nutrition.

Herbage measurements. Herbage availability was monitored by measuring sward heights per plot every 21 days (25 measurements per plot) and a sward height of 4 cm above ground level was taken as minimum target.

Herbage samples were collected from the experimental plots (on day0) for chemical analyses. Samples were collected from 6 randomized areas of each plot by walking in a 'W' transect and cutting at height of ~4 cm above ground level to simulate grazing (Wright *et al.*, 2006). The material cut from each randomized area was labeled and maintained in a plastic bag until processed within 2h. Each sample (6 per plot) was divided in to 2 equal parts. The first half was pooled and sub-samples were taken for chemical analyses. The remaining 6 halves per plot were used to determine the botanical composition of the pastures and were manually separated in to *'sown species', 'seed bank species' and 'dead matter'* (debris). These samples were then dried at 100^{0}C to a constant weight to determine DM content. Samples for chemical analyses were dried at a temperature of 60^{0}C for 48hrs. The latter were milled to pass through 1mm mesh size. Results obtained were expressed on a DM basis.

The concentration of specific minerals was determined by inductively coupled plasma–atomic emission spectrometry (ICP-AES) using Perkin Elmer OES Optima 4300DV. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using the FOSS FiberCap System in accordance with Van Soest (1964). Feed total nitrogen was determined by Kjeldahl technique according to AOAC (1980) and per cent crude protein (CP) was calculated as N x 6.25. Acid hydrolysable ether extract (AHEE) was determined using MAFF (1993). In vivo digestibility was estimated by neutral detergent cellulase and gamannase digestibility (NCGD) using the method of Dowman (1993). Metabolizable energy (ME; expressed in MJ/kg DM) content of feed samples was predicted using the NCGD and AHEE values (Thomas *et al.*, 1988).

Botanical composition was determined on samples to estimate the proportion of sown species relative to dead matter and seed bank species (see *Chapter Four* for details of the latter).

5.2.5 Statistical analyses

Data collected over the experimental days for individual ewes and lambs were re-arranged from calendar date to days relative to entry into experimental plots to account for small variation in achieved lamb age at turnout. Sward height and botanical proportions were compared using descriptive statistics.

FEC and BW of lambs and ewes were analyzed using Restricted Maximum Likelihood (REML) in repeated measures analysis of variance (ANOVA) with power model (city-block metric) for correlation within subject across time which assumes a tailing-off correlation between repeated measurements as time points between the measurements get larger. Nutrient content of forages grazed, ADG of lambs and final BWs of lambs and ewes were analysed with general ANOVA. FEC was transformed via log(FEC+1) prior to statistical analysis and back-transformed means with 95% confidence interval (CI) were reported. Statistical analyses were performed using GenStat 11 (VSN International Ltd., 2008) using a plot as a block. For all measurements appropriate covariates were used when existed and contributed significantly to the model.

5.3 Results

5.3.1 Sward measurements

Sward heights measured on day_0 were (mean ± SEM) 26.5 ± 1.2 cm and 29.3 ± 0.8 cm, for chicory and grass/clover plots, respectively. Mid-way through grazing half of each chicory and grass/clover plots was topped off with a tractor mounted mower at 10 cm height to initiate regrowth whilst ewes and lambs were grazing on the intact part. This was done because the amount of DM on offer was higher than expected and swards showed a tendency to bolt whilst being grazed.

Sward botanical composition (expressed in % on DM basis) is presented in **Figure 5.1**. Sward botanical composition analysis showed both chicory and grass/clover plots represented more than 94 per cent of the sown species. Details on chemical composition of the chicory and grass/clover plots are presented in **Table 5.1**. Chicory has a lower DM content than grass/clover. However, the concentration of most of the nutrients, macro- and trace minerals analysed was higher in its DM content compared to grass/clover. Chicory had also significantly higher CP, NCGD and calculated level of ME content than grass/clover.

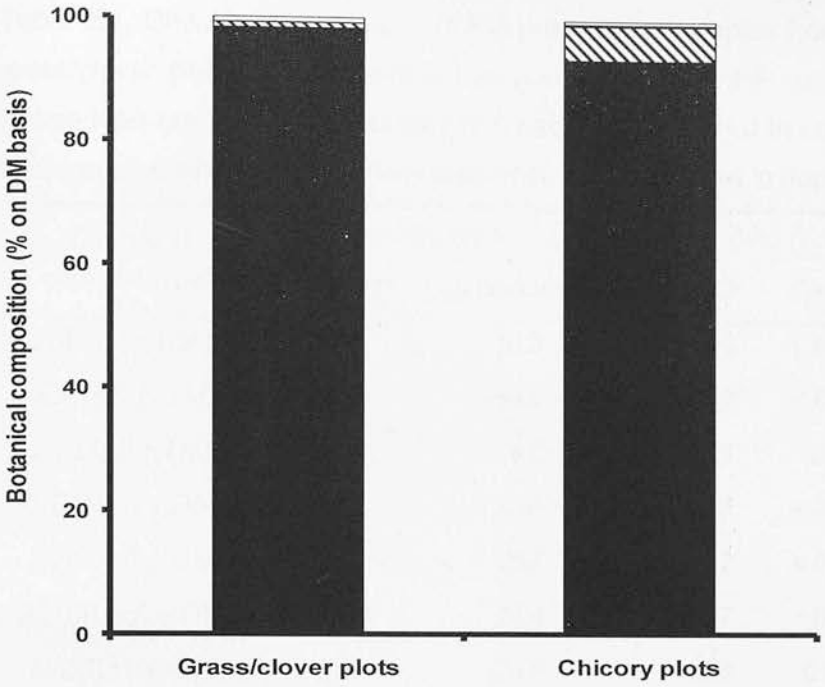


Figure 5.1 Sward botanical composition (% on DM basis) of sown species (■), seedbank species (▨) and dead matter (□) taken on day_0 of the experiment

Table 5.1. Chemical composition of the pre-grazing samples from the chicory and grass/clover plots, and soypass fed as a supplement to HP ewes. Values for the forage type are from a sample size of 6 each and analyzed in duplicates. Soypass was sampled while weighing daily allowances and analyzed in duplicates.

Chemical composition[§]	Forage type		ANOVA		Soypass
	chicory	grass/clover	SED	P-value	
DM (g/kg fresh)	100	213	*4.44*	*< 0.001*	937
CP (g/kg DM)	176	94.0	*6.39*	*< 0.001*	501
Ash (g/kg DM)	126	66.2	*3.75*	*< 0.001*	67.9
NDF (g/kg DM)	284	430	*23.3*	*< 0.001*	256
ADF (g/kg DM)	202	257	*8.72*	*< 0.001*	60.8
AHEE (g/kg DM)	38.6	24.4	*1.97*	*< 0.001*	29.3
NCGD (% DM)	81.7	76.6	*1.73*	*0.017*	86.3
ME (MJ/kg DM)	12.3	11.3	*0.25*	*0.003*	12.8
Macro-minerals (g/kg DM)					
Ca	11.7	4.33	*0.45*	*< 0.001*	4.74
Na	5.89	1.62	*0.56*	*< 0.001*	0.05
Mg	3.04	1.41	*0.09*	*< 0.001*	4.05
K	35.3	20.0	*1.65*	*< 0.001*	25.5
P	5.43	2.84	*0.16*	*< 0.001*	8.65
S	3.54	1.78	*0.08*	*< 0.001*	4.78
Micro-minerals (mg/kg DM)					
Zn	83.0	22.1	*2.30*	*< 0.001*	125
B	30.8	4.81	*0.83*	*< 0.001*	46.2
Cu	12.7	4.36	*0.36*	*< 0.001*	18.4
Mn	145	148	*9.64*	*0.788*	122
Fe	116	55.0	*6.14*	*< 0.001*	233

[§] based on wet chemistry;

Soypass=Xylose treated soybean meal; CP=crude protein (calculated as N X 6.25); NDF=neutral detergent fibre; ADF=Acid detergent fibre; AHEE=acid hydrolysable ether extract; NCGD=neutral detergent cellulase plus gamannase digestibility; ME (MJ/kg DM) calculated as 0.014*NCGD (g/kg DM) + 0.025*AHEE (g/kg DM) (Thomas *et al.*, 1988)

5.3.2 Animal performance

Ewe body weight and BCS. Ewe BW during the experiment is presented in **Figure 5.2a-b**. Over the 70 days of grazing chicory and grass/clover ewe body development was not affected by the three-way interaction effect of time, protein nutrition and forage type (*P=0.26*), the two-way interaction effects of time and protein nutrition (*P=0.99*) and protein nutrition and forage type (*P=0.84*). However, ewe BW was affected by interaction effect of time and forage type (*P=0.014*). As such, it was improved for the first 14 days upon turnout and diverged for the rest of the experimental time with ewes grazing grass/clover losing weight whilst ewes grazing on chicory maintaining their BW. The effect of protein nutrition on ewe BW development tended to be significant (*P=0.09*) with HP ewes remaining heavier than their LP counterparts starting from day$_{14}$ to weaning.

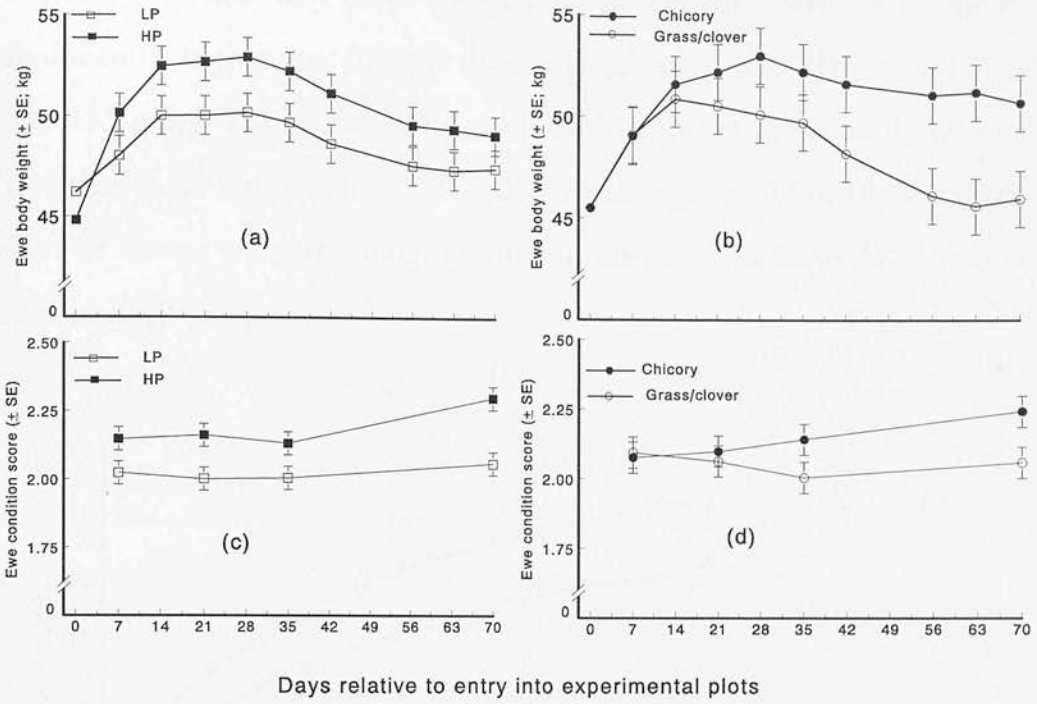


Figure 5.2. Body weight of ewes as affected by **(a)** MP feeding and **(b)** forage type; and BCS of ewes as affected by **(c)** protein nutrition, and **(d)** forage type

Ewe BCS (see **Figure 5.2c-d**) showed a tendency for improvement whilst grazing on chicory compared to grass/clover plots (*P= 0.093*). There was also a tendency for an interaction effect of time and forage type (*P=0.090*) on ewe BCS. Furthermore, protein supplemented ewes had better BCS than non-supplemented ewes (*P=0.026*). All other interaction effects were not significant.

Lamb body weight. Lamb BW is presented in **Figure 5.3**. Lamb BW was affected by the three-way interaction of time, maternal protein nutrition and forage type (*P=0.004*). However, the interaction effect of maternal protein nutrition and forage type on lamb BW was not significant (*P=0.94*). Time interacted with both maternal protein nutrition (*P<0.001*) and forage type

(*P<0.001*) for lamb BW. Over the grazing period, the ADG of lambs was significantly higher on chicory than grass/clover plots (155 *vs.* 117 g/d; SED=13.2 g/d; *P<0.020*). The ADG of LP lambs was not significantly different from that of HP lambs (126 *vs.* 146 g/d; SED=13.0 g/d; *P=0.15*). The interaction effect of forage type and maternal protein nutrition on lamb ADG was not significant (*P=0.94*).

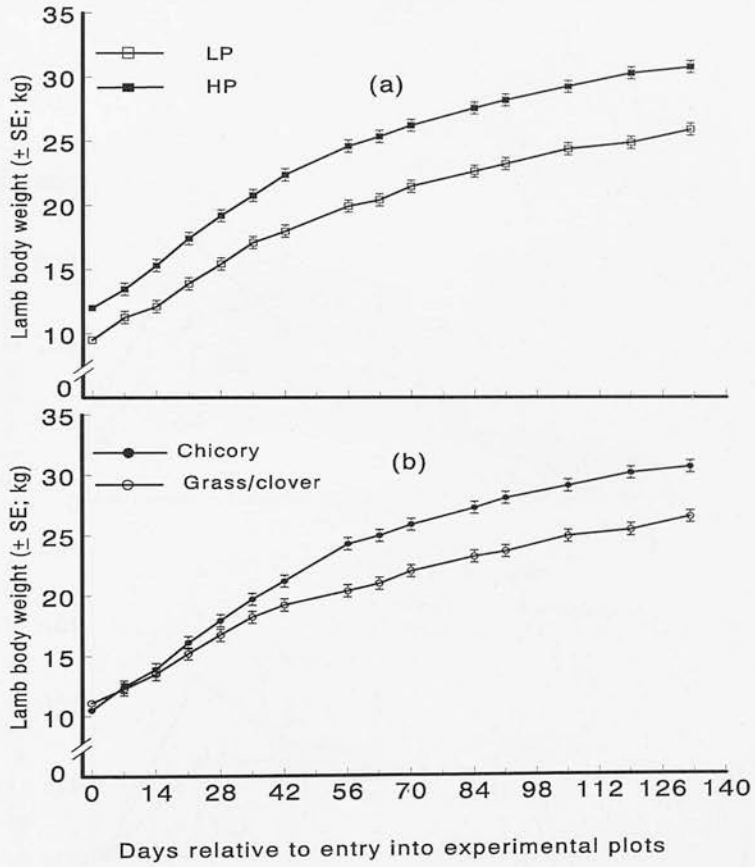


Figure 5.3 Body weight of lambs as affected by **(a)** maternal protein nutrition, and **(b)** forage type

5.3.3 FEC and plasma constituents

Ewe FEC. Backtransformed mean (±95% CI) FEC from ewes is presented in Figure 5.4. During grazing ewe FEC was affected by time (*P<0.001*). As such, the overall mean FEC fell sharply from ~270 epg on day$_7$ to ~55 epg on day$_{42}$. There was not any significant effect of protein nutrition (*P=0.79*) and forage type (*P=0.94*) on ewe FEC. All the interaction effects of the factors involved were also not significant on ewe FEC.

