Interactive effects between genotype, protein nutrition and immune status on the parasite-induced anorexia of sheep

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Dedication

To my parents Georgio and Anthoula

To my brothers Andrea and Theodoro

Declaration

I hereby declare that the work presented in this thesis is product of my own efforts and has not been previously submitted for any other degree or qualification. The work on which this thesis is based is my own and all assistance has been fully acknowledged.

Konstantinos Georgiou Zaralis

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Abstract

Infection with nematode parasites detrimentally affects production efficiency in grazing animals, mainly through a reduction in food intake (anorexia).This thesis describes a series of six *in vivo* experiments designed to investigate the interactive effects of genotype, immune status and protein nutrition on the occurrence of parasite-induced anorexia of sheep. The experiments also investigated the role of the hormore leptin in immune response and anorexia following infection with gastrointestinal nematodes. In general, each experiment involved two breeds of lambs (Suffolk × Greyface, S, and Scottish Blackface, B) or ewes (Greyface cross, G, and Scottish Blackface, B) that are known to differ in their production potential. In each experiment, animals were either infected with the abomasal nematode *Teladorsagia circumcincta* and fed *ad libitum* or non-infected and fed either *ad libitum* or restrictedly..

The first two experiments investigated the effect of a primary and a secondary infection on anorexia and plasma leptin concentrations (PLC) in growing lambs (Chapter 3). The secondary infection started two weeks after the discontinuation of the primary infection. The results showed that lambs of the S breed were more susceptible to nematode infection than B lambs, as judged from the differences in faecal egg counts. Primary infection resulted in anorexia in S lambs but not in B lambs and re-infection tended to affect the food intake of S lambs only. Infection did not result in an acute increase in PLC, but its effect was significant when variation in food intake between treatments was accounted for. These results suggest that anorexia can occur in previously infected lambs, thus the effect of re-infection on anorexia was further investigated (Chapter 4).

Chapter 4 describes a series of 3 experiments with lambs. In these experiments, previously naïve lambs of approximately 3 (experiment I) or 7 (experiment II) months of age were infected with *T. circumcincta* for either 10 or 7 weeks, respectively. Lambs of experiment I were re-infected either 4 or 8 weeks after the end of the primary infection (experiment III). The results showed that the breed differences in resistance to infection were not associated with breed differenced in the degree of anorexia (experiment I) and infection of 7-month old lambs did not result in anorexia. Re-infection of previously infected lambs did also not result in anorexia when lambs were re-infected 4 or 8 weeks after the end of the primary infection. In addition, the results of these experiments showed that nematode (re)infection did not result in an increase of PLC. These results suggest that leptin may be involved in the response of lambs to infection, but it is unlikely that leptin alone is responsible for the parasite induced anorexia in lambs.

The last experiment (Chapter 5) investigated the consequences of protein supplementation on anorexia, and PLC in infected periparturient ewes. Infection resulted in a breakdown of immunity to parasites (PPRI) and a reduction in food intake in both breeds. The breeds differed in the extent of PPRI (G ewes having higher FEC than B ewes), but not in the magnitude of anorexia. Protein supplementation resulted in a reduction in FEC, but had no effect on the magnitude of anorexia. Plasma leptin concentrations changed significantly over time, but were not affected by protein supplementation or infection. It was concluded that infection with *T. circumcincta* in periparturient ewes results in anorexia that is not alleviated by protein supplementation. Leptin is unlikely to be responsible for the anorexia of nematode infection in periparturient ewes.

The outcomes of the above experiments are brought together with the literature in the General Discussion (Chapter 6) and directions of future work, to elucidate the mechanisms as well as the functional significance of anorexia, are put forward.

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List of abbreviations

ADFI	Average daily food intake
B	Scottish Blackface
BCS	Body condition score
BW	Body weight
BWG	Body weight gain
CI	Confidence interval
CP	Crude protein
CV	Coefficient of variation
d	Day
DM	Dry matter
epg	Nematode eggs per gram of faeces
FEC	Faecal egg counts
G	Greyface, Scottish Blackface × Border Leicester
MJ	Mega Joule
MP	Metabolisable protein
MP_I	Intake of Metabolisable protein
MP_R	Requirements of Metabolisable protein
NDF	Neutral detergent fibre
PLC	Plasma leptin Concentrations
PPRI	Periparturient relaxation of immunity
RADFI	Average daily food intake relative to body weight
RIA	Radioimmunoassay
S	Suffolk × Greyface
SEM	Standard error of the mean
Wk	Week

CHAPTER ONE

General Introduction

General Introduction

Gastrointestinal nematode infections of ruminants are a major cause of economic losses in ruminant production systems, especially because such infections result in a decrease in food intake (anorexia). Control of this disease has relied strongly on anthelmintics drugs but the emerging development of anthelmintic resistance of nematodes and increasing consumer concerns about chemical residues entering the food chain have stimulated investigations to find alternative sustainable control strategies.

Some of the main approaches to reduce effects of parasitism on productivity are based on the exploitation of the genetic resistance and improved nutrition (especially protein supplementation) of livestock. Differences in resistance to nematode infections are expected between breeds that differ in their production potential. Whether these breeds differ in the degree (i.e. magnitude and duration) of anorexia following nematode infection, however, is currently not known. In addition, little is known about the occurrence of anorexia in secondary infections, i.e. in the immune lamb or periparturient ewe. Although protein supplementation has a beneficial effect on resilience to nematode infection in periparturient ewes, it is not known how protein supplementation during infection affects anorexia.

The recent literature suggests a link between the immune response and the occurrence of anorexia in infected animals. Evidence suggests that the adipocyte hormone leptin, that is also involved in food intake regulation, plays an important role in the immune response of rodents and a role for leptin in the anorexia of infection and inflammation has, therefore, been proposed It is not known, however, if plasma leptin levels increase during gastrointestinal nematode infections in sheep and if such increases in leptin are associated with parasite-induced anorexia.

This study examined how breeds of sheep that differ in their production potential respond in terms of anorexia, following a primary (previously naïve lambs) and/or a secondary infection (previously infected lambs or ewes) with the nematode *Teladorsagia circumcincta* and how protein nutrition affects the degree of anorexia in those breeds. The study also investigated the possible role of the hormone leptin in the development and expression of immunity in sheep following infection with nematodes.

The introductory chapter of this thesis consists of three sections: the first section is a review of the consequences of gastrointestinal nematode infection in sheep in terms of food intake and immune response. The second part provides information about the effects of genotype, nutrition and immune status of the host on sheep responses to nematode infection, which is relevant for the potential interactions between effects of host genotype and nutrition on the consequences of gastrointestinal parasitism. The last section deals with the available evidence concerning the role of leptin in the food intake regulation and immune response with special focus on the parasitised sheep.

The results of the experiments that were carried out during the course of this study are reported in three Chapters (3 to 5). The first experimental Chapter (3) describes two experiments that investigated the effect of a primary and a secondary infection on anorexia and plasma leptin concentrations in growing lambs of two breeds. In Chapter 4, in addition to the breed effects on the parasite-induced anorexia, the effects of age and immune status were also investigated. The effect of nematode infection on plasma leptin concentrations was also studied. The last experimental Chapter (5) investigated the consequences of protein supplementation on anorexia, expression of immunity and plasma leptin concentrations in parasitised ewes of two breeds. The results of the entire study are discussed in relation to the literature in the General Discussion (Chapter 6).

CHAPTER TWO

Review of the literature

2.1. Gastrointestinal parasites and effects of infection on the host

Ruminants are continuously exposed to infective forms of gastrointestinal parasites through grazing. This inevitable infection of grazing livestock can have numerous effects on the host depending on the parasite genera, infection rate and the immunological status of the animal. *Teladorsagia circumcincta* (previously called *Ostertagia circumcincta*) and *Trichostrongylus* spp are probably the most important nematode species in sheep and goats in temperate climatic zones throughout the world (Encyclopaedia of Parasitology, 2007).