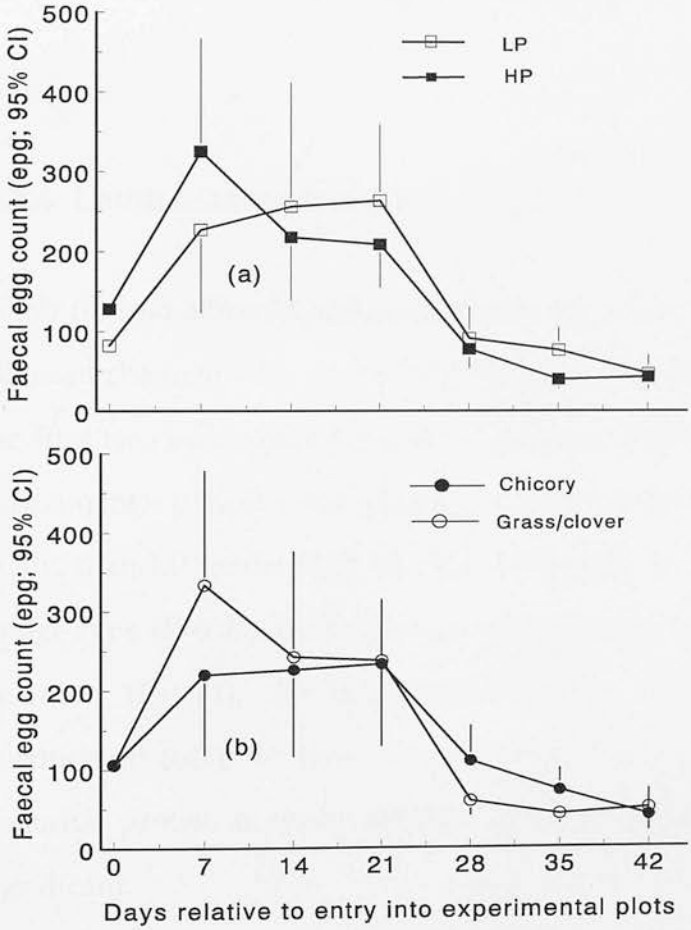


Figure 5.4 Back-transformed mean (± 95% CI) faecal egg count of ewes as affected by: **(a)** protein nutrition, and **(b)** forage type

Lamb FEC. FEC of lambs is presented in **Figure 5.5a-b**. Lambs showed a clear patent infection on day$_{28}$ as expected. FEC remained relatively low until day$_{70}$ and increased with time (*P<0.001*). The three way (time, maternal protein nutrition and forage type) and two-way (maternal protein nutrition and forage type) interaction effects (*P=0.88*) and the effect of maternal protein nutrition (*P=0.13*) on lamb FEC were not significant. The effect of forage type on lamb FEC was highly significant (*P<0.001*). However, forage type tended to interact with time (*P=0.07*) suggesting that the difference in FEC between lambs grazing on chicory and grass/clover diverged with time.

5.3.4 Lamb plasma analysis

Lamb plasma albumin and plasma urea are presented in **Table 5.2**. Plasma albumin changed with time (P<0.001). As such, it showed an increment over the first two measurements (until day$_{56}$) and declined afterwards. Over the experimental period, mean plasma albumin concentration was higher for HP lambs than LP lambs (32.2 vs. 30.3; SED=0.269 g/L; *P<0.001*). The effects of forage type (*P=0.85*), the interaction effects forage type and maternal protein nutrition (*P=0.21*), the interaction effect of time and maternal protein nutrition (*P=0.45*), the three way interaction effects of time, forage type and maternal protein nutrition (P=0.65) on lamb plasma albumin were all not significant.

Plasma urea level tended to be affected by the three way interaction effect of time, maternal MP level and forage type (*P=0.064*). Except for the effect of forage type (*P=0.59*), all other effects including first order interactions were highly significant (*P<0.001*) on plasma urea of lambs.

Plasma pepsinogen is presented in **Figure 5.5c-d**. Plasma pepsinogen changed significantly with time (*P<0.001*). As such, it increased for all treatment groups until weaning, and then after decreased to the end of the experiment. Overall, LP lambs tended to have higher mean plasma pepsinogen than HP lambs (819 vs. 741; SED=28.5 mU/L; *P=0.055*). All other effects including first and second order interactions were not significant (*P>0.15*) on lamb plasma pepsinogen concentration.

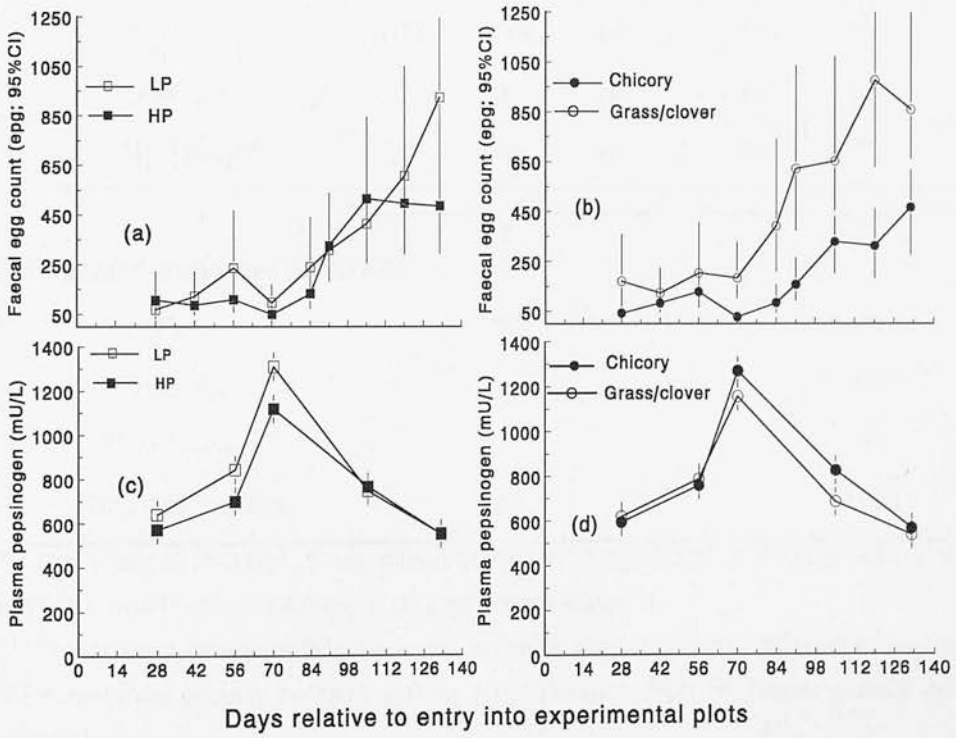


Figure 5.5 Back-transformed mean faecal egg count of lambs (± 95% CI) as affected by: **(a)** maternal protein nutrition, and **(b)** forage type; and mean plasma pepsinogen (± SE) of lambs as affected by **(c)** maternal protein nutrition, and **(d)** forage type

Table 5.2 Plasma albumin and plasma urea of lambs reared by ewes either supplemented with soypass (HP) or not (LP) and grazed either chicory or grass/clover over a period of 132 days

Treatment	Plasma albumin (g/L)			Plasma urea (mmol/L)		
	Day_{28}	Day_{70}	Day_{132}	Day_{28}	Day_{70}	Day_{132}
LP-Chicory	29.2	32.2	26.6	3.56	5.83[a]	6.40
LP-Grass/clover	27.1	32.3	30.1	3.22	7.19[b]	8.22
HP-Chicory	31.8	34.1	29.0	5.48	8.73[c]	7.79
HP-Grass/clover	29.3	34.3	29.6	4.91	7.69[b]	7.81
Average SE	1.23	0.87	1.76	0.288	0.611	0.692
ANOVA†						
MP	0.092	0.056	ns	***	***	ns
Forage	0.099	ns	ns	ns	ns	ns
MP *Forage	ns	ns	ns	ns	**	ns
Repeated measures ANOVA‡						
Time		***			***	
Time *MP		ns			***	
Time*Forage		*			***	
Time*MP*Forage		ns			0.064	

*** significant at P<0.001; ** significant at P<0.01; * significant at P<0.05; NS not significant at P=0.1; and P values between 0.05 and 0.1 are stated

[a,b,c,d] means in a column with different superscripts are significantly different at P=0.05;

MP= maternal protein nutrition (LP or HP); Forage= type of forage grazed (chicory or grass/clover)

†ANOVA at specific time points as repeated measures ANOVA showed interactions with time

‡ Repeated measures ANOVA using samples collected on days 28, 56, 70, 105 and 132 post-turnout

5.4 Discussion

In this experiment the combined effects of maternal protein nutrition with concurrent grazing on chicory relative to grass/clover were compared with the objective of assessing the added benefits of alternative approaches on ewe and lamb performance and parasitic status. It was hypothesized that ewe protein supplementation would improve ewe performance and reduce the extent of PPRI (Coop and Kyriazakis, 1999). The latter is expected to reduce pasture contamination which would further reduce lamb parasitism and improve lamb growth (Coop *et al.*, 1982; Gibson and Everett, 1973). It was also hypothesized that grazing on chicory would improve animal performance and reduce parasitism but these effects would be more pronounced in the LP ewes and their lambs as this group could benefit more from the antiparasitic effects and nutritional qualities of chicory.

5.4.1 Sward characterization

Forage chicory has shown better nutritional quality compared to grass/clover. This was also observed in other experiments; see review by Barry (1998) and reports of Sanderson *et al* (2003). The lower levels of structural components (NDF and ADF), the higher NCGD, ME and CP content of the forage in particular, and the relatively higher levels of micro- and macro-nutrients compared to grass/clover could result in improved performance of animals. These are essential for balanced animal nutrition and make chicory a good candidate for mixing with other conventional forages (Belesky *et al.*, 2001).

5.4.2 Animal parasitism

Whilst grazing, protein supplementation to ewes did not affect FEC. Indeed, the starting FEC at 32 days post-lambing was low and similar between LP and HP ewes. Furthermore, the overall FEC level was reduced to fairly low levels on day$_{42}$, as a result of which FEC was not done afterwards for ewes. This lack of effect of protein supplementation on ewe FEC at the observed low level of FEC suggests that the nutrient intake of ewes from grazing at LP group could have been not limiting; and under the latter condition ewes are not expected to lose their acquired immunity (Coop and Kyriazakis, 1999). The overall low level of FEC may suggest the degree of resistance of the breed to the level of infection (Abbott *et al.*, 1985; Wallace *et al.*, 1996; Zaralis *et al.*, 2009). However, the latter seems to be more obvious as the performance of the ewes, in terms BW and condition score and lamb performance, was affected in the LP group compared to the HP group. Because of this early phase similarity in FEC between LP and HP groups, it is expected that the degree of pasture contamination by ewes from both protein nutrition groups would have been similar and, this would have created similar level of infection for lambs regardless of maternal MP level unless otherwise FEC from the lambs themselves have created differences in the level of pasture infectivity as time went on.

Ewes grazing on chicory, with an already established *T. circumcincta* population did not show a reduction in their FEC compared to grass/clover. Similar justification would hold true here as given for the protein nutrition above, that the nutrient supply from grass/clover might have not been critically limiting for lactating ewes. This is further supported by the sharply falling FEC of both groups of ewes from day$_{28}$ post-turnout. Indeed, the latter

was expected as lambs would have partly shifted to grazing for additional nutrient intake, in addition to suckling. This shift was evident from the patent infection of lambs from grazing, as was seen from the modest level of FEC at this time (see **Figure 5.5**). The former, therefore, had lead to the very low FEC of ewes (mean ~50 epg on day42) and the subtle anti-parasitic effects of chicory could have been masked. Previous work by Athanasiadou *et al* (2007) also indicated the absence of effects of chicory on ewe FEC when the ewes were turned out onto the pasture with an already established nematode parasite infection, mainly *T. circumcincta*.

Lambs showed clear patent infection from grazing and, as such, showed moderate level of FEC on 28 days post-turnout. This was also expected considering the patency period, which is 17 – 21 days, of *T. circumcincta* (Wood *et al.*, 1995). In a similar fashion to that of the ewes, lamb FEC was not affected by their maternal protein nutrition. However, lambs would not have yet developed an acquired immunity at this early age (Smith *et al.*, 1985a), and hence improved maternal milk production was not expected to reduce lamb FEC but to improve lamb resilience (Coop and Kyriazakis, 1999). The above authors suggested that under nutrient scarce situation acquisition of immunity takes priority over growth, whereas during the expression of immunity, immunity gets penalised. This implies that changes in scarce nutrient supply would be expected to affect faecal eggs counts (FEC) and worm burdens only during the phase of expression of immunity.

Lambs grazing on chicory had lower FEC compared to grass/clover throughout the experiment. The results support previous evidence on the anti-parasitic effects of chicory on growing lambs infected with *T. circumcincta* from grazing (Athanasiadou *et al.*, 2007; Kidane *et al.*, 2008). The

variety of chicory used was bred for increased sesquiterpene lactones (Rumball *et al.*, 2003) and the latter are suggested to possess antiparasitic activities (Barrera *et al.*, 2008; Molan *et al.*, 2003) among other biological activities (Picman, 1986). Because lambs used in this experiment were parasite naïve when turned out onto the experimental plots the observed early effects of chicory on lamb FEC partly support the presence of anthelmintic effects of chicory because at this time the differences in the nutritional qualities of the grazed forages were not expected to affect either FEC or worm burden (Coop and Kyriazakis, 1999).

Furthermore, it must also be noted that, all others being equal between chicory and grass/clover, the effects of chicory in reducing larvae survival/migration to the leaf lamina (Marley *et al.*, 2006a) might have contributed the lower initial FEC of lambs. However, in the latter part of the grazing period the effects of forage type on FEC became even stronger (strong tendency for time X forage type interaction) probably suggesting the development of acquired immunity with time and also due to the diverging difference in the lamb FEC which intrun may have contributed to the difference in pasture larave. What mechanism operated could have been partly known if animals were slaughtered for worm recovery and quantification. Furthermore, other works (Marley *et al.*, 2006c; Marley *et al.*, 2006a) indicated that the type of forage could affect larvae development, survival and migration and hence effects of these sort could also account for reduced degree of exposure of lambs to infective larvae whilst grazing chicory (Athanasiadou *et al.*, 2007).

5.4.3 Animal performance

Both ewe and lamb performance was improved by ewe protein supplementation under continuous gastrointestinal nematode parasite challenge. This supports the view that improved nutrition probably leads to improved resilience (Coop and Kyriazakis, 1999), although strictly speaking, a conclusion can not be drawn on resilience without including non-parasitised controls in the experiments

Ewe and lamb performance was also improved by grazing on chicory compared to grass/clover. Such improvement in animal performance when grazing on chicory compared to other conventional forages was also reported by Athanasiadou (2007) and Komolong *et al*, (1992). Ewes finished the experiment with not only improved BW but also with better BCS. These performance parameters are positively correlated with improved reproductive parameters, such as superior conception rates in sheep (Oldham and Thompson, 2004).

The improved performance of ewes and their lambs grazing on chicory compared to the grass/clover plots is primarily attributed to the nutritional superiority of chicory as could be seen from the chemical composition. This was evident from the lower level of structural components and higher levels of ME, CP, micro- and macro-nutrients in chicory compared to grass/clover plots. Such a higher crude protein content and digestible energy yield could usually result in higher DM intakes from forage in sheep (ARC, 1980; Blaxter, 1989) and hence could justify part of the improvement in animal performance. In addition to this, the improvement in performance of ewes and lambs whilst grazing on chicory could have been because of the

apparently reduced parasitism. This is evident from the observed level of lamb FEC, which by itself is an indication of current level of parasitism of lambs and also indication of the concurrent cumulative build up of parasites eggs, and associated potential infective larvae, in the pasture till the end of the experiment. All lambs completed their grazing period on the same experimental field and, therefore, it is reasonable to assume that the degree of exposure of lambs to self-infection relates to the level of nematode egg excretion, the latter being lower for lambing grazing on chicory. Improvements in performance of ewes and lambs were also observed whilst using different grazing strategies that resulted in different levels of pasture contamination (Bailey *et al.*, 2009). In addition, gastrointestinal parasitism is often associated with reduced feed intake (Coop and Angus, 1981). However, to what extent this reduction in feed intake from grazing occurred and as a result contributed to the differences in performance of ewes and lambs between the treatment groups was not clear. In a grazing experiment, where ewes were daily trickle infected with *4000 T. circumcincta* infective larvae, and managed to avoid auto-and cross infection from grazing, Sykes and Juma (1984) observed reduction in feed intake (12%) compared to controls, which was also associated with reduced milk production and lamb growth.