These nematode parasites of the gastrointestinal tract have a direct life cycle (i.e. there is no intermediate host). Adult female worms in the gastrointestinal tract of the sheep lay fertilised eggs that are excreted by the host in the faeces. Eggs, under suitable environmental conditions, hatch within the faeces to produce free-living first larval stage (L1). The L1 develop and undergo a moult to second stage larvae (L2). L1 and L2 are active and feed on bacteria in the faeces. L2 moult to third larvae (L3). The L3 migrate onto the herbage, where they can be ingested by sheep. Within minutes or few hours after ingestion L3 larvae loose their sheath (exsheathment), a fact that marks the transition from the free-living to parasitic phase. L3 larvae move to the abomasums and invade the abomasal gastric glands, where they develop and moult two more times before emerging from the gland as adult (L5) parasite, which may be as early as 8 days post infection (Urquhart et al., 1996). Sexually mature adult worms may then copulate and female worms lay eggs, which in turn are excreted on the pasture though host faeces. The time from ingestion of larvae to appearance of egg laying females is normally about 16 to 21 days (Urquhart et al., 1996).

Although some nematode species differ in the habitat that it occupies and consequently in the damage and the disease that it causes, infections are commonly

characterized by similar clinical signs such as diarrhoea, weight loss, decreased production, rough hair coats, hypoalbuminaemia, dehydration, and in severe infections death. In general, infection of the small intestine with *T. colubriformis* is characterized by villous atrophy and reductions in both saliva and serum phosphate concentrations that presumably reflect impaired phosphorous absorption (Wilson and Field, 1983; Poppi et al., 1986; Bown et al., 1989). Abomasal infections with *T. circumcincta* is frequently associated with reduced functionality of parietal cells, which results in reduced gastric acid secretion and increased abomasal pH and other pathophysiological changes that result in increased circulating gastrin and pepsinogen concentrations and protein loss in the gastric lumen (Anderson et al., 1981; Holmes, 1985; Fox, 1997). Nevertheless, regardless of the parasite species, the main manifestation of nematode infections in sheep is the temporary depression in voluntary feed intake i.e. anorexia (Sykes and Coop, 1977; Symons et al., 1981), which undoubtedly contributes to reduced production efficiency of the nematode infected sheep (Coop and Sykes, 2002).

The most common criterion for the reduced productivity of parasitised sheep is the reduction in body weight gain of the host. Lambs infected with 37,500 or 120,000 *T. circumcincta* larvae per week showed anorexia and had reduced growth rates of approximately 35% or 53% compared to non-infected counterparts during the first 12 weeks of infection, respectively (Symons et al., 1981). Reductions in bodyweight gain of 20% to 60% have been also recorded in lambs infected with the same or other nematode species (Sykes and Coop, 1976, 1977; Abbott and Holms 1983; Steel et al., 1980). In periparturient ewes, which may also show anorexia following infection with *T. circumcincta*, reductions in performance were reflected in significant weight loss and reductions in milk production and wool growth (Leyva et al., 1982). In addition to changes in body weight, alterations in body composition also occur in sheep since infected animals have lower deposition of fat, protein

and skeletal calcium and phosphorus compared to uninfected controls (Sykes and Coop, 1976, 1977).

However, comparisons of the performance of parasitized lambs with that of uninfected controls offered the same amount of feed (pair-fed animals) have shown that changes in animal metabolism and in the efficiency of food utilization can also account for impaired productivity (Coop and Holmes, 1996; van Houtert and Sykes 1996; Coop and Sykes 2002). In lactating ewes, which were infected weekly (for 12 weeks, starting 6 weeks before parturition) with 2,500 L3 larvae of *Haemonchus contortus*, milk production was 23% lower compared to milk production of uninfected control animals, despite the absence of anorexia in the infected ewes (Thomas and Ali, 1983). It has been therefore suggested that anorexia alone is unlikely to be totally responsible for the reduced production observed in parasitized sheep (Coop and Holmes, 1996; van Houtert and Sykes 1996; Fox, 1997; Sykes and Coop, 2001; Coop and Sykes, 2002). Bown et al., (1991) have suggested that losses of epithelial cells, plasma and extracellular fluids that are associated with nematode infections of the gastrointestinal tract have to be replaced, at a cost. In addition, the animal has to mount an immune response, involving local inflammatory responses, epithelial cell secretions and antibody production (Sykes and Coop, 2001; Greer, 2008). The cellular and humoral responses that the host needs to develop and/or maintain following nematode infections are likely to be nutrient demanding, particularly in sulphur-containing amino acids (Macrae et al., 1993; Sykes and Coop, 2001; Colditz, 2002; Greer, 2008). It is suggested that the detrimental consequences of gastrointestinal parasitism on animal productivity are mainly due to both anorexia and increased nutrient demand for immune response (i.e. acquisition and/or maintenance) and tissue integrity restoration. The aspects of anorexia and immune response during the gastrointestinal nematode infection in sheep, are described in more details below.

2.1.1. Anorexia during gastrointestinal infections in sheep

General. Reductions in voluntary food intake of 10% to 30% are typically observed in parasite naïve lambs following infection with abomasal (Sykes and Coop, 1977; Coop et al., 1982) or small intestine nematodes (Steel et al., 1980, 1982; Kyriazakis et al., 1996a,b) they are observed less frequently in large bowel nematode infections. The magnitude of anorexia can range in severity from as little as 5-10% to complete anorexia within 1-3 weeks of challenge (Sykes, 1991; Coop and Sykes, 2002) and is likely to be related to the number of larvae ingested, within certain limits. For example, in *T. colubriformis* infections the degree of anorexia was directly related to larval doses of 3000, 9500, and 30,000 per week, with the last dose reducing consumption by about 55%, but no reduction in food intake was observed at dose rates of 300 or 950 larvae per week (Steel et al., 1982). Similarly, in *T. circumcincta* infections, Coop et al., (1982) showed that the reduction in food intake was greater in lambs given 35,000 L3 per week than those receiving 15,000 L3, which in turn was greater than those dosed with 7,000 L3 larvae per week. The timing of the occurrence of anorexia following infection with *T. circumcincta* or *T. colubriformis* differs between these parasite species. Anorexia following infection with *T. circumcincta* develops as early as the second week of the onset of infection (Sykes and Coop, 1977; Symons et al., 1981), whereas anorexia following *T. colubriformis* infection develops after the fourth week of the onset of infection (Steel et al., 1980; Kyriazakis et al., 1996b). Food intake seems to recover once animals have become immune to parasites (Kyriazakis et al., 1994, 1996b), although this is likely to depend on the rate of development of the immunity, depending on the parasite species (Sykes and Coop, 1977; Coop et al., 1982; Kyriazakis et al., 1994, 1996b).

Factors that may affect anorexia in sheep. The occurrence of parasite-induced anorexia in sheep has been studied extensively over the past thirty years almost solely in parasite naïve crossbred lambs; comparisons between breeds on the degree of anorexia following nematode infection are not available so far. However, according to a recently

developed model for predicting feed intake of growing animals during exposure to pathogens, differences in anorexia are expected between breeds of animals that differ in their production potential (Sandberg et al., 2006). In addition, the occurrence of anorexia in secondary infections, i.e., in the immune host, has not been studied extensively and there is scant evidence whether re-exposure to infection of previously infected sheep results in renewed anorexia or not. For example, there are only two studies available to report results on anorexia in periparturient ewes. Leyva et al., (1982) found that periparturient ewes can show a reduction in voluntary food intake of around 16% during lactation, following infection with *T. circumcincta*; however, in the study of Thomas and Ali (1983) there was no significant difference in intake between control and infected ewes with *Haemonchus contortus* during the periparturient period, although infection had detrimental effects on ewe milk production. The outcomes of secondary infection on food intake are more consistent in studies with barren ewes (i.e. non-pregnant, non-lactating), and these studies report that anorexia is not likely to occur in re-infected mature sheep (Kimambo et al., 1988; Greer et al., 2005). In occasions in which re-infection may result in anorexia, Symons (1985) suggests that the causes and effects are unlikely to be different from those of primary infections.