The original hypothesis that lambs and ewes would accrue extra benefit from combining the maternal protein nutrition and grazing on chicory vs. grass/clover in terms reduced parasitism and improved performance was not observed in this study. This suggests that the combined effect of the alternative approaches is as good as the added effects of each individual approach. In previous studies where protein supplementation and quebracho tannin feeding were combined in anticipation that the effects on the approaches on parasitism to be on different aspects of defence to

parasites, no added or interactive effects were observed in lambs infected with intestinal parasite *Trichostrongylus colubriformis* (Butter *et al.*, 2000). The lack of any interactive effects in the present study may have been attributed to the fact that the ewes were already 5 weeks post-parturient by the time grazing started probably missing the peak periparturient increase in the FEC which has been shown to start from around 4–6 weeks prior to parturition and to last 6–8 weeks into lactation (Barger, 1993). It could also be that the antiparaitic effects might have been subtle and/or HP may have not been more than adequate.

The current ever increasing awareness of public health, increasing share in and demand for organic system of production (Helga *et al.*, 2008), sustained global nematode parasite challenge and emerging anthelmintic resistance for the existing class of drugs necessitate the need for options that could reduce the degree of use of anthelmintics without compromising animal productivity and welfare. The combination of the alternative approaches described above produced an outcome without any extra benefit of the combination but with the individual effects still maintained. Further work with the alternative approaches targeting at the time immediately pre- and post-lambing would provide an added knowledge on if any extra benefit could be accrued from combination of the alternative approaches.

Implications. The ewe and lamb performance data and the FEC of lambs while grazing on chicory strongly support the view that the use of chicory in a grazing system can maintain satisfactory levels of productivity, relative to grazing on grass/clover, whilst reducing reliance on anthelmintics especially if the latter is informed through increased FEC and impaired performance.

CHAPTER SIX

The combined effects of maternal protein nutrition and chicory (*Cichorium intybus*) grazing on sheep performance, parasitism and lamb drench requirement on *Teladorsagia circumcincta* infested pasture

Abstract

Thirty-six, 4 to 5 years old, twin rearing Scottish Mule ewes fed either protein supplemented (high protein; HP) or not (low protein; LP) with their twin lambs (Suffolk crosses) were turned out onto a Teladorsagia circumcincta infested chicory or grass/clover pasture, at 32 days post-parturition, in a 2 X 2 factorial design. Ewes grazed the plots for 70 days whilst lambs finished the experiment at 139 post-turnout. Since parasitized ewes and parasite naïve lambs were exposed to the already existing infection from the contaminated pasture, the initial effects of ewe supplementation were expected to be on (1) ewe performance and PPRI and (2) lamb resilience through increased milk production. Grazing ewes and lambs on chicory was further expected to improve animal performance and reduce parasitism but these effects were expected to be larger for protein non-supplemented ewes and their lambs. Ewe performance was improved by HP feeding and chicory grazing in an additive manner. This improvement was further accompanied by improved body condition score. Similarly, at the end of the experiment, lambs reared by HP ewes and lambs grazing on chicory were 20 and 25% heavier than lambs reared by LP ewes and lambs grazing on grass/clover, respectively. Ewe FEC was reduced by protein supplementation and the overall FEC was reduced to very low levels after 56 days of turnout. The effect of forage type on ewe FEC was not significant. Lamb FEC was biased by the involvement of strategic anthelmintic "rescue" drench. Maternal protein supplementation reduced this drench requirement to 69% relative to lambs coming from un-supplemented ewes. Similarly, grazing lambs on chicory reduced lamb drench requirement to 60% relative to lambs grazing on grass/clover. This extra drench required by lambs reared by LP ewes and lambs grazing on grass/clover did not restore weight gain and final body weight of lambs to their treatment contrast groups. In terms of days on grazing, this difference in the lamb final body weight showed a lag period of about 70 and 90 days in the LP and grass/clover lambs

compared to their respective treatment groups. The results clearly suggest that the combination of these alternative approaches along with strategic use of conventional drugs could improve animal health and productivity. Secondly, the reduced selective and targeted use of anthelmintics in the presence of the alternative approaches, in the long-run, can reduce the degree of evolution of anthelmintic resistance by nematode parasites.

***Key words**: metabolizable protein; faecal egg count; body weight; lamb growth; lamb drench requirement*

6.1 Introduction

The development of anthelmintic resistance by parasites is highly associated to the frequency of the use of anthelmintics in the treatment of parasitized animals (Barton, 1983; Martin *et al.*, 1984). However, the use of anthelmintics has been extensive so as to maintain animal productivity and prevent mortalities (Gill and LeJambre, 1996). This is increasingly less sustainable in the face of the evolution of drug resistance in parasite populations (Jackson, 1993; Prichard, 1990). The latter necessitates reduction in the treatment frequency and involvement of additional novel non-chemical methods of worm control strategies in order to slow down the spread of resistance and maintain animal productivity (Athanasiadou *et al.*, 2008; Gill and LeJambre, 1996; Kaplan, 2004; Silvestre *et al.*, 2002; Waller, 1997).

Reduction of the use of anthelmintics without adversely affecting animal productivity may safely be achieved if the degree of parasite exposure is reduced, especially for susceptible classes of animals. Metabolizable protein (MP) supplementation used during periparturient period has reduced faecal egg count (FEC) in parasitized ewes (Houdijk *et al.*, 2006), which implies a reduced pasture contamination for their parasite naïve lambs. Grazing on chicory has reduced lamb FEC (Athanasiadou *et al.*, 2007), worm burden (Athanasiadou *et al.*, 2007; Marley *et al.*, 2003b; Tzamaloukas *et al.*, 2005) and improves lamb performance (Athanasiadou *et al.*, 2007; Komolong *et al.*, 1992) when compared to other conventional forages.

In the experiment reported earlier (***Chapter Four***), where the above alternative approaches were combined in a sequential manner, periparturient maternal protein supplementation and finishing lambs on chicory improved

lamb performance and reduced parasitism in an additive fashion. However, it was questioned if these results would replicate when: (1) both these approaches were applied at the same time, (2) ewes and their lambs were turned onto initially parasite infested pasture, and (3) lamb exposure to parasites was prolonged. Therefore, the objective of this experiment was to assess the combined effects of periparturient ewe protein nutrition and grazing ewes and lambs on chicory compared to grass/clover on: (1) ewe and lamb performance, (2) ewe and lamb parasitism and (3) lamb drench requirement on an already infested pasture.

6.2 Materials and Methods

6.2.1 Animals and pre-grazing history

Thirty-six Mule ewes (Scottish Blackface X Bluefaced Leicester), all four to five years old, with their twin lambs (Suffolk cross) were used in the experiment. Ewes and their lambs came from a pen experiment. During the pen experiment, all ewes were fed 1/3 medium quality hay and 2/3 concentrates for the last 24 days of pregnancy and the first 32 days of lactation. During this time, ewes were fed at 0.9 times their assumed metabolizable energy requirement, and either 0.8 (LP; n=18) or 1.3 (HP; n=18) times their estimated MP requirement (AFRC, 1993).

As the ewes were 4 to 5 years old and previously grazed on natural pastures, they were expected to have substantial prior exposure to gastrointestinal nematode parasites through grazing. Therefore, they were treated at housing on day$_{-56}$ with levamisole (Levacide 3%; Norbrook Laboratories, GB Limited) and ivermectin (Oramec, Merial, Harlow, UK) at the rate of 7.5 and 0.2 mg/kg body weight (BW), respectively, to clear any resident worms. They were trickle infected with the abomasal nematode parasite *T. circumcincta* at a dose of 10,000 third stage infective larvae (L_3) three days a week (on Mon-Wed-Fri basis) from 41 days before to 32 days after lambing. The *T. circumcincta* used was Moredun ovine susceptible isolate that has been maintained in the laboratory for several years and passed one generation through parasite naïve donor lambs. Whilst grazing, ewes and lambs were exposed to an already infected pasture from previous year grazing by sheep infected mainly with an abomasal parasite *T. circumcincta* and no further artificial

infection was expected to be needed. [This was later confirmed by the level of initial FEC of lambs on day$_{28}$ post-turnout].

6.2.2 Grazing, supplementation and lamb drenching

Thirty-two days post-lambing, the ewes with their lambs were turned out onto experimental plots. In the experimental plots, animals grazed either chicory cv. Puna II, *(Cichorium intybus)* or a pasture sown with 80:20 grass : clover mixture (*Lolium perenne/Trifolium repnes*), 6 plots each forage type (3 ewes with their twin lambs in a plot), maintaining maternal LP and HP nutrition, initiated during housing. The chicory and grass/clover plots did not differ in their starting pasture strongyle larvae count (mean 13,967 *vs* 15,042; SED 5230; *P=0.465*) in the respective arder. The LP and HP feeding treatments were achieved through leaving LP ewes non-supplemented on pasture, whilst HP ewes were offered ~420 g of xylose-treated soybean meal (Soypass) per ewe per day for about 70 days supplied on a feed trough that was raised from the ground level to restrict access for lambs. This amount of supplementation was used based on the estimation that was used for housed ewes (Kidane *et al.*, 2009) where such a quantity of Soypass on top of a low protein basal diet increased the MP intake of twin-rearing ewes from 0.8 to nearly 1.3 times their estimated MP requirement calculated according to AFRC (1993).

Once lambs started grazing and the self-infection became patent, they were monitored for any clinical signs of nematodiasis. Lambs were given a rescue drench with levamisole (Levacide 3%; Norbrook Laboratories, GB Limited) at the recommended dose of 7.5 mg levamisole hydrochloride per kg BW when

they showed diarrhoea (breech soiling), a negative BW gain or stasis over weekly measurement and/or high FEC (equalling or in excess of 1000 epg). The number of drenches given to the lambs was recorded to calculate individual drench requirement as total number of drenches used per lamb throughout the experiment. Lambs were weaned on day$_{70}$ post-turnout and continued to graze the experimental plots until day$_{139}$. Each group was grazed on an experimental plot with an area of 0.24 ha. Water was supplied *ad libitum* to all animals. Animals were checked visually at least twice daily for any clinical signs of parasitism.

6.2.3 Measurements

Animal measurements. Lamb and ewe BW was assessed once every week until weaning. Afterwards, lambs were weighed fortnightly. The body condition of the ewes was scored regularly, by lumbar palpation on a 0 to 5 scale, and to an accuracy of a quarter, as described in Russel (1984).

Faecal egg count (FEC) expressed in eggs per gram fresh faeces (epg) was assessed weekly in ewes and fortnightly in lambs. A modified flotation methodology was used to perform the FEC (Christie and Jackson, 1982) with the use of polyallomer centrifuge tubes to collect the nematode eggs from the meniscus.

Blood samples were taken from the jugular vein of the lambs into heparinised vacutainers on the first day of FEC, 14 days before weaning, at weaning, 42 days post weaning and at the end of the experiment. The plasma was separated by centrifuging for 15 min at 1340 X *g* at 4^{0}C and stored at –

20^0C pending analyses. The plasma samples were later analyzed for plasma urea (*m*mol/L), plasma pepsinogen (expressed in *IU**1000 per liter as *mU/L*) and plasma albumin (g/L) concentration. Plasma pepsinogen was expected to show the level of infection and hence an associated abomasal permeability (Kidane *et al.*, 2009; Valderrabano *et al.*, 2002); plasma albumin and plasma urea were expected to reflect achieved level of protein nutrition.

Herbage measurements. Herbage availability was monitored by measuring sward heights in each plot using the Hill Farm Research Organization (HFRO) sward stick (Murphy *et al.*, 1995). Herbage samples were collected from the plots (on day_0) for chemical analyses. Samples were collected from 6 randomized areas of each plot by walking in a '*W*' transect and cutting at height of ~4 cm above ground level to simulate grazing (Wright *et al.*, 2006). The material cut from each randomized area of a plot was labeled and maintained in a plastic bag until processed within 2h. Each of the 6 samples per plot was partitioned into 2 equal parts. The first half was pooled per plot and sub-samples were taken for chemical analyses. The remaining 6 halves per plot were used to determine the botanical composition of the pastures and were manually ramified in to *'sown species'*, *'seed bank species'* and *'dead matter'* (debris). These samples were then dried at 100^0C to a constant weight to determine DM content. Samples for chemical analyses were dried at a temperature of 60^0C for 48hr. The later samples were milled to pass through 1mm mesh size. Results obtained were expressed on a DM basis.

At about mid-way through grazing half of both chicory and grass/clover plots were topped-off with a tractor mounted mower at about 10 cm from the ground level to remove the reproductive stem and initiate regrowth whilst ewes and lambs in each plot were grazing on the intact part. The plots were

not fertilized in the current growing/grazing season.

The concentration of specific minerals was determined by inductively coupled plasma–atomic emission spectrometry (ICP-AES) using Perkin Elmer OES Optima 4300DV. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using the FOSS FiberCap System in accordance with Van Soest and Wine (1967). Total nitrogen was determined by Kjeldahl technique according to AOAC (1980) and % crude protein (CP) was calculated as N x 6.25. Acid hydrolysable ether extract (AHEE) was determined using MAFF (1993). *In vivo* digestibility was estimated by *in vitro* neutral detergent cellulase and gamannase digestibility (NCGD) using the method of Dowman (1993). Metabolizable energy content (ME; expressed in MJ/kg DM) was predicted using the NCGD and AHEE values (Thomas *et al.*, 1988).

6.2.4 Statistical analyses

Data collected over the experimental days for individual ewes and lambs were re-arranged from calendar date to days relative to entry into experimental plots (day_0). For sward measurements (sward height and botanical proportions) descriptive statistics was used. FEC and BW of lambs and ewes were analyzed using Restricted Maximum Likelihood (REML) in repeated measures analysis of variance (ANOVA) with power model (city-block metric) for correlation within subject across time which assumes a tailing-off correlation between repeated measurements as distance between time points gets larger. Lamb drench requirement was square root transformed to normalize variance and analysed with general ANOVA.

Nutrient content of the forage types grazed, ADG of lambs and final BW of lambs and ewes were analysed with general ANOVA. FEC was transformed via log(FEC+1) prior to statistical analysis and back-transformed means (with 95% confidence interval, CI) were reported. [For FEC from lambs that were not drenched at all, only arithmetic mean was presented, see **Figure 6.8,** as the data showed inconsistency in terms of animal numbers and drop-outs due to drench involvement]. Statistical analyses were performed using GenStat 11 (VSN International Ltd., 2008) using a plot as a block. For all measurements appropriate covariates were used when existed and contributed significantly to the model.

In almost all statistical analyses performed, no significant interaction effects were observed between the main factors (i.e., MP level and forage type) and, therefore, only the effects of the main factors are presented in the graphs for this chapter.

6.3 Results

6.3.1 Sward characterization

Sward botanical composition (% on DM basis) is presented in **Figure 6.1**. On measurements taken on day$_0$, mean (± SEM) sward height from chicory and grass/clover plots were 14.4 ± 2.5 cm and 24.7 ± 1.9 cm, respectively.

Sward botanical composition analysis showed chicory and grass/clover plots represented more than 80 and 94 % of the sown species in their respective plots. Seed bank species, (which consisted mainly of annual ryegrass and a very small proportion Chickweed and Thistle) and debris were recovered in small quantities.

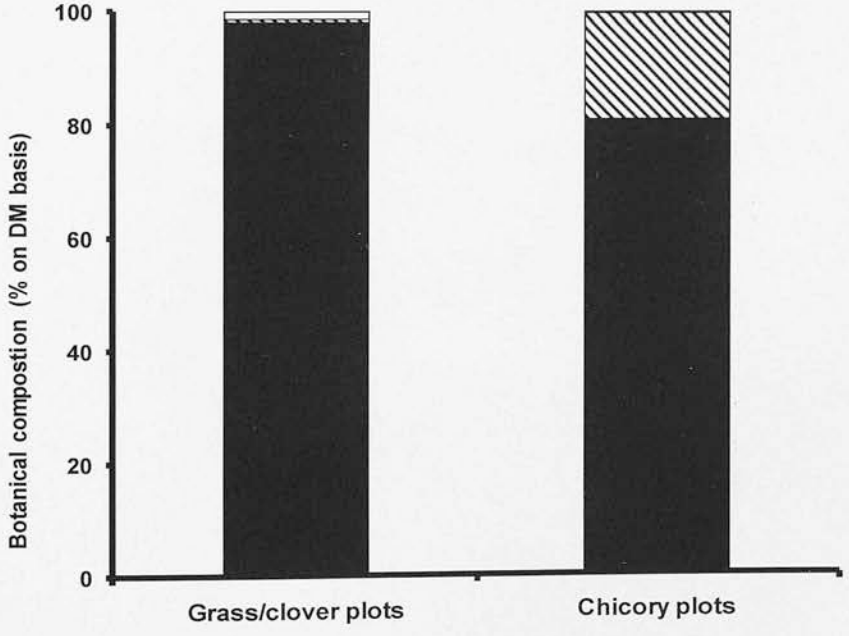


Figure 6.1 Sward botanical composition (% on DM basis) of sown species (■), seed bank species (▨) and dead matter (□) taken on day$_0$ of the experiment

Chemical composition of representative samples collected from the chicory and grass/clover plots is presented in **Table 6.1.** Chicory plots had higher CP, ME, macro- and micro-minerals contents than grass/clover plots. Furthermore, chicory plots had significantly lower NDF and ADF contents than grass/clover plots. However, chicory plots had significantly lower DM content compared to samples from grass/clover plots.

Table 6.1. Chemical composition of the pre-grazing samples from the chicory and grass/clover plots, and soypass fed as a supplement to HP ewes. Values for the forage type are from a sample size of 6 each and analyzed in duplicates. Soypass was sampled while weighing daily allowances and analyzed in duplicates.

Chemical composition§	Forage type		ANOVA		Soypass
	chicory	grass/clover	SED	P-value	
DM (g/kg fresh)	111	220	*4.55*	*<0.001*	937
CP (g/kg DM)	229	89.5	*7.26*	*<0.001*	501
Ash (g/kg DM)	106	54.8	*3.82*	*<0.001*	67.9
NDF (g/kg DM)	270	419	*14.8*	*<0.001*	256
ADF (g/kg DM)	211	244	*9.78*	*0.007*	60.8
AHEE (g/kg DM)	45.5	26.6	*1.44*	*<0.001*	29.3
NCGD (% DM)	82.0	80.7	*1.20*	*0.30*	86.3
ME (MJ/kg DM)	12.6	12.0	*0.20*	*0.009*	12.8
Macro-minerals (g/kg DM)					
Ca	8.92	4.71	*0.58*	*<0.001*	4.74
Na	4.99	2.36	*0.61*	*0.002*	0.05
Mg	2.68	1.40	*0.20*	*<0.001*	4.05
K	30.8	16.6	*1.35*	*<0.001*	25.5
P	5.23	2.84	*0.18*	*<0.001*	8.65
S	3.50	1.61	*0.17*	*<0.001*	4.78
Micro-minerals (mg/kg DM)					
Zn	80.6	20.5	*4.10*	*<0.001*	125
B	24.8	4.95	*0.80*	*<0.001*	46.2
Cu	12.1	6.00	*2.60*	*0.042*	18.4
Mn	125	142	*10.7*	*0.136*	122
Fe	220	64.0	*24.6*	*<0.001*	233

§ based on wet chemistry;

Soypass=Xylose treated soybean meal; CP=crude protein (calculated as N X 6.25); NDF=neutral detergent fibre; ADF=Acid detergent fibre; AHEE=acid hydrolysable ether extract; NCGD=neutral detergent cellulase plus gamannase digestibility; ME (MJ/kg DM) calculated as 0.014*NCGD (g/kg DM) + 0.025*AHEE (g/kg DM) (Thomas *et al.*, 1988)

6.3.2 Animal performance

Ewe body weight and BCS. Ewe BW development during the experiment is presented in **Figure 6.2a-b.** Over the grazing period, ewe body development was not significantly affected by the interaction effect between time, protein nutrition and forage type (*P=0.97*), between time and protein nutrition (*P=0.13*), between time and forage type (P=0.48) and between protein nutrition and forage type (*P=0.27*). The effect of protein nutrition on ewe BW was significant (*P<0.001*) with HP ewes remaining heavier than their LP counterparts starting from day$_{14}$ to weaning. Similarly, ewes grazing on chicory were significantly *(P=0.011)* heavier than ewes grazing on grass/clover starting from day$_{42}$ to weaning.

Ewe BCS is presented in **Figure 6.2c-d**. Body condition score was also significantly higher (*P=0.001*) for HP ewes than LP ewes. Similarly, grazing ewes on chicory improved BCS (*P=0.027*) compared grazing to grazing on grass/clover. The interaction effect of protein nutrition and forage type (*P=0.47*), and the interaction effect of time, protein nutrition and forage type (*P=0.53*) were not significant. With time, ewe BCS changed significantly (*P<0.001*) and this was observed as an improvement for HP ewes (time X MP level; *P=0.053*) and chicory ewes (time X forage type; *P=0.019*).

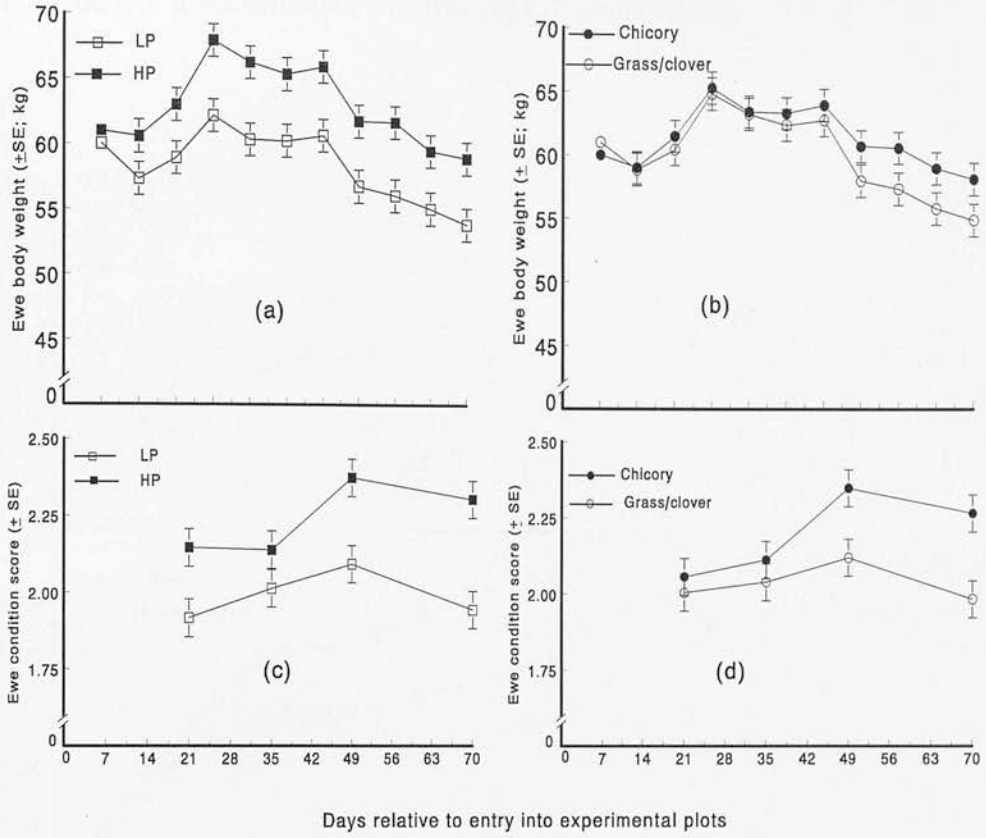


Figure 6.2. Body weight of ewes as affected by **(a)** MP level and **(b)** forage type; and BCS of ewes as affected by **(c)** MP level and **(d)** forage type

Lamb body weight. Lamb BW development is presented in **Figure 6.3**. Lamb BW was not affected by the interaction effect of time, maternal MP level and forage type (*P=0.71*) and maternal MP level and forage type (*P=0.95*). Time interacted with both maternal MP level (*P<0.001*) and forage type (*P<0.001*) for lamb BW. As such, starting from day28 post-turnout, the difference in BW of lambs grazing chicory *vs.* grass/clover, and lambs from LP *vs.* HP ewes diverged. Over the grazing period the ADG of lambs was higher on chicory than grass/clover plots (170.2 *vs* 102.3 g/d; SED=9.50 g/d; *P<0.001*). This tended to be higher for lambs reared by HP ewes than LP ewes (147.6 *vs.* 128.2 g/d; SED 9.38 g/d; *P=0.072*). The interaction effect of forage type and

maternal MP level on ADG was not significant (*P=0.59*).

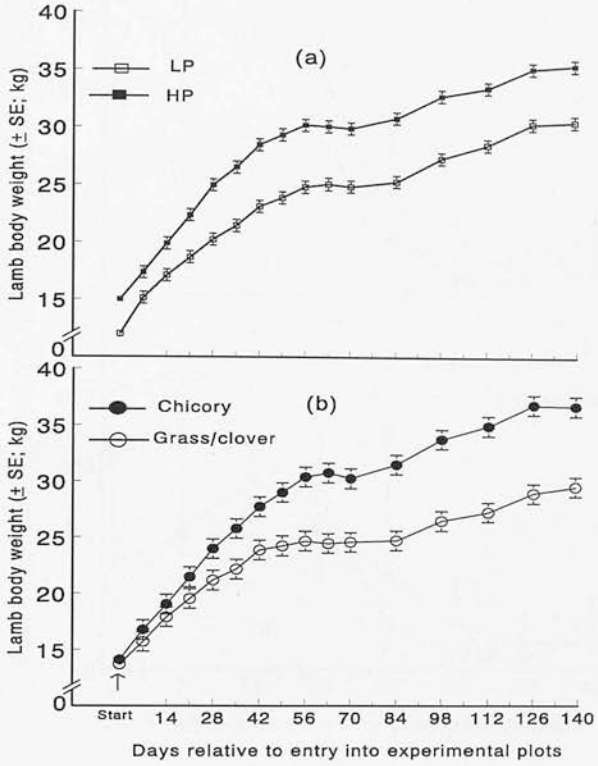


Figure 6.3 Body weight of lambs as affected by (**a**) maternal MP level and (**b**) forage type

6.3.3 FEC, plasma constituents, and lamb drench requirement

Ewe FEC and plasma pepsinogen. During grazing ewe FEC significantly changed with time (*P<0.001*). Faecal egg count were lower under MP supplementation (*P < 0.001*) but the effects of MP level were dependent on time with a significant time and MP level interaction effect (*P=0.020*). The mean FEC peaked on day14 for both groups of ewes (**Figure 6.4**). The effect of forage type (*P=0.14*), the interaction effect of time with forage type (*P=0.08*) and MP level with forage type (*P=0.82*) and the three-way interaction effect

of time with MP level and forage type (*P=0.46*) were all not significant.

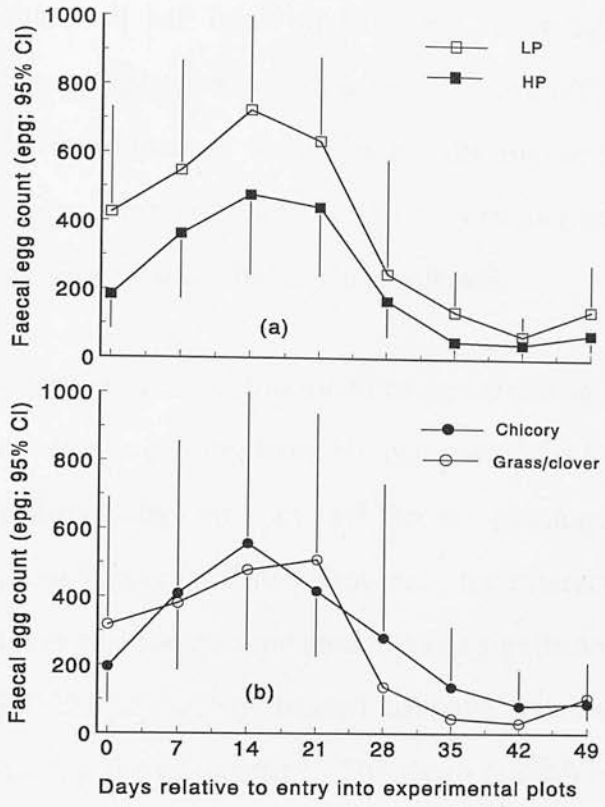


Figure 6.4 Back-transformed mean (± 95% CI) faecal egg count of ewes as affected by: **(a)** MP level and **(b)** forage type

Mean plasma pepsinogen taken on day$_{14}$ was higher for LP ewes than HP ewes (1622 vs. 1072 mU/L; SED 251 mU/L; *P=0.022*). The effects of forage type (*P=0.91*) and the interaction effect of forge type and MP level (*P=0.91*) were not significant.

Lamb FEC and drench requirement. The FEC of lambs is presented in **Figure 6.5a-b**. Lambs showed a clear patent infection from grazing on day$_{28}$. The FEC remained low until day$_{70}$ and increased with time (*P<0.001*). The three way interaction effect of time X maternal MP level X forage type

(P=0.74) and two-way interaction effect of maternal MP and forage type (P=0.65) on lamb FEC were not significant. The effects of forage type and maternal MP level on lamb FEC were not significant (*P>0.10*). However, forage type interacted with time (*P<0.001*). As such, FEC for grass/clover lambs remained were numerically higher than that of chicory lambs until day112. However, for the last two measurements FEC of grass/clover lambs was lower than that of chicory lambs.

Lamb drench requirement, as presented in **Figure 6.6**, was reduced by 31% for lambs coming from HP compared to LP ewes (*P=0.013*). Similarly, it was reduced by 40% in the group grazing on chicory compared to the grass/clover (*P=0.001*). However, the interaction effect between maternal MP level and forage type tended to be significant (*P=0.054*) such that lambs from HP-chicory group tended to have the lowest mean drench requirement during the experiment. The mean (±SEM) numbers of drenches received by lambs starting from the lowest were 0.65 ±0.17 for HP-chicory, 1.59 ±0.23 for LP-chicory, 1.76±0.16 for HP-grass/clover and 1.94±0.25 for LP-grass/clover.