The effect of dietary protein intake on the degree of anorexia has not been studied extensively and the available studies in this respect are summarised below. Coop and Sykes (2002), in their review about the interactions between parasitism and nutrition, report that the degree of anorexia is probably influenced by the intake of dietary protein or phosphorus (P) or both. Relevant to these suggestions is the observation that individually penned sheep infected with *T. colubriformis* and offered a choice between isoenergetic foods with a high (206 g/kg DM) or a low (86 g/kg DM) protein concentration, consumed a higher proportion of the high-protein diet than uninfected controls (Kyriazakis et al., 1994, 1996b). In the study of Kyriazakis et al., (1996b) lambs fed solely on the high protein diet had an overall reduction in intake of about 11%, while the reduction in intake in the low protein fed lambs

was approximately 17%, but the interaction between parasitism and protein intake was not statistically significant. Similar were the results of the study of Bown et al., (1991), who found that the reduction in intake in protein or glucose supplemented lambs (abomasal infusion of casein or glucose) following deliberate infection with *T. colubriformis* averaged 20%, while in infected unsupplemented lambs the reduction in intake was approximately 30%. Reduced food intake was observed in some lambs fed restrictedly on a low (88 g CP per kg DM)- but not on a high (170 g CP per kg DM)-protein diet, following a single dose of *H. contortus* infection (Abbott et al., 1985), but no quantitative figures on food intake were given. It is not clear from the study of Abbott et al., (1985) whether the high-protein group would have shown some degree of anorexia if it had been fed ad libitum instead of the 1000 g per day. The only other study that investigated effects of food quality on anorexia reports that the degree of anorexia following trypanosome infection in goats was similar in animals that received either a high or a low protein food (vanDam et al., 1997). Particular studies on the effect of protein on anorexia in periparturient ewes are completely lacking, although the effects of protein supplementation during the periparturient relaxation of immunity have been studied extensively. It is evident that further investigation into the role of food quality on parasite induced anorexia, especially in the periparturient ewe, is warranted.

Underlying mechanisms of parasite-induced anorexia in sheep. Despite the importance of the factors (i.e. breed, protein supply, immune status) that may affect the occurrence of anorexia in parasitised sheep, underlying mechanisms that may be involved are not yet fully understood. Such knowledge however, is essential in order to understand better the interactive effects between breed, nutritional regime and immune status on the parasite induced anorexia in sheep. In this paragraph, the current knowledge on the reasons and the underlying mechanisms that may be involved in the parasite-induced anorexia in sheep, are reviewed.

At first sight, the occurrence of anorexia seems somewhat paradoxical as it appears as though animals have evolved a mechanism in which nutrient intake is reduced at a time when demands for energy and protein for the development of immune function and repair of damaged tissue are increased. However, it has been argued that anorexia has an evolutionary and ecological significance and some authors proposed that it has to be beneficial for the host, particularly early in disease, but, agreed that if prolonged it adversely affects the host (Symons, 1985; Hart, 1998; Kyriazakis et al., 1998). The most convincing support for the role of anorexia during disease comes from the experiments of Murray and Murray (1979) on mice infected with the bacterium *Listeria monocytogenes*. Force feeding of infected mice to a "normal" energy intake (i.e. that of their uninfected controls) led to an increased mortality and shortened survival time, compared with infected mice that were allowed to become anorexic. In addition, if food deprivation is employed two to three days before the bacterial infection, survival rates in mice can be increased (Wing et al., 1982).

With regards to the parasite-induced anorexia in sheep, Kyriazakis et al., (1998) in a comprehensive review have discussed several functional hypotheses to account for the reduced feed intake in parasitized animals. These authors, in view of the available knowledge, concluded that the most plausible hypothesis is that anorexia should be considered as a disease-coping strategy that has evolved in order to help animals to promote an effective immune response. This hypothesis certainly fits with evidence that increased intakes of trace elements, and particularly of iron and zinc, may reduce immune responses to bacterial diseases (Chandra 1993). The occurrence of anorexia results in reduced changes of raising plasma concentrations of iron from iron-containing foodstuffs, but, such reasoning is more applicable for carnivores that can take in more iron in a short period of time rather than grazing ruminants (Hart, 1998).

In contrast, reduced intakes of protein (Houdijk et al., 2003; Kahn et al., 2003) or energy (Valderrabano and Uriarte, 2003) do not seem to promote an effective immune response as shown in studies with sheep fed restricted amounts of food, compared to their adequately fed counterparts. Therefore, rather than being viewed as a disease coping strategy, the reduction in food intake during gastrointestinal parasitism may be considered a consequence of the developing immune response. Direct evidence for this suggestion, at least for the *T. circumcincta* or *T. colubriformis* species, comes from a recent study which investigated the role of the immune response per se in the depression of appetite caused by these parasites during the first 10 weeks of infection (acquisition phase) in previously naive lambs (Greer et al., 2005). In this study, growing lambs were fed ad libitum and were either non infected or infected with 2000 larvae of *T. colubriformis* per day or similarly infected but concurrently immunosuppressed by glucocorticoids. The food intake of infected sheep was reduced by 30% compared to the non-infected counterparts, while lambs that were infected but immnnosuppressed did not show any reduction in feed intake. These results allowed the authors to conclude that the effect of infection on food intake is the consequence of the activation by the parasite of the host immune response rather than being simply the physical damage to the gastrointestinal tissue alone.

Earlier studies on the possible causes of anorexia in parasitised sheep supported that food intake is depressed in ruminants by reduced flow of digesta as a result of increase in gastrin (Fox et al., 1988, 1989) and abnormal gut motility (Bueno et al., 1982). However, there were inconsistent changes in serum gastrin in sheep parasitized by an isolate of *T. circumcincta* in which larval development takes place almost exclusively in the pylorus and there were periods of anorexia without any increase in serum gastrin (Simcock et al., 1999). Much interest was also developed in earlier years about the role of the neuropeptide cholecystokinin (CCK; a hormone of the gastrointestinal tract) on food intake regulation as food intake increased in parasitized lambs given a CCK receptor antagonist that acts in the

hypothalamus (Dynes et al., 1998). As mentioned earlier, the recent finding that parasite-induced anorexia in young sheep can be completely abolished if an effective immune response is prevented (Greer et al., 2005), provided support of the view that anorexia is directly related to the immune system activation. A number of earlier studies in mice and rats suggest that anorexia during infection is caused by the action of the pro-inflammatory cytokines in the hypothalamus (Spurlock, 1997; Plata-Salaman, 1998; 2004). Cytokines are a diverse group of small proteins that induce responses in the cells that produce them or in other cells via specific receptors (Janeway, 2001). Recent studies (Pernthaner et al., 2005, 2006) provided novel evidence that immune response to nematode infection in sheep depends on pro-inflammatory cytokine production, as suggested earlier for parasitised murine models (Roberts et al., 1999; Maizels and Yazdanbakhsh, 2003). However, it is currently not known whether pro-inflammatory cytokines contribute to the anorexia of nematode infections in sheep. In addition, although specific evidence from sheep is lacking, there is increasing evidence, admittedly from other animal species that the components of the cytokine cascade in the early phase of the immune response are up-regulated by leptin expression (Grunfeld et al., 1996; La Cava and Matarese, 2004; Fantuzzi, 2005; Matarese et al., 2005). Further elucidation of the possible role of cytokines and leptin on anorexia the parasite induced anorexia is reviewed later in this chapter (part 2.3.2.)

2.1.2. Immune response to nematode infections

General aspects. Lambs do not appear to be born with a natural non-specific (innate) immunity to gastrointestinal nematode infections (Smith et al., 1985), but they acquire immunity following continuous exposure to gastrointestinal nematodes. Following the first contact with gastrointestinal worms, hosts become sensitised by recognising the parasite antigens (macromolecules such as proteins and polysaccharides) as non-self and gradually develop immunity against them (Balic et al., 2000). Generally, in parasite-naive hosts, as the protection develops, animals first acquire the ability to inhibit the development of infective

larvae, then become able to inhibit the establishment of incoming larvae, to reduce the fecundity (egg-laying capacity) of adult female worms and finally to expel adult worms (Seaton et al., 1989). This phase of acquisition of immunity to gastrointestinal parasite is relatively long compared, for example, to bacterial or blood-born protozoan infections (Janeway, 2001). Development of lamb immunity against *T. circumcincta* or *T. colubriformis* parasites may take between 4 to 8 weeks of continuous and repeated challenge (Seaton et al., 1989; Dobson et al., 1992; Barnes and Dobson, 1993; Coop et al., 1995). Although many aspects of the immunisation process and acquisition of immunity are not yet fully understood (Balic et al., 2000), this delay in the development of immunity may occur due to the stage-specific nature of immunity and the changing of antigenic profile of developing/moulting infective larvae (Keith et al., 1990; Harrison et al., 2003), or may reflect the loose degree of contact between parasites and the host tissue (Wakelin, 1996). Once animals have acquired immunity to gastrointestinal nematodes, they can be successfully protected from subsequent challenge infections. In rodent models and ruminants, the first evidence of developing protective immunity to a primary infection and acquired protective immunity following challenge infection is usually a decreasing and no egg output in faeces, respectively. Although faecal egg count (FEC; eggs per gram faeces, epg) is the only parasitological parameter of immunity that can be obtained sequentially and regularly in the same animal in the course of an infection, it does not strictly reflect the fecundity of the female worm population (Claerebout and Vercruysse, 2000), as other factors may affect the faecal egg count such as the amount of food intake and the digestibly of the food. However, a very good correlation between faecal egg counts and the number of eggs in utero was found in *C. oncophora* (Claerebout and Vercruysse, 2000) and *O. circumcincta* (Stear et al., 1995) infections in calves and sheep respectively.

The acquired immunity to parasites is likely to diminish with time if challenge is withdrawn (Dineen and Wagland, 1966; Jackson et al., 2004) or it can break down to varying

degrees during the periparturient period. The periparturient relaxation of immunity (PPRI) is particularly evident for abomasal nematode infections and is manifested by a high rise in FEC (Barger, 1993). The timing of PPRI is variable, but, in general, the immune status of the ewe is lowered from about 2 to 3 weeks before and up to 6 to 8 weeks after parturition (McAnulty et al., 2001). The causes of PPRI are still subject to debate, but there is supporting evidence that it may have a nutritional basis (this aspect is reviewed later).

Mechanisms of immune response. Regulation of gastrointestinal nematode populations in small ruminants has been shown to involve effector mechanisms that affect key biological processes such as establishment, growth, reproduction and persistence of the parasite within the host (Barger, 1988). These mechanisms involve the stimulation of both cellular and humoral (immunoglobulin (Ig) based) responses that provide protection that is specific to both stage of development and parasite genera (Balic et al., 2000). Such mechanisms responsible for the resistance against parasitic infections are: (i) the mucosal mast cell and globule leukocyte hyperplasia, (ii) the proliferation of eosinophils in both blood and local mucosal sites, (iii) the increase of mucus production and appearance in mucus of substances inhibitory to parasites and (iv) the production of nematode specific IgGl, IgG2, IgE and IgA antibodies (Huntley et al., 1992, 1998; Harrison et al., 1999, 2003; Balic et al., 2003).

To help explain the observations involved in cellular immunity, the phenomenon has essentially been split into two types of response, either a T-helper1 (Th)-1 response, which is generally considered to be an inflammatory and "innate" action of the immune system, or a Th-2 type response which is consider as specific, or "acquired" immune reaction to antigens (Maizels and Yazdanbakhsh, 2003). Nematode infections invoke a Th2 response, which is associated with secretion of IL-4, IL-5, IL-9, IL-10 and IL-13 which promote the growth and differentiation of mast cells and eosinophils as well as production of IgE, which is believed

to mediate the activation of these cells (Jankovic et al., 2001). The majority of our understanding of cell mediated immune response towards gastrointestinal infections has been derived from murine and human studies (Maizels and Yazdanbakhsh, 2003). However, recent investigations of cytokine production profiles in the intestinal lymph of sheep have shown increased cytokine expression in lambs selected for enhanced immunity during infection with *T. colubriformis* (Pernthaner et al., 2005, 2006).

In addition to the changes in cellular immunity, parasite-induced antigenic stimulation causes a pronounced increase in the production of an array of nematode-specific immunoglobulins. The most abundant of these in ruminants is immunoglobulin-A (IgA), which has been implicated in the regulation of worm length and fecundity of populations of *T. circumcincta* (Smith et al., 1983; Stear et al., 1999; Strain and Stear, 2001), and levels of which in lymph draining the abomasum can indicate protection against *T. circumcincta* (Smith et al., 1987; Strain and Stear, 2001). Although the role of IgG is unclear, phenotypic correlations of up to 0.62 for FEC with serum IgG levels have been observed (Douch et al., 1995). In comparison, IgE is believed to mediate the maturation of mast cells and subsequent production of globule leukocytes (Huntley et al., 1992). Consequently, serum IgE levels are negatively associated with worm burden, since the expulsion of worms from the gut would result in reduced mast cell turnover that would reduce the requirement for IgE, thus causing a rise in serum levels (Thatcher et al., 1989).

In summary, the immune response that is invoked by gastrointestinal nematodes is multifaceted and very complex. As a consequence, there are still many gaps in our knowledge regarding the ovine immunological cascade and the role of each component (Gasbarre et al., 2001; Meeusen, 1999). Furthermore, these complex immunoregulatory responses are relatively costly for the host to maintain since they require continuous production of mucins, antibodies, inflammatory mediators and other effector cell products in

addition to the loss of endogenous proteins associated with hypersensitivity reactions (Sykes and Coop, 2001; Jackson et al., 2004; Colditz, 2008). In addition, experimental evidence has shown that expression of immunity to parasites is greatly influenced by the host genotype, nutrition, immune status and age of the host (Gray et al., 1992; Coop and Kyriazakis, 1999; Coop and Sykes 2002; Colditz, 2002; Colditz, 2008), which are reviewed in the following section.

2.2. Factors affecting sheep response to nematode infection

Two terms have been used to describe the response of the host to parasitic infections; resistance and resilience. Resistance has been used to define the ability of the host to initiate and maintain immune responses in order to limit larval establishment and the longevity or fecundity of adult worms since, in the main, resistance has been judged by the host's ability to limit faecal egg count (Woolaston and Baker, 1996; Baker, 1998; Coop and Sykes, 2002). Resilience has been used to describe the ability of an animal to function or maintain normal productivity in the face of larval challenge or nematode egg count in faeces (Woolaston and Baker, 1996; Baker, 1998; Coop and Sykes, 2002). However, with the increasing knowledge of the nutritional implications on infection, the above definitions cannot be independent and may well prove to be unhelpful (Sykes and Coop, 2001).

Attributes of the host and the environment that influence both the strength of an immune response (i.e. resistance) and the outcome of an infection (i.e. resilience) include genotype, age, gender, passive immunity, prior exposure to the pathogen, capacity to recall the antigen, concurrent infections, physiological status, nutritional status, day length and presence of concurrent stressors (Colditz, 2008). Of these attributes, the most relevant for the purposes of the present thesis is host genotype (i.e. production potential or genetic resistance), and host nutrition, both of which are described below.

2.2.1. Host genotype

It has been recognized for many years that different breeds of sheep vary in their resistance to nematode infection. The variation in resistance to infection between breeds is very likely to be associated with the variation in breed production potential (McEwan et al., 1992; Morris et al., 1996; Bisset et al., 2001), although, it appears to be no general

relationship between resistance to parasites and productivity within a breed (Bishop and Stear, 1999). In a comprehensive review, Rauw et al., (1998) presented more than a hundred references on undesirable correlated effects of selection for high production efficiency, with respect to increased immunological susceptibility and other physiological and behavioural problems in livestock production systems. Evidence for such effects in gastrointestinal infections in sheep can be observed from breeds that have been selected for increased growth rates or fleece weight and have been found to have higher FEC than randomly bred animals (McEwan et al., 1992, Morris et al., 1996, Bisset et al., 2001). In addition, breeds of sheep showing resistance to nematode infection often display a reduced PPRI when compared with susceptible or unselected sheep (Barger, 1993). For example Courtney et al., (1984) reported differences between the extent of the PPRI in exotic breeds when compared with the PPRI in US domestic breeds. This, reflected differences between breeds in resistance to both existing and newly acquired infections. A difference in the extent of the PPRI between Merino and Merino×Border Leicester ewes was also noted by Donald et al., (1982) with Merinos being more susceptible. Conversely however, the tropical breeds that have been shown to be substantially more resistant to gastrointestinal nematodes than European breeds, when compared under the same conditions, tend to be less productive in temperate countries (Woolaston and Baker, 1996; Bisset et al., 2001).

In practice, increased resistance would be expected to lead to improved resilience or performance in the face of incoming larval challenge (Bishop and Stear 1997). In this respect, simulation of the epidemiological relationships between productivity and resistance to gastrointestinal nematodes in young lambs estimated that the reduced pasture contamination as a result of selecting animals with a low FEC would result in large increases in bodyweight gain and a favourable (negative) genetic correlation between FEC and bodyweight (Bishop and Stear 1997). However, selection studies for improved resistance have generally not resulted in increases in resilience (McEwan et al., 1997; Williamson et

al., 1995; Morris et al., 1997). For example Morris et al., (2000) have found that performance of reproductive ewes has been only slightly increased or not increased at all (Morris et al., 1997) in breeds selected for low FEC. In addition, selection for low FEC affected negatively the efficiency of bodyweight gain in post-weaning lambs (Watson et al., 1992; Morris et al., 2000; Bisset et al., 2001).