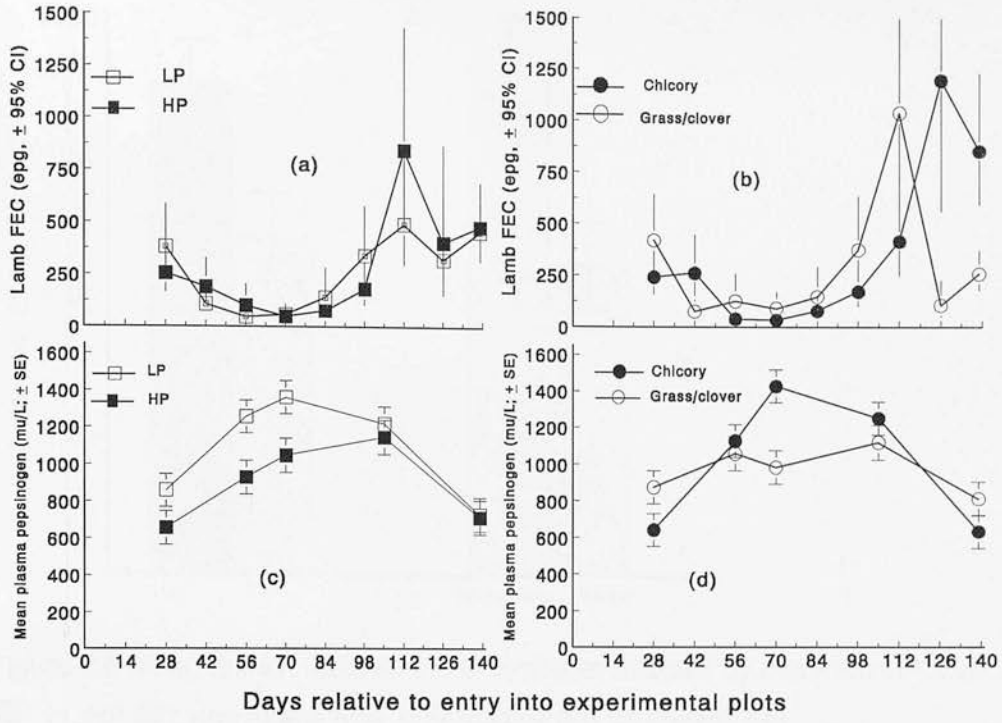


Figure 6.5 Mean faecal egg count of lambs as affected by: **(a)** maternal MP level and **(b)** forage type; mean plasma pepsinogen of lambs as affected by: **(c)** maternal MP level and **(d)** forage type

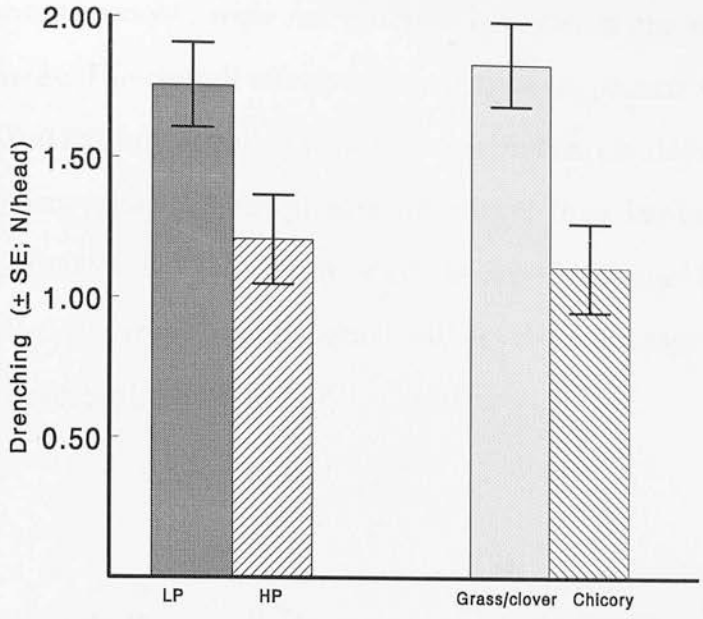


Figure 6.6 Mean drench requirement of lambs as affected by maternal MP level: LP (■) *vs.* HP (▨); and forage type: grass/clover (□) *vs.* chicory (▧)

Lamb plasma constituents. Lamb plasma albumin changed with time (*P<0.001*). There was a significant interaction effect of time and maternal MP level (*P=0.022*), and a tendency for time and forage interaction (*P=0.07*) on lamb plasma albumin (see **Figure 6.7**). As such, on day$_{28}$ plasma albumin was higher for lambs grazing chicory and also lambs coming from HP ewes. However, these differences disappeared on day$_{56}$, and peaked for all treatment groups on day$_{70}$. The interaction effect of maternal MP level and forage type (*P=0.93*) and that of time, maternal MP level and forage type (*P=0.53*) were not significant.

Similarly, lamb plasma urea increased with time (*P< 0.001*) with a significant time and maternal MP level (*P<0.001*) and time and forage (*P=0.033*) interaction effects. Thus, lambs reared by HP ewes had higher plasma urea concentration than lambs reared by LP-ewes up until day$_{70}$. The latter two

measurements were not different between lambs reared by the LP and HP ewes. The overall effects of forage type on plasma urea were not significant (*P=0.86*) but at the end of the experiment, on day$_{139}$, lambs that grazed on chicory had higher plasma urea level than lambs grazed on grass/clover (*P<0.001*). The interaction effects between maternal MP level and forage type (*P=0.70*) and time, maternal MP level and forage type (*P=0.27*) were not significant.

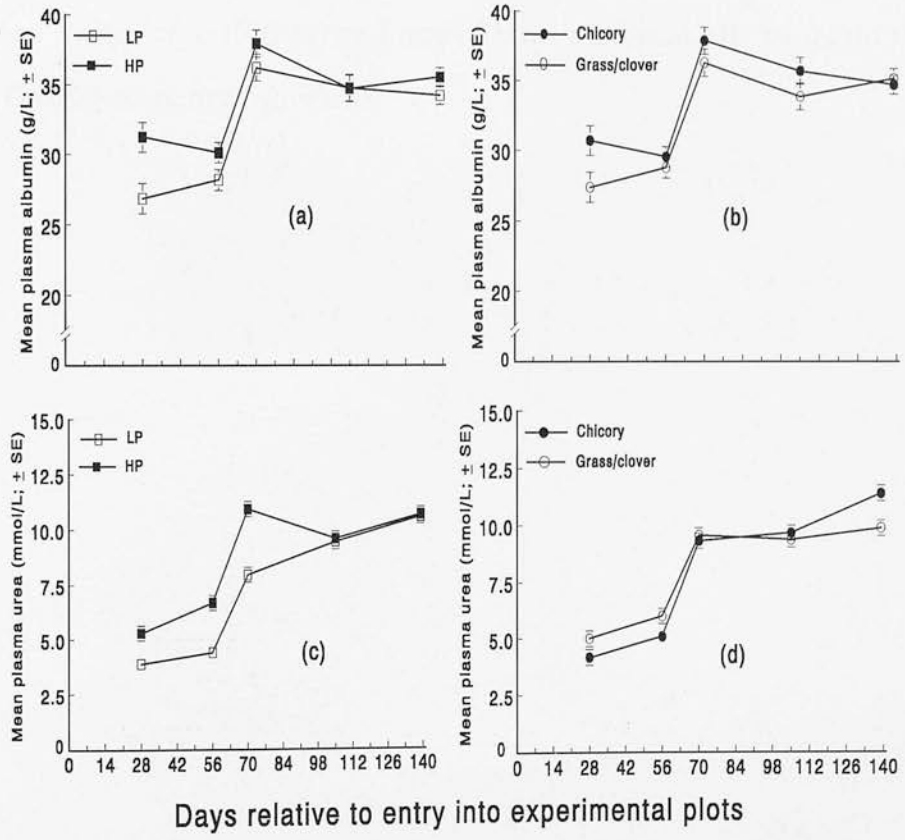


Figure 6.7 Plasma albumin of lambs as affected by: **(a)** maternal MP level and **(b)** forage type; and plasma urea as affected by **(c)** maternal MP level and **(d)** forage type

Plasma pepsinogen, as presented in **Figure 6.5c-d**, changed significantly with time (*P<0.001*). The effect of maternal MP level on lamb plasma pepsinogen concentration was dependent on time (*P<0.001*). Thus, lambs from LP ewes had consistently higher level of plasma pepsinogen, which was significantly higher until weaning. Similarly, the effect of forage on lamb plasma pepsinogen depended on time with significant interaction effect (*P=0.002*) and, as such, lambs grazing chicory showed strong temporal change in plasma pepsinogen whilst the apparent change for lambs grazing on grass/clover was minimal. The interaction effects between maternal MP level and forage type (*P=0.81*) and that of time, maternal MP level and forage type (*P=0.83*) were not significant.

6.4 Discussion

In this experiment, the combined effects of maternal MP feeding with ewe and lamb grazing on chicory *vs.* grass/clover were investigated when both the ewes and their twin lambs were turned out onto a mainly *T. circumcincta* infested pasture. Lambs coming from the two maternal MP feeding backgrounds grazed either chicory or grass/clover plots and monitored for performance and parasitism. As ewes and parasite naïve lambs were exposed to the already existing infection from the contaminated pasture, the initial effects of ewe supplementation were expected to (1) improve ewe performance, (2) reduce the degree of PPRI and (3) improve lamb resilience through increased ewe milk production. Consequently, the reduced PPRI form supplemented ewes could have led to reduced pasture contamination, in addition to the existing contamination, for the lambs following the supplemented ewes. It was further expected that grazing ewes and lambs on chicory relative to grass/clover would reduce the effects of parasitism, drench requirement and improve performance in lambs mainly but these effects would be more pronounced for lambs from the LP ewes as these could have been under restricted nutrient supply. Here the findings are discussed in relation to the current knowledge on the use and effects of the above alternative approaches.

6.4.1 Sward characterization

Sward botanical composition analysis showed that chicory and grass/clover plots represented more than 80 and 94 % of the sown species in their respective plots. This was an improvement in contrast to the proportion obtained in the first year of establishment where chicory and grass/clover plots had nearly 50 and 90 % of the sown species, respectively, at maximum (Kidane *et al.*, 2010a). This could probably be due to the dominance of annual weeds in refugia in the first year which could have been reversed by a well established sown species in the second year. Annual weeds usually grow up quickly in the spring and are more of a problem in establishing pastures, but are said to be rarely a problem once the sown pasture is established (OMAFRA Staff, 2000). Increased representation of chicory in the stand over years was also reported by Hume *et al.* (1995) for different mixtures with other conventional forages under sheep grazing conditions. Furthermore, the observed low level of seed bank species was likely to have very limited effects and these effects, if existed, would have not been different between the grazing groups.

Chemical composition of the forages compared here indicated that chicory had higher concentration on most of the desirable nutrients compared to grass/clover. This better nutritional quality was consistent and in agreement with the results reported earlier (see ***Chapters Four and Five***) and also reported in other works (Belesky *et al.*, 2001), where chicory was compared to other forages for its mineral composition.

6.4.2 Animal parasitism

FEC and plasma pepsinogen concentration were used as a proxy to indicate the level of parasitism. Upon turnout, the FEC of ewes increased over a period of 21 days and then declined dramatically. The observed increased FEC from LP ewes compared to the HP ewes at the early stages of grazing was accompanied by significantly elevated level of plasma pepsinogen on day_{14} suggesting lower worm burden (Murrell *et al.*, 1989) in the later group. The eventual fall of FEC of ewes was expected and can be primarily attributed to the degree of ease on nutrient demand by the ewes (Houdijk *et al.*, 2001b) as lambs shifted more to grazing which was evident with lamb patent self-infection during this time (see **Figure 6.5**). In a pen experiment, such a reduction in nutrient demand brought about by removing a lamb from twin-rearing ewes had resulted in reduced FEC within days of the experiment (Houdijk *et al.*, 2006). The elevated level of FEC for the LP ewes at the early stages of grazing, therefore, suggests that indeed twin-rearing ewes were under nutrient scarcity whilst grazing which is in agreement with the view of Sykes and Kyriazakis (2007). This could have created differences in the amount of infective larvae between the forage types and hence between the grazing groups.

The lack of effect of chicory on the ewe overall FEC and plasma pepsinogen (day_{14}) was not expected. In a grazing experiment designed to assess the effects grazing a bioactive forage on the different stages of the parasite with lambs, Tzamaloukas *et al.*(2005) reported reduction in worm burden in the group that grazed chicory. In the latter work, parasite naive, 3-month old, grazing lambs were artificially infected with 8000 infective larvae of *T. circumcincta* on day one of the experiment and on 21 days post-infection

lambs were allocated to either onto chicory or the control grass/clover forage. On day 35 lambs were killed for worm recovery to investigate the effects of forages on the viability of adult worms in the lambs. Indeed, the ewes used here had previous exposure to nematode parasites and were also trickle infected for about 10 weeks when in fact in the cited work lambs were parasite naïve at start and had just two infections. The observation reported here is in agreement to the reports of Athanasiadou *et al.* (2007) where grazing ewes with an already established nematode parasite (mainly *T. circumcincta*) infection on chicory compared to grass/clover did not affect FEC. Indeed, chicory ewes likely had lower levels of larval intake based on lower pasture larval count and chicory structure (Kidane *et al.*, 2010a; Marley *et al.*, 2006a). However, the observation of Kidane *et al* (2009) would suggest that variation in larvae intake may not necessarily correlate with variation in FEC in these partially immune animals. Thus, an absence of FEC in ewes may not necessarily mean similar levels of ewe parasitism.

In lambs FEC was used as one of the parameters used for rescue drenching. Therefore, effects of maternal protein nutrition and forage type on FEC were biased by the drench involvement. Maternal protein supplementation had no effect on lamb FEC. As FEC is positively skewed (Barger, 1985) drenching few animals with higher FEC in a group could have affected the overall image of the data as observed in other works (Cringoli *et al.*, 2009). Therefore, it can not be discounted that lambs grazing on grass/clover might have carried higher worm burden than chicory lambs even in the absence of difference in FEC. The lack of difference in FEC of lambs reared by LP and HP ewes could be mainly due to the increased rescue drenching in the LP group (*see next section*). However, from the higher early FEC of LP ewes, elevated plasma pepsinogen of lambs reared by LP ewes and reduced ewe

and lamb performance of the group it can suggested that, indeed, the lambs reared by the LP ewes had higher level of infection (Coop *et al.*, 1982). Furthermore, it was observed (see ***Chapter Four***) that chicory plots had lower larvae counts than grass/clover plots after being grazed for a similar period by ewes and lambs that had similar FEC at the start of grazing.

To assess how drench involvement affected the trend in lamb FEC, data from those lambs that did not require any drench, and those that required but data collected until the first drench was given to a lamb is plotted in **Figure 6.8**. It must be noted that this does not, in any way, represent the true picture of FEC as in the complete absence of drench. The reason is that a drench would likely be given for a lamb that is either not resistant and/or resilient. Both of these parameters were used in the drench criteria of this experiment and, therefore, FEC of lambs here is still biased and would look like a selected group of resistant/resilient lambs. Yet, it is clear from the graph that FEC data without drench involvement appears higher for lambs grazing grass/clover compared to lambs grazing chicory. By day 112 of the experiment every lamb grazing grass/clover received at least once drench and hence FEC is not indicated in the graph.

However, in the whole data, i.e., data including drenched lambs, similar trend was observed except the last two weeks of the experiment where the FEC of the lambs from grass/clover plots remained lower than that of lambs from chicory. The latter was due to the fact that all lambs in the grass/clover were drenched at least once by this time.

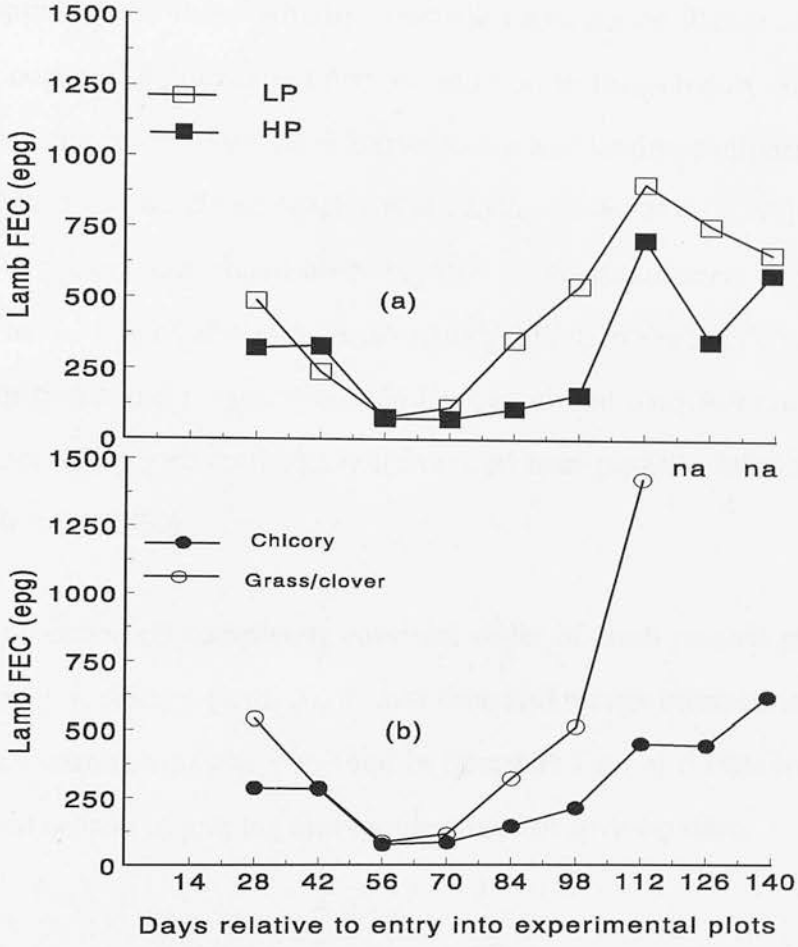


Figure 6.8 Mean faecal egg count from lambs until the first drench was give and/or from lambs that were not drenched at all as affected by: **(a)** maternal MP level and **(b)** forage type [**na** *stands for data not available as all lambs in the grass/clover plots were drenched at least once by this time*]

Overall, the low level of FEC of lambs in the chicory plots agrees to previous related work where lamb FEC was reduced by grazing on chicory compared to other forage species (Athanasiadou *et al.*, 2007). This further supports the

reported anthelmintic-like effects of chicory (Tzamaloukas, 2006) as the cultivar of chicory used here was bred for increased sesquiterpene lactones (Rumball *et al.*, 2003). The difference in the nutritional quality of the forages grazed would not be expected to have an effect on the establishment of parasites on these initially parasite naïve lambs (Coop and Holmes, 1996; Coop and Kyriazakis, 1999). In addition to this, chicory might have limited the migration of infective larvae to the leaf lamina compared to grass/clover (Marley *et al.*, 2006c; Marley *et al.*, 2006a; Scales *et al.*, 1995) and hence lambs on chicory may have been exposed to lower infective larvae (see ***Chapter Four***). Lastly, the forages do widely differ in their CP, macro- and micro-nutrients and it may very well be argued that over extended grazing period such difference could have influenced host-parasite relationship (Coop and Holmes, 1996).

The observed completely reversed order of lamb plasma pepsinogen whilst grazing chicory (with significant time and forage interaction; see **Figure 6.5c**) at weaning was also observed in ***Chapters Four*** and ***Five*** with lambs at their first season of grazing and requires further investigation.

6.4.3 Lamb drench requirement

The frequency of drench between individual lambs and grazing groups differed during the grazing period. As such, lambs from HP ewes required 31% less drench compared to lambs from LP ewes over the grazing period. Similarly, this drench reduction was 40% for lambs grazing on chicory compared to grass/clover. This could be due to improved resilience of the lambs from HP ewes and also lambs grazing on chicory as improved host

nutrition was expected to lead to improved resilience (Coop and Kyriazakis, 1999) at this early age and also resilience was one of the drench triggers. Furthermore, it could also be that the anthelmintic-like effects of chicory could have played a role in the latter (Tzamaloukas *et al.*, 2005) and as indicated by the initial lower FEC and lower plasma pepsinogen of the chicory lambs. The FEC of lambs between grazing groups would have not differed at the beginning of grazing had it not been for this effect of anti-parasitism because the lambs were parasite naïve on entry to the experimental plots and hence were not assumed to have any acquired immunity to nematode parasites. The results for chicory agree well to the work of Hoskin *et al.* (1999) where grazing deer on chicory compared to grazing grass/clover suggested the potential of chicory for reducing anthelmintic use in farmed weaner deer.

The observed drench reduction under such a reasonably long grazing period for lambs and such a condition that is true to most of the current sheep farms, supports the view that current global problem which demands reduction in the use of drugs (Kaminsky, 2003; Waller, 1997) could be targeted with a combination of options. The need for reduced (frequency of) drench use is repeatedly stressed but such a reduction in drench requirement whilst using alternative approaches is actually very rarely reported.

6.4.4 Animal performance

Maternal MP supplementation and grazing on chicory did not show any interactive effect on ewe or lamb performance from the combination, but the effects were all additive. As such, ewe and lamb performance was improved

by maternal MP supplementation. Similarly, grazing on chicory improved ewe and lamb performance compared to grazing on grass/clover. The BW improvement of ewes was accompanied by improved BCS at weaning. The improvement whilst grazing chicory could be due, at least, to two reasons. Firstly, the better nutritive value of chicory which could be seen from the lower level of structural carbohydrates (NDF and ADF), the higher ME and CP content and the relatively higher levels of macro- and micro-nutrients compared to grass/clover. Similar result was also observed with lambs finished on chicory compared to grass/clover (see ***Chapter Four***). Other works also reported improved performance by ruminants while grazing chicory compared to grass/clover or other conventional forages (Komolong *et al.*, 1992; Marley *et al.*, 2003b). Secondly, this improved performance whilst grazing on chicory could suggest that chicory could have reduced the level of parasitism (Tzamaloukas *et al.*, 2005) as the degree of impairment on performance of grazing animals could be related to the level of parasitism (Coop *et al.*, 1982). Bailey *et al.* (2009) reported improvement in performance of ewes and lambs whilst using different grazing strategies that resulted in different levels of pasture contamination. Given that HP ewes and chicory lambs seeded less nematode eggs in their respective plots it could, therefore, be perceived that these ewes and lambs were exposed to reduced degree of parasitism. The latter again would have served to reduce the plasma pepsinogen of lambs reared by HP ewes and lambs grazing on chicory (at early stage) compared to their respective treatment counterparts as increased plasma pepsinogen suggests increased gut permeability due to increased level of infection (Kidane *et al.*, 2009; Murrell *et al.*, 1989; Nogareda *et al.*, 2006; Valderrabano *et al.*, 2002; Yakoob *et al.*, 1983).

Under a grazing condition, where ewes were daily trickle infected with *4000 T. circumcincta* infective larvae and managed to avoid auto- and cross-infection from grazing, Sykes and Juma (1984) observed reduction in feed intake (12%), milk production (8%) and lamb growth rate (11%) by infected animals compared to controls. Butter *et al.*,(2000) reported that subclinical infection with *T. colubriformis* reduced lamb performance (~20 %), compared to non-infected controls, in the group fed a diet low in CP (97 g/kg) whilst this difference was compensated for in the groups fed high CP (222 g/kg). The authors suggested that at least part of this compromised performance of lambs was through reduced feed intake. In the current experiment no control group was included and feed intake was not measured. However, it can not be ruled out that the low level of performance of ewes and lambs from both LP group and grass/clover plots of this experiment could have been partially attributed to factors like reduced feed intake and reduced efficiency of use of nutrients (Coop *et al.*, 1982) from field infections.

The increased drench requirement of lambs reared by LP-ewes and lambs that grazed on grass/clover did not restore their performance to a similar level to those lambs coming from HP-ewes and grazing on chicory respectively. These results agree to those reported by Coop *et al.* (1982) where lambs daily trickle infected with 5000 *T. circumcincta* larvae and drenched every 21 days did not restore BW gain to the level of non-infected controls or those infected with 1000 *T. circumcincta* larvae. Similar results were reported by Gibson and Parfitt, (1976) where lambs trickle infected with *T. circumcincta* and drenched at different frequencies, the most frequent being weekly, during the course of infection none of the anthelmintic treatment regimes completely prevented BW loss compared to the non-infected control group. However, in contrast to the above reports, Sykes and Juma (1984)

indicated that lactating Coopworth ewes trickle infected daily with 4000 *T. circumcincta* and drenched at 14 days intervals appeared to have completely restored performance compared to non-infected controls. This discrepancy between the latter cited works could be due to different reasons. For instance, the level of larval challenge to which the degree of damage may be proportional, the drenching interval employed, the class of animal and thus the level of immunity (ewes *vs* lambs) involved and the nutritional environment under which the experiments were conducted are some to mention. In support of the nutritional environment argument, in Scottish Blackface sheep infected with *Trypanosoma congolense* and fed high (176 g protein per kg DM) or low (81 g protein per kg DM) protein, Katunguka-Rwakishaya (1996) reported that treatment with a trypanocidal drug (i.e.; isometamidium chloride) improved killing out percent and live-weight relative to non-infected controls only in the high protein group suggesting that nutritional environment can affect the response to treatment. This suggests that once the damage is induced by (sub-)clinical parasitism, even over a short period, these effects could only be reversed at a cost of increased nutrient supply depending on the degree of damage inflicted by parasites.

The data from this experiment did not show any interaction effects between maternal MP supplementation and grazing on chicory on almost all parameters assessed except for the slight tendency in the lamb drench requirement. It is not clear why this was the case. However, in other work in which protein supplementation and Quabracho tannin (QT) feeding were combined, with expected nutritional effect of the former and antiparasitic effect of the latter, the combination did not show any added benefit in lamb performance and parasitism when lambs were infected with intestinal

parasite *T. colubriformis* (Butter *et al.*, 2000). The authors reported that for lambs fed high protein with QT supplementation, faecal egg output was similar to low protein fed QT-non-supplemented infected lambs where it was suggested the effects of QT and protein feeding were neutralized. However, based on the current experiment and a parallel experiment conducted with a different breed (***Chapter Five***) the alternative approaches showed an additive effect on most of the parameters described in this paper. This suggests that either the achieved level of protein intake from the combination of soypass supplementation and grazing chicory was below maximum requirement for the twin rearing ewes, or the antiparasitic effects of chicory could have been limited.

Implications. The current ever increasing awareness of public health, increasing share in and demand for organic system of production (Helga *et al.*, 2008) with its restriction on the prophylactic use of chemical drugs and emerging anthelmintic resistance for the existing class of drugs demands the need for alternative approaches. The lack of evidence for synergetic effects between the alternative approaches means that the approaches can be employed either independently or in combination. Which way, single or combined, is viable may need cost benefit analyses over a longer period of time. The reduced drench requirement, reduced period of exposure to infective larvae due to faster ADG of lambs and overall improved animal performance whilst using the alternative approaches point in the right direction for the current suggested selective and targeted use of anthelmintics (Athanasiadou *et al.*, 2008) in the aim of reducing selection pressure on parasites (Barger, 1985) and reducing the degree of evolution of

anthelmintic resistance by nematode parasites over a long term.

CHAPTER SEVEN

General discussion, conclusion and future directions

7.1 Introduction

Whilst comprehensive and relevant discussion has been provided within each experimental chapter of this thesis, some points are revisited and summarized here as an overview of the whole work. This chapter completes the thesis by providing brief conclusions based on the experiments conducted and highlighting future directions for related works in the area of parasite control in sheep at the time when reduced use of chemicals is being recommended for many reasons (see ***Chapter one and introductory section of each chapter***).

7.2 General discussion

Nematode parasitism has become a global concern and the conventional method of anthelmintics is no more sustainable. In order to control parasitism and maintain productivity, there have been strong suggestions for integrating alternative management practices (Athanasiadou *et al.*, 2008; Thamsborg *et al.*, 1999; Waller, 1997). The latter are meant to reduce the degree of exposure of animals to parasites and, further reduce reliance on anthelmintics (Abbott *et al.*, 2004; Vlassoff *et al.*, 2001) and increase the percentage of worms in refugia (Coles, 2002) to delay the evolution of resistance by nematode parasites.

Parasitism causes a reduction in food intake (Kyriazakis *et al.*, 1998), reduced utilization efficiency of nutrients (Parkins and Holmes, 1989) and

severe pathology of the gastrointestinal tract with subsequent reduction in performance, reproductive inefficiency and death under severe conditions (Sykes and Coop, 1976; Sykes and Coop, 1977). These effects of parasites are more profound in young and growing lambs which are naïve to parasites, and hence have higher susceptibility (Dobson *et al.*, 1990; Smith *et al.*, 1985b), resulting in compromised welfare, extra cost for care and treatment, extended feeding period before marketing and reduced benefit from the sector. Furthermore, there is an escalating problem of the evolving anthelmintic resistance by nematode parasites (Van Wyk *et al.*, 2006) necessitating for alternative approaches in integrated parasite control strategy. To this end, this thesis has produced substantial information in terms of combing some of the alternative approaches and the outcomes thereof both under pen and field experimental conditions.

All experiments executed in this project involved twin-bearing and –rearing ewes with their suckling lambs as both of these are very important with regard to parasite epidemiology for a susceptible host (Abbott *et al.*, 2009; Vlassoff *et al.*, 2001).

The first experiment (***Chapter Two***) reported results from a pen experiment conducted to assess whether effects of periparturient protein supplementation would differ at varying degree of parasite infection pressure under pen conditions. This was done under two different metabolizable feeding treatments combined in three different levels of experimental parasite infections (1000, 5000 and 10,000 L_3), in reproducing ewes with substantial previous parasite exposure history. The hypothesis was that metabolizable protein supplementation to periparturient ewes

would reduce the degree of PPRI and improve performance, with such expected effects to increase with increasing level of infection.

Similarly, ***Chapter Three*** was a pen experiment with the level of infection suggested by experiment one that compared two different breeds with a carefully designed experiment for parasite resistance under two different metabolizable protein feeding treatments. This experiment challenged previously reported works on differences between genotypes that differ in reproductive output and degree of parasite resistance under similar environmental/feeding conditions (Good *et al.*, 2006; Wallace *et al.*, 1995; Wallace *et al.*, 1996; Zaralis *et al.*, 2009) and provides information on whether preferential feeding strategy/management is needed for parasite control that takes the degree of susceptibility and hence the degree of response to supplementation into account. This was tested on two British breeds of sheep (Blackface and Mule ewes) with contrasting differences in their production potential and purported resistance to nematode parasites. It was hypothesized that if the Blackface ewes were indeed more resistant to nematode parasites than Mule ewes then the degree of PPRI in Blackface ewes would be lower than that of Mule ewes under a feeding condition that takes into account the level of productivity of each breed.

The last three grazing experiments (***Chapters Four, Five*** and ***Six***) were designed to investigate the interactive effects of ewe periparturient metabolizable protein nutrition and grazing on chicory around weaning (***Chapter Four)*** or from turnout (32 days from lambing) to finishing (***Chapters Five*** and ***Six***) on animal performance, parasitism (***Chapters Four, Five*** and ***Six***) and lamb drench requirement (***Chapter Six***) over a reasonably long period. All grazing experiments lasted for at least 20 weeks in contrast

to previous short period grazing experiments with bioactive forages and lamb parasitism (Tzamaloukas, 2006) to further extend the results from earlier works to farm situations. The underlying hypotheses in the combination were that if chicory were to offer both better nutritional and antiparasitic benefits to grazing ewes and lambs (see proposed pathways in **Figure 7.1**) the effects of grazing chicory were expected to be bigger for protein non-supplemented animals relative to the protein supplemented animals (see **Figure 7.2**). This assumes that a linear plateau for additional nutrient utilization efficiency (i.e., protein in this case).

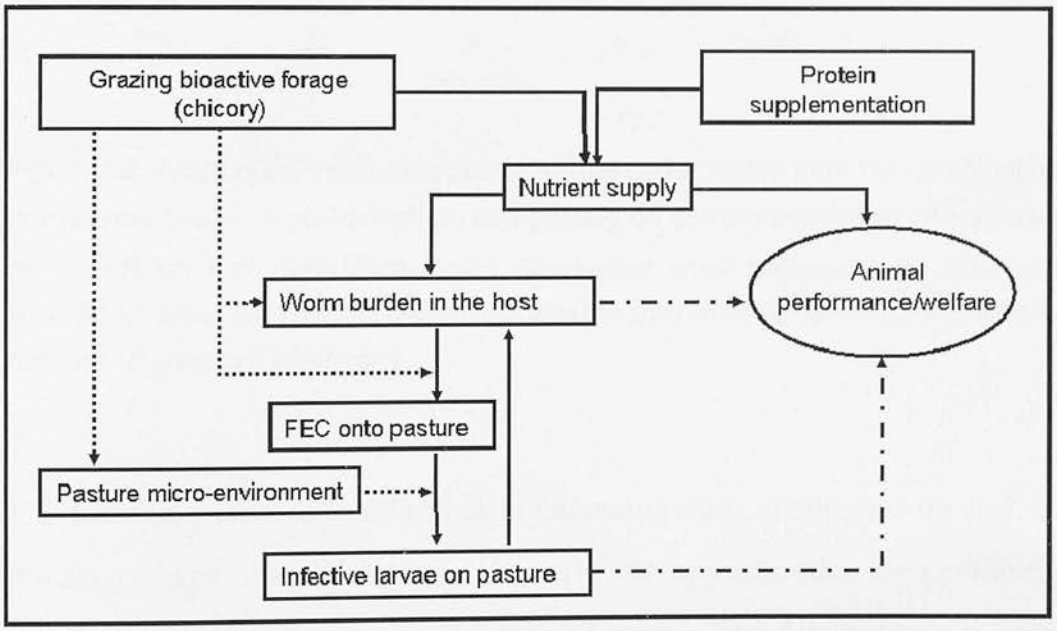


Figure 7.1 Suggested mechanisms for the effects of protein supplementation and grazing a bioactive forage (chicory) relative to conventional forages (⟶ direct benefits of nutrient supply; ⋯⋯▸ additional non-nutritional benefits from chicory in the presence of parasitism; and – · – · ▸ negative effects of parasitism) on animal performance