Comparison of immune function between breeds that have been selected for genetic resistance with unselected breeds suggests that the genetic variation in response to infection is likely the result of the mechanisms that regulate the prioritization of nutrient use between immune defence and production traits (Coop and Kyriazakis, 1999; Sykes and Coop, 2001; Colditz 2002; Colditz 2008; Greer, 2008). This is not surprising given the evidence that genetically resistant sheep have greater numbers of mast cells and globule leucocytes in the abomasal and intestinal mucosa (Stankiewicz et al., 1995; Douch et al., 1996) and greater production of parasite specific immunoglobulins during infection with the intestinal nematodes (Pernthaner et al., 2005, 2006), components that are proteinaceous in nature (Bown et al., 1991; Sykes and Coop, 2001).

The accumulating evidence that breed production efficiency is negatively correlated with the host resistance (i.e. ability to initiate and/or maintain immunity) to infection and the finding that anorexia following nematode infection is a direct result of the immune response to infection (Greer et al., 2005), suggests that differences in the magnitude of anorexia between breeds that differ in their production potential are expected. In a recently developed model Sandberg et al., (2006) postulated that susceptible to infection genotypes will show a greater reduction in food intake during the course of a primary nematode infection than resistant genotypes. However, experimental evidence for this assumption has not been provided yet.

2.2.2. Host nutrition and its interaction with genotype

It has long been recognized that nutrition can influence the outcome of exposure of sheep to nematode parasites (Gibson 1963), although only during the last twenty years research activity in this area has established that nutrition enhances host resistance and resilience to gastrointestinal infections (Coop and Holmes, 1996; Coop and Kyriazakis, 1999; Sykes and Coop, 2001). This is mainly mediated through acquired immunity, and thus nutrition has the potential to affect the rate of acquisition and/or the degree of expression of immunity (van Houtert and Sykes, 1996; Kyriazakis and Houdijk, 2006). Most research into the effects of nutrition on immunity to gastrointestinal nematodes in sheep has concentrated on metabolizable protein (MP). This seems sensible because many components of the immune effector responses are highly proteinaceous in nature (Bown et al., 1991; Coop and Holmes, 1996; Sykes and Coop, 2001). In addition, damaged epithelial cells and losses of plasma and extracellular fluids as a result of the activity of the established nematodes in the gut have to be replaced, imposing an increased cost of protein synthesis (Sykes and Coop, 2001). Consequently, the proteinaceous nature of the immune response and the maintenance and/or restoration of tissue integrity and function impose an increased protein demand for the host, especially in the view of the reduced food intake as result of infection (Coop and Holmes, 1996; Coop and Kyriazakis 1999; Sykes and Coop, 2001). The immune system, like any bodily function, would also have requirements for energy and micronutrients like vitamins and minerals, and increased availability of each of these resources can be expected to improve immunity to gastrointestinal nematodes provided that they are first limiting (Kyriazakis and Houdijk, 2006). However, the focus of the current section will be on the role of MP nutrition on immune response against gastrointestinal nematodes; moreover, moderate changes in energy nutrition do not appear to greatly affect gastrointestinal parasitism (Bown et al., 1991; Donaldson et al., 1998)

Effects of MP supply on resistance to gastrointestinal nematodes can be viewed within a nutrient-partitioning framework, which accounts for the partitioning of nutrients between somatic tissue and the immune system during different phases of the growth cycle (Coop and Kyriazakis, 1999). From the available information in the literature, the framework argues that the poor growth of lambs during early infection occurs because acquisition of immunity is more important for survival and would have priority over gain in body protein and hence the high susceptibility to infection. The partitioning framework suggests that responses of the immune system to protein supplementation would be small during this early acquisition phase but would be more apparent later in a parasitic infection, when the host is expressing a degree of immunity. In contrast, in the reproductive female it appears that immunity is foregone in the interests of reproduction (Coop and Kyriazakis, 1999)

With regards to the effect of protein supplementation during the early acquisition phase, evidence in the literature supports the view that protein supplementation enhances the rate the animal can "acquire" and the degree it can express immunity against a parasitic challenge, rather that it influences initial parasite establishment in naive sheep (Coop and Holmes, 1996; Coop and Sykes, 2002). For example, Abbott et al., (1988) showed that FEC of an established *H. contortus* infection could be reduced by approximately 30% when sheep were fed a high-protein diet (169 g CP kg−1 dry matter (DM)) in comparison with animals offered a low-protein ration (88 g CP kg−1 DM). In addition, approximately three times as many worms were recovered at post-mortem from the sheep that were offered the low-protein ration (Abbott et al., 1988). Experimental studies with *T. colubriformis* infection in growing sheep have demonstrated that provision of additional protein, either as a direct infusion into the abomasum (Bown et al., 1991) or fed as a dietary supplement (Kambara et al., 1993; van Houtert et al., 1995), can lower the fecundity and/or increase the rate of expulsion of parasites from the host. The decrease in FEC or in the apparent rate of worm expulsion appears to be influenced by the level of protein supplementation offered (van

Houtert et al., 1995; Van Houtert and Sykes, 1996). Enhanced immune expression in response to protein supplementation has also been reported in studies with *T. circumcincta* (Coop et al., 1995) and mixed *T. circumcincta* and *T. colubriformis* infections, (Smith et al., 1996). In the study, of Coop et al., (1995), the development of immunity was assessed by observing trickle-infected lambs, supplemented with bypass protein for 8 weeks. Animals were then treated with anthelmintic and given a single challenge dose of *T. circumcincta* larvae. Supplemented animals had lower worm burdens, increased globule leukocyte and mucosal mast cells numbers, higher concentration of mast cell proteases and a significantly higher proportion of inhibited larval development, allowing the authors to conclude that the provision of by-pass protein accelerated the development of immunity in these lambs (Coop et al., 1995).

The beneficial effects of protein supplementation on the degree of the acquisition of immunity in parasitised lambs are likely to be more pronounced in breeds that are more susceptible to infection (Coop and Holms, 1996). Considerable evidence in support of this view comes from the studies of Abbott et al., (1985) which compared Finn-Dorset and Scottish Blackface lambs that are known to differ in their susceptibility to *H. contortus* infection (i.e. Finn-Dorset being more susceptible; Altaif and Dargie, 1978). The results indicated an additive detrimental effect of genetic susceptibility and poor diet but also showed that the expression of genetic superiority in terms of disease resistance was not compromised by poor protein nutrition. A similar study using lambs of a relatively resistant breed, Scottish Blackface, showed that within this breed protein supplementation did not influence the FEC and worm burdens whilst in the lambs of the susceptible breed (Hampshire Down lambs) the higher protein diet (173 g CP/kg DM vs. 98 g CP/kg DM) did reduce the FEC (Wallace et al., 1996). These results clearly show that the benefits of a superior genotype are not lost on a low protein diet whilst a high protein diet can help overcome the disadvantages of an inferior genotype (Coop and Holmes, 1996).

In the case of the periparturient relaxation of immunity (PPRI) results of several studies support the view that the occurrence of PPRI has a nutritional basis (Coop and Kyriazakis, 1999). An experiment undertaken by Donaldson et al., (1998) demonstrated that protein supply appears to be more important than energy supply in the host- parasite interactions in the ewe, a finding similar to that reported for young growing animals (Bown et al., 1991). Subsequent studies showed that supplementation with protein in late pregnancy or early lactation or both can reduce the faecal nematode egg output and/or worm burdens in ewes (Donaldson et al., 2001; Houdijk et al., 2000, 2001a; Kahn et al., 2003), goats (Chartier et al., 2000) and rats (Houdijk et al., 2005a), while protein scarcity exaggerates the extent of PPRI. The relaxation of immunity is influenced by nutritional demand, being greater in ewes carrying or rearing twin lambs compared with singles (Houdijk et al., 2001b), and that the response will be moderated by the extent of body protein reserves (Houdijk, et al., 2001a). In addition, the breakdown of immunity was reversed when the reproductive effort of the dam was reduced, by the removal of one of the lambs (Houdijk et al., 2006). These data and those from recent experiments in rats (Houdijk et al., 2003, 2005a) support the view that an increase in MP supply or a reduction in MP demand can ameliorate PPRI to *T. circumcincta* during periods when there is a scarcity of MP. However, in contrast to studies with growing lambs, the interactions between nutrition and genotype in breeding ewes have been not studied extensively and these interactions merit further research (Kahn et al., 2000; Coop and Sykes, 2002). In addition, whether protein supplementation can affect the degree of anorexia in nematode infected ewes during the periparturient period in a similar way to its effects on PPRI it is not known.

Nevertheless, despite the view that sheep response to gastrointestinal parasitism is influenced by the host genotype, nutrition and their interactions, the underlying mechanisms responsible for the occurrence of anorexia are not yet fully understood. In an attempt to elucidate the mechanism responsible for the occurrence of anorexia in sheep following

nematode infections, the present study investigates the implication of the adipocyte hormone leptin. Thus, the role of leptin in the regulation of food intake and immunity is reviewed in the next section.

2.3. The role of leptin in immunity and food intake regulation

General. The discovery of the *ob* gene and its encoding protein leptin (Zhang et al., 1994), has renewed the interest in the regulation of food intake and how intake is integrated with metabolism, accumulation of body energy stores, reproduction and immunity (Friedman and Halaas, 1998; Flier, 1998; Ingvartsen and Boisclair, 2001; La Cava and Matarese, 2004; Tolkamp et al., 2007). Leptin is mainly produced by the cells of the adipose tissue and, at lower levels, by tissues such as stomach, skeletal muscle, placenta and mammary gland (Friedman and Halaas, 1998; Chilliard et al., 2001; Chilliard et al., 2005). Based on anatomic and functional data, it appears that leptin exerts its effects on food intake regulation and energy balance mainly by acting in the brain, as leptin receptors are abundantly present in the hypothalamic regions in humans, rodents (reviewed by Ahima and Flier, 2000) and sheep (Thomas et al., 2001). However, short leptin receptor isoforms are expressed in peripheral tissues, such as kidney, liver, lung, and gonads, where they may serve a transport and/or clearance role (Ahima and Flier, 2000). Total leptin deficiency, although very rare, causes severe obesity in both rodents (Zhang et al., 1994) and man (Montague et al., 1997); administration of recombinant leptin reduces food intake and body weight of leptin-deficient (*ob/ob*) and diet-induced obese mice but not in leptin-resistant (*db/db*) obese mice (Campfield et al., 1995). The fact that the initial leptin's function appeared to be resisting obesity and promoting leanness led to the choice of the name "leptin" (Campfield et al., 1995), from the Greek root *leptos*, meaning thin.

The biology of leptin has been studied extensively in rodents and humans (Friedman and Halaas 1998; Flier, 1998; Ahima and Flier, 2000; Faggioni et al., 2001; Matarese et al., 2002; Kershaw and Flier, 2004; Fantuzzi, 2005; Maratos-Flier, 2008). The biology of leptin in domestic animals has been progressed less rapidly (Houseknecht et al., 1998; Ingvartsen

and Boisclair, 2001; Chilliard et al., 2001, 2005) due to difficulties encountered for the development of specific tools to study leptin gene expression and plasma leptin variations in ruminants (Chilliard et al., 2001; Chilliard et al., 2005). Preliminary results for plasma leptin concentrations in ruminants were obtained by the use of a commercial "multi-species" RIA kit (e.g.: Bocquier et al., 1998; Chilliard et al., 1998; Soliman et al., 2001). However, the multi-species commercial RIA kit provided estimates of plasma leptin in sheep and cattle that are low and unresponsive to changes of nutrition or adiposity (Bocquier et al., 1998; Chilliard et al., 1998; Ehrhardt et al., 2000). Ruminant specific RIAs, and in particular ovine-specific RIAs for leptin determination have been developed since 2000 (Delavaud et. al., 2000; Marie et. al., 2001; Ehrhardt et al., 2000; Blache et. al., 2000). The slow rate of development of ovine specific RIAs seems to be due to the low immunogenicity of ruminant leptin, since efficient antibodies were obtained either in rabbits or in birds immunized with high doses of recombinant ruminant leptin during several months (Chilliard et al., 2005; Dr. Alastair Wylie, personal communication). Thus, the role of leptin on nutritional behaviour and particularly on the immune response in farm animals has not yet been adequately investigated (Ingvartsen and Boisclair, 2001; Kulcsar et al., 2005). In this section, the available literature on the biology of leptin in ruminants, with special focus on the food intake regulation and immune responses in sheep, is reviewed. However, because in many instances information from ruminant studies is not available the review includes examples from rodent and human studies as well.

2.3.1. Effects of body fatness and food intake on leptin production

Leptin synthesis is influenced by the status of energy stores in fat, as evidenced by increased adipose *ob* mRNA expression and plasma leptin concentrations. Adipocyte size is an important determinant of leptin synthesis, as larger adipocytes secrete more leptin than smaller adipocytes in the same individual (Ahima and Flier, 2000). Plasma concentrations of leptin correlate with body fatness in all species studied to date, including humans (Considine

and Caro, 1996), rodents (Maffei et al., 1995), sheep (Blache et al., 2000; Delavaud et al., 2002, Tokuda et al., 2003), cattle (Ehrhardt et al., 2000; Ren et al., 2002) and camel (Chilliard et al., 2005). The interactions between body fatness and breed on leptin production has not been studied extensively in sheep, but available studies from beef and dairy cattle suggest that breed differences in body fatness are positively associated with breed differences in leptin mRNA expression in adipose tissue, with leaner breeds having lower plasma leptin concentrations (Ren et al., 2002; Higashiyama et al., 2003; Bellmann et al., 2004). However, Delavaud et al., (2002) showed that in cattle, breed differences in plasma leptin concentrations disappear when leptin values are corrected for individual differences in subcutaneous adipocyte size, suggesting that plasma leptin reflects primarily differences in body fatness (Chilliard et al., 2005).

Plasma concentration of leptin is also affected by acute changes in food intake. In rams, complete food deprivation causes a rapid fall in plasma leptin concentrations which are positively associated with plasma insulin, and negatively correlated with non-esterified fatty acids (NEFA), both between meals and during fasting (Marie et al., 2001). Long-term restricted feeding decreases the plasma leptin concentration in adult ewes (Delavaud et al., 2000), adult rams (Blache et al., 2000), pregnant ewes (Thomas, et al., 2001), and lambs (Morrison et al., 2001; Ehrhardt, et al., 2000). Contrary to observations in rodents (Ahima and Flier, 2000) plasma concentration of leptin did not change (Blache, et al., 2000) or increased only slightly (Marie et al., 2001; Kadokawa et al., 2003) within a few hours after meal intake in sheep. Nevertheless, circulating leptin levels changed within 48 hours, in parallel with the changes in dietary intake (i.e. from moderate to high, or high to moderate) in pregnant ewes (Thomas, et al., 2001). Furthermore, the concentration of leptin in plasma increased approximately two-fold within 5 days after increasing the plane of nutrition in adult rams (Blache et al., 2000) and cattle (Delavaud et al., 2002). However, in evaluating the consequences of feeding level on plasma leptin concentration, it is important to consider

the duration of the treatment as differences in plane of nutrition eventually will create differences in body fatness (Delavaud et al., 2000; Ehrhardt, et al., 2000). In general, plasma leptin response to feeding level is strongly dependent on body fatness (Chilliard et al., 2005). In Scottish Blackface ewes the effect of fasting (for 48 hours) was twice as great in very fat than in moderately fat animals (Daniel et al., 2002). Similarly, no effect of fasting (for 32 hours) was observed on plasma leptin concentrations in lean (15% lipids per BW) contrary to fat (35% lipids per BW) ewes (Henry et al., 2004). In growing sheep, leptin secretion was highly sensitive to feeding level, but again this effect was thrice as great in lambs with 15% compared to 5% lipids per BW (Ehrhardt, et al., 2003). It has been therefore suggested that long-term effects of feeding factors are predominant (probably via body fatness) on leptin expression in ruminants and interact with mid/short-term effects (Chilliard et al., 2005).