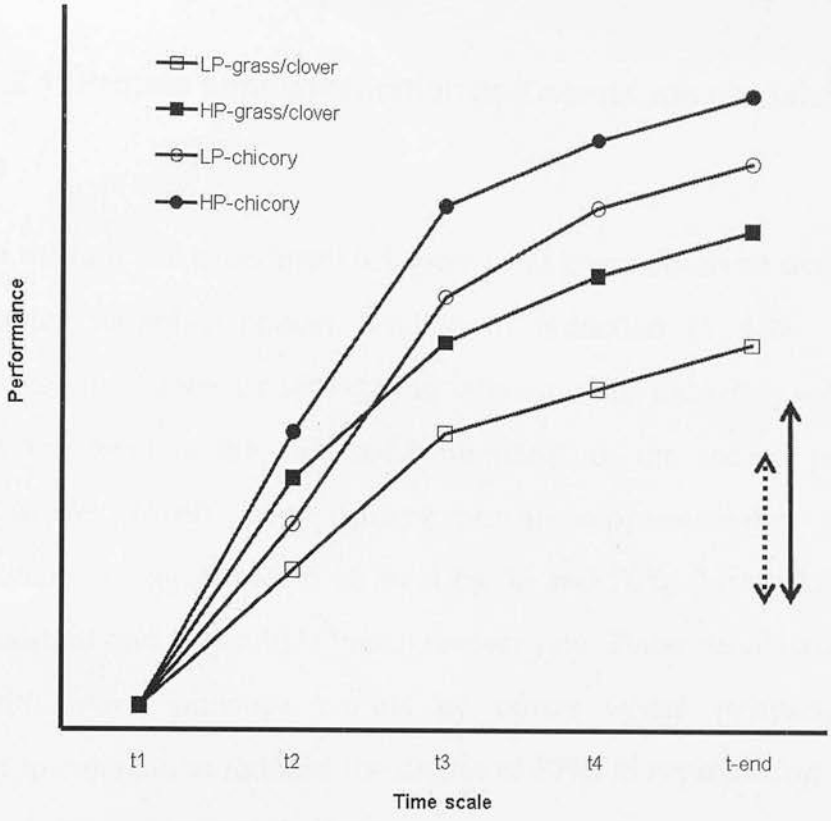


Figure 7.2 Hypothetical trend expected in animal performance from the combination of maternal protein supplementation and grazing on chicory relative to grass/clover on performance of parasitized lambs *[the broken arrow designating the difference between HP-grass and HP-chicory is shorter than the solid arrow designating the difference between LP-grass and LP-chicory]*

The following sections briefly discuss the main points challenged by each of the above experimental chapters. As such, the opportunities for exploiting existing alternative approaches, i.e. using between-breed difference, protein nutrition and grazing on chicory, and paired combinations of these approaches, at a time for parasite control and improved performance are discussed first. Lastly, challenges during this work and future recommendations are highlighted.

7.2.1 Protein supplementation and nematode parasite resistance

In the first pen experiment (***Chapter Two)*** it was observed that periparturient protein supplementation resulted in reduction in daily nematode egg excretion of ewes by 65% during lactation. This reduction was independent of the level of infection used. Similarly, in the second pen experiment (***Chapter Three***), periparturient protein supplementation reduced daily nematode egg excretion of ewes by 35 and 70%, during lactation, from a resistant and susceptible breed, respectively. These results are in agreement with many previous reports by others where periparturient protein supplementation reduced the degree of PPRI in reproducing ewes (Houdijk *et al.*, 2006; Zaralis *et al.*, 2009).

As host resistance to gastrointestinal nematodes is largely mediated through acquired immunity (Coop and Kyriazakis, 1999), the observed results support the view that host nutrition has the potential to affect gastrointestinal nematodes through its effect on the degree of expression of immunity. The latter could be manifested as expulsion of adult parasites and reduction in worm length, decrease in female worm fecundity, failure of infective larvae to establish and arrested development of larvae (Onah and Nawa, 2000; Vercruysse and Claerebout, 1997) and is highly dependent on specific nutrient availability (Calder and Yaqoob, 1999; Cunningham-Rundles, 2001).

Such a reduction in nematode egg excretion during periparturient period brought about by maternal protein supplementation is important in reducing

pasture contamination for parasite naïve lambs in their first encounter of grazing. Therefore, a parasite control strategy targeted at ewes during periparturient period would be crucial in sheep production.

In experiment one, in contradiction to the original expectation, FEC of ewes showed an inverse relationship to the level of infection with *T. circumcincta* in the range of larvae dosing used. The observed FEC was negatively correlated to the level of plasma pepsinogen, whilst litter and ewe performance tended to decrease with increasing pressure. As plasma pepsinogen correlates positively to the level of infection/worm burden (Iposu *et al.*, 2008; Nogareda *et al.*, 2006; Valderrabano *et al.*, 2002), and furthermore, as animal performance is negatively correlated to the level of infection, the observed results suggest the occurrence of density-dependent effect for *T. circumcincta.* This phenomenon is observed in rats infected with *Strongyloides ratti* (Bleay *et al.*, 2007; Paterson and Viney, 2002) and reported to depend on the quantitative increment in Th2 response by hosts to the degree of parasite challenge affecting worm life history traits (for instance, worm fecundity). Furthermore, these responses are dependent on host immune status at the time of infection and as such are not expected at early stages primary infections and in immuno-suppressed hosts (Paterson and Viney, 2002). However, in ***Chapter Three*** where different batch of *T. circumcincta* was used, a positive correlation was observed between FEC and plasma pepsinogen.

It has been reported by many workers that between-breed difference in resistance to nematode parasites exists under pen experiments or grazing conditions (Abbott *et al.*, 1985; Good *et al.*, 2006; Zaralis *et al.*, 2009). In all the reported works the different breeds were either fed *ad lib* or co-grazed in the field which did not discriminate the breeds on the quantity of nutrients fed.

However, these breeds practically differ in their productivity and hence in their level of protein demand. Such differences in demand for protein are suggested to drive the degree of breakdown of immunity, at least for the periparturient ewes, to nematode parasites (Houdijk, 2008). In ***Chapter Three,*** this reported difference between breeds has been further refined with feeding treatments which discriminated between the two breeds involved, based on level of production, and the results strongly indicated that only part of the difference in periparturient relaxation of immunity between the breeds involved could be explained by MP scarcity. As such, strong interaction effects were noted between protein supplementation and breed, with the former reducing the degree of PPRI to larger extent in Mule ewes whilst this effect was minimal in the Blackface ewes, as protein scarcity did not significantly reduce periparturient resistance in the latter breed.

The mechanism as to how protein supplementation showed strong effects in a susceptible breed while these effects are not so pronounced in a more resistance breed is not clear from this work. However, strong response to *T. circumcincta* by a more resistant breed may cause elimination of adult worms, arrested development of established larvae or expulsion of infective larvae before they can establish (Vercruysse and Claerebout, 1997), whilst these responses require adequate level of MP in the more susceptible breed. The non-specific inflammatory responses caused by release of mediators from either mucosal mast cells, eosinophils or other cell types may have also mediated the observed effects most of which are genetically controlled (Vercruysse and Claerebout, 1997) and influenced by host MP nutrition (Houdijk *et al.*, 2000; Houdijk *et al.*, 2005). However, it could also be due to the differences in the nutrient partitioning and prioritization of the breeds involved as some breeds differ in the way they were selected for specific

production purpose, and with such selections some unwanted but correlated traits may have been selected for the highly productive breed (Rauw *et al.*, 1998).

The results from ***Chapter 3*** suggest that only at times of MP scarcity, resistance to gastrointestinal parasites was lower in highly productive Mules than in the less productive Blackface ewes. Although this experiment was not designed to compare genotype per se, as there was insufficient information on the number of sire groups involved to conclude with confidence that observed effects are truly breed differences, the results suggest that genetic differences in parasite resistance may be more pronounced when MP is scarce. Estimated breeding values (EBV) for FEC may describe within-breed variation in parasite resistance, and outside the direct remit of this thesis, an experiment was carried out to test whether FEC are indeed positively correlated with EBV for FEC at times of MP scarcity only (Kidane *et al.*, 2010b). Pure-bred periparturient Suffolk ewes with an EBV for FEC ranging from -0.37 to +0.91, were trickle infected with *T. circumcincta* larvae, and fed at different levels of MP requirements. Pearson's correlations between their EBV and mean lactational FEC were indeed significant for ewes fed at 0.8 times MP requirements ($r=0.75$; $P<0.05$) but not for those fed at 1.3 times MP requirements ($r=-0.12$; $P>0.35$). These observations support the view that genetic superiority in terms of resistance to parasites may only be observed at times of protein scarcity.

The results from ***Chapter Three,*** therefore, further suggest the need for different parasite control strategies that take the breed's level of resistance to nematode parasites and the response to supplementation into account. If the results from such a pen experiment translate into grazing conditions, it

would very likely be that grazing a susceptible class/breed of animal with/after a more resistant breed of sheep (Abbott *et al.*, 2009) would help in minimizing the degree of risk of residual parasite infection for lambs (Uriarte *et al.*, 2003) and, thereby, reduce the ill effects of parasitism on lamb growth/performance (Sykes *et al.*, 1977; Sykes and Coop, 1976).

7.2.2 Effects of grazing on chicory on performance and parasitism

In each of the three grazing experiments carried out, grazing ewes and their lambs on chicory consistently showed improved animal performance and reduced lamb parasitism as indicated by proxies (FEC and drench requirement). These results agree well to earlier reported works on chicory with lambs (Athanasiadou *et al.*, 2007; Komolong *et al.*, 1992; Scales *et al.*, 1995). Lambs finishing on grass/clover showed a lag time of 60 days (***Chapter Five;*** Blackface breed) and 90 days (***Chapter Six;*** Mule X Suffolk cross) in contrast to lambs finishing on chicory over aperiod of grazing for about 140 days. This lag in time or observed cost in BW of lambs whilst grazing grass/clover was not compensated by the extra drench grass/clover lambs required compared to the lambs on chicory (***Chapter Six***). The latter is in agreement with other works where under the effects of continuous larvae challenge of lambs drenching frequently did not restore final BW of lambs to that of a non-infected control group (Coop *et al.*, 1982). The observed effects, therefore, strongly suggest the need for cleaner grazing environment and grazing management for parasite control (Coop *et al.*, 1982) emphasizing on the old adage *'prevention is better than cure'*.

Furthermore, grazing lambs on chicory consistently reduced FEC in all experiments over the two years trials. However, these effects were not observed in ewes which were turned out onto the experimental plots with an existing established parasite infection. The latter could probably be due to, as observed in ***Chapter One*** and reported by others (Athanasiadou *et al.*, 2007), density-dependency effects on these mature partially immune ewes where the effects could have been masked (Bleay *et al.*, 2007; Blood *et al.*, 1979; Paterson and Viney, 2002). It could also be due to the reason that the effects of chicory could have been minimal on an already established parasite, and this means that ewes would continue to have the initial level of FEC (from the already existing adult parasite population) even if the degree of current infection subsides.

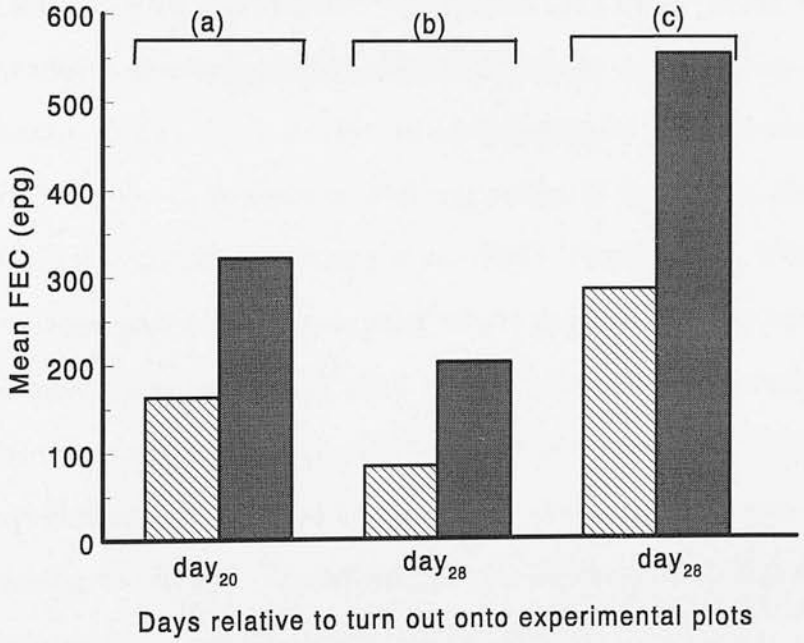


Figure 7.3 Arithmetic mean faecal egg counts from lambs grazing on either chicory (▨) or grass/clover (■) at early stages of the experiments *[**(a)** and **(b)** were on initially clean pastures grazed in the years 2007 and 2008 as reported in **Chapters Four** and **Five**, respectively, and **(c)** was on an initially parasite contaminated pasture grazed in the year 2008 as reported in **Chapter Six**]*

A few reasons could be mentioned here for the observed effects of chicory. First, chicory had significantly higher levels of macro- and micro-nutrients than grass clover plots. Secondly, it had higher CP and lower structural carbohydrate content than grass/clover plots (**Tables 4.1, 5.1 and 6.1**). Furthermore, the cultivar used here was originally bred for higher levels of plant secondary metabolites (Rumball, 2003) and the latter are said to have anti-parasitic effects (Barrau *et al.*, 2005; Barrera *et al.*, 2008; Molan *et al.*, 2003) through which parasitism may have been limited. In support of the latter argument, the FEC of initially parasite naïve lambs over the first four weeks of grazing from the three grazing experiments reported in this study is shown in **Figure 7.3**. The suggestion is that, since parasite naïve lambs (***Chapters Five*** and ***Six***) had only grazed for a short period at this time point, the observed effects could be due to either the anthelmintic-like properties of chicory or the effects of chicory on the pasture micro-environment, at least, affecting the development and migration of larvae to the grazing height (Marley *et al.*, 2003b; Marley *et al.*, 2003a; Marley *et al.*, 2006a). However, in the latter part of the experiment, where expression of acquired immunity to parasites is expected to play a role (Coop and Kyriazakis, 1999), the observed even stronger effects of chicory on lamb FEC in all grazing experiments, is an indication of the enhanced immune response whilst grazing the forage (Tzamaloukas *et al.*, 2006) mediated by the differences in the nutritional qualities of the forages grazed.

Outcomes of the above type are clearly needed if any non-chemical parasite control strategy is to be viable. The advantages here are many and include, among others, early lamb finishing for better price, reduced input (in terms

of feed, drench and labour reducing production cost), reduced residual larvae in the field for subsequent season due to short grazing period and lower FEC, and in the long-run delayed evolution of anthelmintic resistance by parasites.

Overall, reduced parasitism by initially parasite naive lambs whilst grazing chicory, compared to grass/clover, as indicated by consistently lower FEC in all grazing experiments or reduced drench requirement based on clinical signs (***Chapter Six***) suggests that chicory can be used for finishing lambs over a relatively longer grazing period in contrast to the recommendation of Tzamaloukas (2006) only for short term grazing. Nonetheless, short term effects can also be beneficial; the results from ***Chapter Four*** clearly suggest that weaning lambs on chicory compared to grass/clover would benefit in attaining early market weight regardless of previous maternal protein nutrition level and reduced residual pasture infectivity for subsequent grazing.

Lastly, these benefits of chicory can be utilized either by growing the crop alone (***Chapters Four, Five and Six***) or in a mixed stand (Hogh-Jensen *et al.*, 2006). The latter could also be seen from one of the experiments in this study (***Chapter Four***) where the proportion of chicory was nearly 50% on DM basis, the rest representing weeds from seed bank, and yet its benefits in terms of improved performance and reduced parasitism were evidently observed. For example, the CP content of the grazed herbage (***Chapters Four and Six***) was regressed against the proportion of chicory in the stand and the results (**Figure 7.4**) clearly suggest that the effects of the crop could also be observed under mixed cultivation with other forages (Hogh-Jensen *et al.*, 2006) and help as a supplement of the most expensive nutrient in the diet of animals

(i.e, CP). The combination would further benefit improved DM yield from the companion crop as chicory is low in DM content as observed in all the grazing experiments.