It is frequently repeated throughout the literature that leptin has satiety effects. Administration of leptin reduces food intake and increases energy expenditure in several animal models. For example both central (intracerebroventricular) and peripheral injections of recombinant leptin reduce food intake and increase energy expenditure in *ob/ob* mice (Campfield et al., 1995; Pelleymounter et al., 1995) and in wild-type rodents (Barrachina, et al., 1997), but also in monkeys (Tang-Christensen et al., 1999) and pigs (Bard et al., 1998) in a dose-dependent manner. However, it is of interest that leptin intracerebroventricular injection inhibits food intake and decreases adiposity more potently than peripheral leptin administration, which is probably due to the higher expression of leptin receptors in the hypothalamus rather than in peripheral tissues (Ahima and Flier, 2000). As in other species, the clear anorexic effects of leptin are observed in ruminants also by administration of recombinant leptin. In ovariectomized ewes, administration of human leptin for 3 days decreased the voluntary dry matter intake to approximately a third of the preinfusion intake (Henry et al., 1999). Similarly, lambs that were centrally administrated with leptin exhibited anorexia (Morrison et al., 2001), however, the anorexigenic effects of leptin were lost when

growing and adult sheep were fed restrictedly (Morrison et al., 2001; Henry et al., 2001). Although it is well established that leptin has clear anorexic effects and its concentrations in plasma are highly correlated with the body fat mass and the plane of nutrition in mammals, leptin secretion interacts also with a variety of other factors. The most relevant to the purpose of the present thesis are that of the immune response to infection, which is described below.

2.3.2. Leptin, immune response and anorexia

Recent evidence has shown that leptin plays an important role in the regulation of immune responses (La Cava and Matarese, 2004; Fantuzzi, 2005; Matarese et al., 2005), in addition to its role in food intake inhibition and stimulation of energy expenditure. Leptin is a member of the helical cytokine family with a structure resembling the cytokines IL-2, IL-6, and IL-15 (Faggioni et al., 2001; Matarese et al., 2002). In addition, the leptin receptor is a cytokine receptor and belongs to the class I cytokine receptor family that includes the common signal-transducing component for the IL-6-related family of cytokines (Faggioni et al., 2001; Matarese et al., 2002). This section reviews the existing evidence from murine and farm animal studies on the role of leptin in the regulation of immune responses and anorexia.

A number of studies in mice have shown that leptin affects both innate and adaptive immunity and this effect of leptin appears to be both direct and indirect, i.e., via modulation of central or peripheral pathways (Matarese et al., 2002; La Cava and Matarese, 2004). Leptin deficiency increases susceptibility to infectious and inflammatory stimuli and is associated with dysregulation of cytokine production (Faggioni et al., 2001). Experimental evidence on leptin implication in adaptive immune response comes from the study of Lord et al., (1998). This study showed that fasting induced immuno-suppression can be abolished by an increase in blood leptin (i.e. leptin administration), which shows the importance of high blood leptin for the immune response. In addition, studies from rodents and humans have

established that leptin promotes the activation of phagocytosis by monocytes and enhances the secretion of pro-inflammatory cytokines such as IL-6, IL-2 and TNF-α, important mediators of the APR and stimulates expression of adhesion molecules (La Cava and Matarese, 2004). These products are all involved in the regulation of inflammation and therefore markedly influence the immune response (La Cava and Matarese, 2004). Furthermore, plasma leptin concentrations are acutely increased by inflammatory and infectious stimuli such as lipopolysaccharide (LPS), turpentine, and cytokines (Grunfeld et al, 1996; Faggioni et al., 1998) and the increase in leptin production during local and systemic inflammation is modulated in a manner similar to the cytokine response to infection and injury (Faggioni et al., 2001). In experiments with nematode infected rats, Roberts et al., (1999) have suggested *Nippostrongylous brasiliensis* infection results in elevated leptin concentrations during the first 48 h of infection. Similarly, Mercer et al., (2000) observed that elevated leptin concentrations occurred only in those rats with a continuing infection, and were not observed in uninfected controls. Increased leptin levels also occur during experimental peritonitis in mice (Moshyedi et al., 1998) and in *Escherichia coli* intestinal infection in rats (Barbier et al., 1998). In the majority of these studies there was an undoubted positive correlation between the elevated circulating leptin levels and reduced intake, thus a role for leptin in the anorexia of infection was proposed (Barbier, et al., 1998; Grunfeld et al., 1996; Sarraf et al., 1997; Mercer et al., 2000; Niswender et al., 2001). However, on a per gram basis, leptin has been shown to be more weakly anorexigenic than IF-a, IL-1b, and TNF-a as these cytokines produced anorexia of greater magnitude with lower doses (Faggioni et al., 1997; Kaibara et al., 1998). Currently, it is unclear if and to what extent leptin participates in the anorexia of infection in ruminants, since leptin's role in the regulation of immunity during infectious diseases has been studied less extensively in farm animals. Even less studies are available about the role of leptin on the gastrointestinal parasitism of ruminants.

In lactating dairy cows, experimental endotoxin or natural outbreak of *mastitis* do not result in systematic and acute change in plasma leptin concentrations within 72 hours following infection (Kulcsar et al., 2005; Soliman et al., 2002). Similarly, acute puerperal *metritis* did not alter plasma leptin concentrations in dairy cows (Kulcsar et al., 2005). These results are in contrast with those found in laboratory rodents and primates, allowing the authors to conclude that the profile (and possibly the regulation) of endotoxin-induced leptin is probably species-related. Soliman et al., (2001) also showed that intravenous challenge with endotoxin failed to elevate plasma leptin concentrations in sheep, in spite of the marked anorexia and fever observed. Plasma leptin concentrations did not differ also between controls and *S. typhimurium*-infected pigs (Jenkins et al., 2004). Barb et al., (2001) studied the effects of acute endotoxin challenge in ad libitum fed pigs, thereby removing potential confounding effects of fasting on leptin expression and immune suppression. These authors concluded that leptin regulation in infected animals is under the control of two major opposing mechanisms: stimulation by inflammatory mediators, and inhibition due to inflammation-induced changes in energy metabolism (i.e. anorexia) and related hormones such as insulin. A similar mechanism may be operating during the gastrointestinal infections in sheep (Kulcsar et al., 2005). The study of Valderrabano, et al., (2006) suggests that leptin may be involved in the protective immune reactivity in periparturient ewes, but these results are not supported by those from Fox et al., (2006) in infected lambs. Although these studies are the first to test hypotheses in relation to plasma leptin concentrations during the gastrointestinal parasitism of sheep the results are difficult to interpret because of the absence of non-infected controls animals. In addition, there are no available studies on whether leptin is implicated in the parasite-induced anorexia in sheep. The possibility that the leptin is involved in the development of immunity and anorexia in parasitised sheep requires further investigation.

2.4. Objectives of the thesis

The present thesis used *in vivo* experimentation to test the interactions between genotype, immune status and nutrition on the consequences of the gastrointestinal parasitism of sheep, with special focus on the occurrence of anorexia. So far, grazing and indoors experiments have established that anorexia is a prominent feature of parasitic infections especially in previously naïve young lambs, but information on whether breeds differ in the magnitude of anorexia following infection, and whether anorexia occurs following re-exposure to infection is not available. With regards to the reproductive ewes, although the phenomenon of the periparturient relaxation of immunity (PPRI) is well established, there is scant evidence whether periparturient ewes show anorexia, and more interestingly, whether the degree of anorexia is affected by the dietary protein supplementation, which has been shown to reduce the extent of PPRI. The thesis examines how breeds of sheep that differ in their production potential respond in terms of anorexia, following a primary (previously naïve lambs) and/or a secondary infection (previously infected lambs or ewes) with the nematode *T. circumcincta*; and how protein nutrition affects the degree of anorexia in those breeds. It should be noted that an early occurrence of anorexia in sheep following experimental infection was preferred. This was because the hypotheses are related not only to the degree of anorexia in terms of magnitude but also in terms of duration. Thus, because anorexia occurs earlier in *T. circumcincta* infections, this parasite model was considered more appropriate for testing the hypotheses of the present study and was used throughout the experiments of the thesis.

The aim of this thesis is also to provide further knowledge on the role of leptin on the sheep response to nematode infections and therefore the thesis examines the possible implication of the hormone leptin in the development or expression of immunity in

parasitised sheep following infection with nematodes. In order to address the hypotheses developed for the effect of infection in plasma leptin concentrations, the experiments used a novel design which accounted for confounding effects of nutrition and/or body fatness between the infected and the control animals.

The specific hypotheses that have been tested in this thesis are described in details in each chapter, respectively. However, the overall adjectives of the present thesis can be summarised as follows:

1. To investigate whether breeds of different intrinsic capacity for growth differ in the magnitude and/or duration of anorexia following a primary infection with *T. circumcincta.*
2. To examine whether a secondary nematode infection results in renewed anorexia in lambs following re-exposure to infection; and whether breed differences are as apparent as during the primary infection.
3. To investigate whether or not ewes develop anorexia during the periparturient relaxation of immunity and whether the degree of anorexia is affected by breed; to examine whether protein nutrition can affect the degree of anorexia.
4. To test whether or not gastrointestinal infection with nematodes results in elevated plasma leptin concentrations in sheep during the acquisition or expression of immunity.