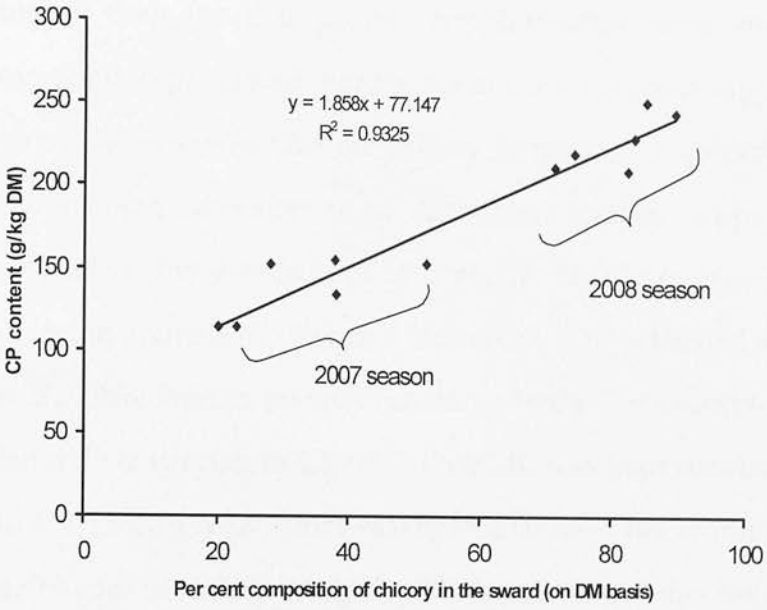


Figure 7.4 The relationship between the proportion of chicory in the stand and the CP content of the of the herbage

7.2.3 Synergism between protein supplementation and grazing on chicory

Interactive effects were expected between maternal protein nutrition and grazing of chicory. These meant that animals under scarce protein nutrition (in this case, protein non-supplemented) were expected to respond to grazing on chicory to a greater extent than animals that have been fed under adequate or more than adequate protein whilst under infection. The reasons for this expectation was that chicory was expected to provide better

nutritional qualities (for example higher level of CP compared to grass/clover (Athanasiadou *et al.*, 2007; Tzamaloukas *et al.*, 2005)), reduce the degree of exposure of grazing animals to larvae in the pasture (Marley *et al.*, 2006a) and render direct anthelmintic-like effects (Tzamaloukas, 2006). For the non-supplemented ewes and their lambs the above effects of chicory would be bigger than for that protein supplemented ewes and their lambs. This expectation presumed that the benefit from protein supplementation to ewes would diminish whilst on chicory compared to grass/clover plots. Such a diminishing response to an additional protein supply whilst the energy content of the diet is kept at constant supply or vice-versa is common in growing animals (Gabler and Heinrichs, 2003; Hegsted and Neff, 1970; Kuan *et al.*, 1986; Kyriazakis and Emmans, 1992). For example, in Holstein heifers fed a diets varying in CP whilst the ME was kept constant, increasing the CP to ME ratio (g/Mcal) from 45.0 to 69.4 showed linear improvement in average daily gain of heifers whilst further increment of this ratio to 77.3 g/Mcal did not improve heifer growth (Gabler and Heinrichs, 2003). Indeed, the works of Hegsted and Neff (1970) showed that protein utilization efficiency was essentially constant over an appreciable range of intakes from the maintenance level to those that allow near maximum rates of growth. In this project, the ME content of the diet (grazed forage with or without protein supplement) of both the protein supplemented and non-supplemented ewes was nearly constant (~12.0 MJ per kg DM). Assuming a constant ME and fairly similar daily DM (2 kg/day/ewe) intake and further assuming a simple substitution effect of soypass supplementation on DM intake from grazed forage, the mean daily CP to ME intake ratio (g CP per MJ ME) probably varied between grazing groups from 8.53 in LP-grass/clover to 19.10 in HP-chicory (***Chapter Five***) and from 7.61 in LP-grass/clover to 21.90 in HP-

chicory (***Chapter Six***). The lamb average daily gain drawn against estimated maternal CP to ME intake ratio is shown for the above experiments (**Figure 7.5**). Data from both chapters were best represented by a linear equation with a similar slope between the fitted lines. When combined, data from the two experiments still maintained similar trend and slope. This *post hoc* analysis gives a suggestion for the lack of interaction effect between maternal protein supplementation and grazing on chicory on performance and parasitism parameters assessed. This could very well have arisen from the fact that the level of protein intake attained by ewes from the combination (chicory grazing and soypass supplementation) might have not been more than adequate or might have not been at a level to result in diminishing response to the addition protein intake from chicory.

Furthermore, it could also be that the anthelmintic-like effects of chicory could have been subtle or the measured parameters are not very sensitive to the degree of changes in nutrient supply and parasitism at the achieved level of nutrient intake. In the last grazing experiment (***Chapter Six***), this could have further been complicated with the number of drenches lambs received at varying levels affecting animal performance, daily nematode egg excretion, and subsequently the level of larvae in the field and therefore, degree of infection pressure.

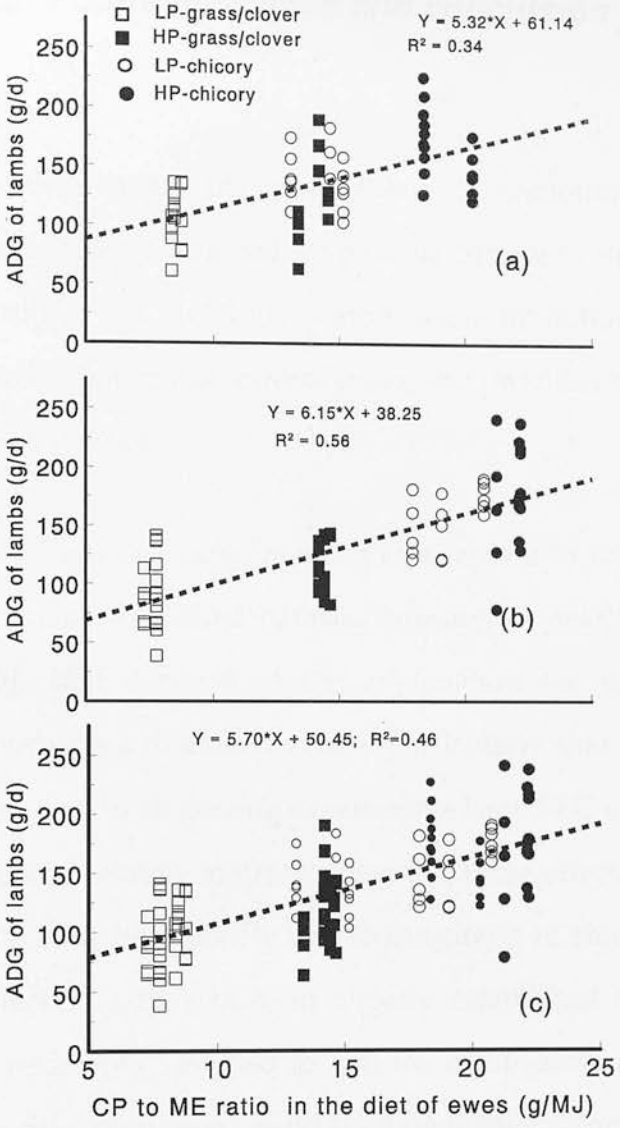


Figure 7.5 The relationship between lamb mean daily body weight gain and the CP to ME ratio in the diet of ewes (**a**) data extracted from ***Chapter Five***; (**b**) data extracted from ***Chapter Six*** and (**c**) data from '**a**' and '**b**' combined. *[Fitted lines are all linear as other combinations/forms did not improve the R^2 of the equations]*

7.3 Future directions and conclusion

In this thesis, the possibilities of combining some of the alternative approaches developed for parasite control in sheep were assessed. However, through out the study period, some limitations have been observed. This section points out some of these, and provides suggestions for future work in related areas.

In all grazing experiments, ewes went into the experimental plots with an existing established parasite infection of nearly 10 weeks trickle infections. This had constrained the explanation for the effects of the alternative approaches described above, particularly that of the effects of chicory. For instance, in all grazing experiments lamb FEC was reduced whilst grazing on chicory relative to grass/clover but these effects were not observed in ewes. This may be probably due to the effects of chicory on the contemporaneous infection rather than an already established parasite infection. Therefore, experiments designed to link the commencement of infections and grazing on chicory in time would provide further information if the effects observed in lambs could be also true for ewes.

In all three grazing experiments, even though the perennial ryegrass: clover ratio was 80:20 at sowing, the observed levels of clover and consequently the CP content of grass/clover plots were very low. This warrants further research in a condition where the grass/clover ratio represents farm situations.

Lack of adequate resources to run full factorial experiments including control (non-infected) group in both pen and grazing experiments had somehow limited explanations for the observed outcomes. Indeed, comparison with non-infected controls was not part of the overall objectives but with hind sight could have been useful.

During the grazing experiments, mainly that of ***Chapters Five and Six,*** animals finished the experiments after grazing for about 20 weeks on the same experimental plots. As such, moderate level of feed shortage was observed in the above experiments towards the end of the grazing season as judged by the sward height. However, it could be assumed that the observed differences between chicory and grass/clover grazing groups could have been bigger had the experiments been under regular rotations instead of the set stocking used here. The latter assumption comes from the point that under the stocking rate of the reported experiments, it was chicory rather than grass/clover that declined rapidly as reported in ***Chapter Four*** (and also visual observation during experiments reported in ***Chapters Five and Six***).

In all experiments, ewe and lamb parasitism was inferred from proxy measures like FEC, plasma pepsinogen levels and clinical signs (like diarrhoea) and BW change over a period of measurement. Indeed, the reported work was not intended to investigate the underlying mechanisms of either protein nutrition and/or grazing bioactive forages on parasitism and performance as these have been extensively done and reported (Athanasiadou *et al.*, 2005; Houdijk *et al.*, 2000; Houdijk *et al.*, 2003a; Houdijk *et al.*, 2005; Houdijk *et al.*, 2006; Tzamaloukas *et al.*, 2005; Tzamaloukas *et al.*, 2006). However, inclusion of strategic slaughter points for examinations of worm burdens and local (mucosal) immunity indicators could have

substantiated whether the observed effects were due to reduced worm fecundity, worm establishment rate, and/or on improved immune responses from the diets.

Future works involving the combination of alternative approaches would provide information on profitability of the use of chicory if works involve also economic analysis. This will help in translating the benefits observed under such works to farmers as farmers would not adopt something which is economically not viable. It must be mentioned here, however, that there are other benefits that can not easily be put into economic aspects in the short term for farmers. For instance, reduced anthelmintic use would slow the evolution of drug resistance but it would be difficult to translate this into farm economics.

The combined effects of ewe MP supplementation and grazing on chicory, which were not significant in almost all parameters in this study, would better be examined in an experiment where non-infected controls groups were included with more than two levels of protein nutrition (instead of the LP and HP used here). The inclusion of non-infected controls would provide a benchmark for the scale of improvement whilst using alternative approaches, and a degree of penalty from the observed level of infection whereas the inclusion of graded levels of protein supplementation would show clear trends over the range of supplementation.

In conclusion, MP supplementation can be used for improved resilience/resistance of periparturient ewes and hence improved lamb growth. The degree of observed effects was dependent on the breed of sheep used suggesting a breed dependent nutritional management of

periparturient ewes. Grazing chicory reduced parasitism and drench requirement and improved both ewe and lamb performance. Therefore, chicory can be incorporated in nematode parasite control with apparent economic and expected long-run benefits on delaying the degree of evolution of gastrointestinal nematode parasites to the existing anthelmintics. The combined additive effects of periparturient ewe metabolizable protein nutrition and grazing on chicory were consistent across the project years and experiments. This shows that the alternative approaches could be used either in combination or independent of each other as none of the effects were seen to be antagonistic.

The observed inverse relationship between level of infection and FEC (***Chapter Two***) in partially immune ewes needs further work. Lastly, the reported effects of chicory on lamb plasma pepsinogen suggest that the latter may not be a good indicator of the degree parasitism in lambs whilst grazing on chicory and also call for further work.

Furthermore, this work was limited to the findings on experimental infections with an abomasal parasite of small ruminants *T. circumcincta* and further work with parasites of other species may provide a broad view on the effects of combinations of the alternatives approaches used here.

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Publications

I. Papers in refreed journals:

1. ***Kidane A.,*** *J.G.M. Houdijk, S. Athanasiadou, B.J. Tolkamp, and I. Kyriazakis. 2009 Consequences of infection pressure and protein nutrition on periparturient resistance to Teladorsagia circumcincta and performance in ewes.* ***Veterinary Parasitology.*** *163:78-87.* ***(Chapter Two)***

2. ***Kidane A.,*** *J.G.M. Houdijk, S. Athanasiadou, B.J. Tolkamp, and I. Kyriazakis (2010). Effects of maternal protein nutrition and subsequent grazing on chicory (Cichorium intybus) on parasitism and performance of lambs.* ***Journal of Animal Science. 88:****1513-1521.* ***(Chapter Four)***

3. ***Kidane, A.,*** *J.G.M. Houdijk, S. Athanasiadou, B.J. Tolkamp, and I. Kyriazakis Nutritional sensitivity of periparturient resistance to nematode parasites in two breeds of sheep with different nutrient demands.* ***Accepted for publication in the British Journal of Nutrition.*** ***(Chapter Three)***

II. Short papers in conference proceedings:

4. ***Kidane A.,*** *Z. Parisi, J.G.M. Houdijk, S. Athanasiadou, B.J. Tolkamp, and I. Kyriazakis (2009). Chicory (Cichorium intybus) as promising bioactive forage for sheep production.* ***Grassland Science in Europe.14****: 394-397.* ***(Extracted from Chapters Five and Six)***

5. ***Kidane A.,*** *J.G.M. Houdijk, S. Athanasiadou, B.J. Tolkamp, and I. Kyriazakis (2009). Breed dependent nutritional sensitivity of periparturient relaxation of immunity to nematode parasites in sheep. In: 13th Seminar of the FAO-CIHEAM Sub-Network on Sheep and Goat Nutrition. Leon, Spain* ***(In press).*** ***(Extracted from Chapter Three)***

III. Abstracts published in conference proceedings:

6. ***Kidane A.,*** *J.G.M. Houdijk, S. Athanasiadou, B.J. Tolkamp, and I. Kyriazakis (2008). Effects of periparturient metabolizable protein nutrition on ewes subjected to different infection pressures with the abomasal nematode Teladorsagia circumcincta. Proceedings of the British Society of Animal Sciences; P.94.* ***(Extracted from Chapter Two)***

7. ***Kidane A.,*** *S. Athanasiadou, J.G.M. Houdijk, B.J. Tolkamp, and I. Kyriazakis (2008). The interactive effects of maternal nutrition and subsequent grazing on bioactive forage on lamb performance and parasitic status. Proceedings of the British Society of Animal Sciences; P. 97.* ***(Extracted from Chapter Four)***

8. ***Kidane A.,*** *J.G.M. Houdijk, B.J. Tolkamp, S. Athanasiadou, and I. Kyriazakis (2009) Are Scottish Blackface ewes more resistant to nematode parasite infection*

than Mule ewes? Proceedings of the British Society of Animal Sciences; P. 28. ***(Extracted from Chapter Three)***

9. ***Kidane A.****, J.G.M. Houdijk, S. Athanasiadou, B.J. Tolkamp, and I. Kyriazakis (2009). Interactive effects of protein nutrition and breed difference on periparturient immunity to parasites in sheep. Proceedings of the 22nd International Conference of the World Association for the Advancement in Veterinary; P.71.* ***(Extracted from Chapter Three)***

10. ***Kidane A.,*** *J.G.M. Houdijk, S. Athanasiadou, B.J. Tolkamp, and I. Kyriazakis (2009). Consequences of ewe protein nutrition and grazing on chicory (Cichorium intybus) on animal performance and parasitism. Proceedings of the 22nd International Conference of the World Association for the Advancement in Veterinary Parasitology; P.16.* ***(Extracted from Chapter Six)***