CHAPTER THREE

Changes in food intake and circulating leptin due to gastrointestinal parasitism in lambs of two breeds

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3.1. Abstract

A reduction in food intake is a prominent feature of many infectious diseases. However, the underlying mechanisms of parasite-induced anorexia in sheep are poorly understood. This study tested the hypotheses a) that the degree of parasite-induced anorexia in lambs is influenced by their growth potential and b) that nematode infection results in elevated plasma leptin concentration in lambs. The hypotheses were tested with Suffolk × Greyface (S) and Scottish Blackface (B) lambs that are known to differ in their growth potential (S lambs are of higher growth potential than B lambs). During a primary parasite infection, 24 out of 48 lambs per breed were trickle infected with 7,000 infective *Teladorsagia circumcincta* larvae per day, 3 days per week, for a period of 12 weeks (experiment I). The lambs were then de-wormed and after a 2 week interval, half of the 24 lambs per breed that were previously infected were re-infected for another 12 weeks with the same parasite and dose as used in the primary infection (experiment II). In both experiments, infected lambs were fed grass pellets ad libitum while non-infected lambs were fed grass pellets either ad libitum or restrictedly. Lambs of the S breed, were more susceptible than B lambs to nematode infection as judged from the differences in fecal egg counts ($P = 0.007$). Parasitized lambs of the more susceptible breed (S) showed anorexia, i.e. a decrease in intake of 13% compared to uninfected controls ($P = 0.01$), while no significant reduction in food intake was observed in lambs of the more resistant breed (B). Re-exposure to nematode infection of previously infected animals tended to result in renewed anorexia in S lambs but not in B lambs ($P = 0.08$) in a similar extent as during primary infection. Plasma leptin concentrations did not differ between ad libitum fed infected and control lambs, but were higher in infected than in non-infected lambs at a similar level of food intake during both the primary ($P = 0.02$) and the secondary parasitic infection ($P = 0.004$) in both breeds. The

results show that leptin may be involved in the response of lambs to infection, but that it is unlikely that leptin alone is responsible for the parasite induced anorexia in lambs.

3.2 Introduction

Anorexia associated with gastrointestinal nematode infections has a significant impact on productivity in livestock grazing systems. However, the underlying mechanisms that induce and maintain anorexia in infected sheep remain unclear. The recent literature suggests that anorexia following nematode infection in lambs is likely to be related to the development of the immune response (Greer et al., 2005). Recent evidence from studies in rodents indicates that the adipocyte hormone leptin plays an important role in the regulation of immune responses. Plasma leptin concentrations (PLC) are associated with ADFI (Henry et al., 1999) but also increase during infection and inflammation in many models of disease (Faggioni et al., 2001). Whether PLC increase during nematode infections in sheep, and if such increases in leptin are associated with parasite-induced anorexia, is not known.

Sandberg et al. (2006) postulated in a recently developed model that a genotype more susceptible to parasitic infection, as assessed by fecal egg count (FEC), is likely to exhibit a higher degree of anorexia compared to a resistant genotype. Host immune responses to infection can be compromised by the needs for maintenance and growth (Coop and Kyriazakis, 1999) and, therefore, differences in resistance to nematode infection can be expected between breeds that differ in their intrinsic capacity for growth. However, whether breeds of sheep that differ in their growth potential differ also in the degree of anorexia following infection is not known.

In the current study the following hypotheses were tested i) nematode infection will result in a greater extent and longer duration of anorexia in lambs of high growth potential than in lambs of lower growth potential and ii) infected lambs will have elevated PLC

compared to non-infected lambs at a similar level of food intake. Both hypotheses were tested during a primary and a secondary trickle infection in two consecutive experiments.

3.3 Materials and Methods

The experiments took place at the facilities of the Scottish Agricultural College after approval of the experimental protocol by the Animal Experiments Committee (ED AE07/2004) and under Home Office license for experimental infection and blood sampling (PPL 60/3004).

3.3.1 Experiment I (Primary Infection)

Animals, husbandry procedures and housing. Ninety-six weaned lambs, 48 Suffolk × Greyface crosses (S) and 48 Scottish Blackface (B) were used. The lambs were approximately twelve weeks of age and half of them were male and half female within each breed. All lambs were born indoors and, soon after birth, were transferred along with their mothers (as a single flock) onto a pasture which was newly ploughed and seeded and had never been grazed before in an attempt to maintain it parasite-free. Before lambing the ewes had been orally drenched with a combination of fenbendazole (Panacur 10 %; Hoechst Roussel Vet Ltd, Milton Keynes, Bucks., UK) at a rate of 0.2 mL/kg BW and levamisole (Nilverm Gold, Schering-Plough, Welwyn Garden City, UK) at a rate of 7.5 mg/kg BW, to remove worm burdens and ensure that lambs would not become infected from contact with the ewe derived parasites deposited onto the pasture. The lambs remained on this pasture until weaning at around 10 weeks of age. After weaning, the lambs were brought into a naturally illuminated and ventilated shed and housed in individual pens, measuring 2.0 by 1.5 m, until the end of the experiment. All pens contained a food trough that allowed measurement of individual ADFI and a water bowl that gave animals continuous access to water. Fresh food was supplied in 2 daily portions (early morning and late afternoon). Lambs were acclimatized for a period of 3 weeks prior to the start of experimental observations. At the end of this period, S and B lambs had mean initial BW of 30.1 ± 0.32 kg and 21.3 ± 0.43

kg, respectively. Four additional wether sheep were housed separately and used as larvae donors during the experiments.

Experimental design. Lambs were assigned randomly to treatments on the basis of their breed, sex and initial BW, ensuring an equal number of males and females of similar BW within both breeds on each treatment. To ensure the availability of sufficient animals for the re-infection experiment (see below), half of all lambs (24 of each breed) were assigned to the infection treatments. Of the remaining control lambs, half (12 of each breed) were fed ad libitum to allow estimation of anorexia in infected lambs. To measure effects of ADFI on PLC, the remaining control lambs were assigned to 1 of 3 restricted feeding levels (4 lambs of each breed per feeding level). All lambs in the infection treatment (treatment INF) were dosed with 7,000 infective third-stage *Teladorsagia circumcincta* larvae (L_3) every Monday, Wednesday and Friday. The trickle infection started on day 0 and ceased after twelve weeks (d 84). Each infective dose was suspended in 10 mL of water and was administered orally using a syringe. The L_3 originated from an anthelmintic susceptible strain, which had been donated by the Moredun Research Institute (Edinburgh, UK). Larvae were harvested every 14 days from faeces of a monospecifically infected donor sheep using a standard Baerman procedure. Following harvesting, L_3 were stored at 4 °C in tap water (700 L_3/mL) and used within 3 weeks of collection. All control lambs were given a similar volume of water ("sham" infection) at the same time, thus undergoing the same amount of handling stress as the infected animals. On day 84, INF lambs were drenched with a combination of fenbendazole and levamisole (same dose as above), to remove worm burdens. These lambs remained in the shed and were used in experiment II, while non-infected lambs were returned to stock. Lambs received grass pellets with an average composition per kg as-fed of 935g DM, 186g CP, 516g NDF and 9.5 MJ metabolisable energy according to feed supplier analyses. Infected lambs were fed ad libitum throughout the experiment while non-infected lambs were fed either ad libitum (treatment C_{al}; n = 12) or, restrictedly, at 90% (treatment

C_{90}; n = 4), 80% (treatment C_{80}; n = 4) or 70% of ad libitum (treatment C_{70}; n = 4). For the first 3 weeks of dietary restriction, food allowances for each of the restricted treatments were calculated using the ad libitum intake of each individual animal recorded during the previous 2 weeks. Thereafter, allowances were altered on a weekly basis so that the increase in intake by restrictedly fed animals was always proportional to the increase in intake relative to BW observed in C_{al} animals. The design of experiment I and the timing of the procedures are set out in Table 3.1. The design allowed measurement of the effects of nematode infection on voluntary ADFI by comparing ad libitum fed treatment groups, and on plasma leptin levels at similar intakes by including the data obtained from restrictedly fed lambs.

3.3.2. Experiment II (Secondary Infection)

Animals, housing and experimental design. Forty-eight of the previously infected lambs, 24 S and 24 B, were used in experiment II. At the start of the experiment all lambs were approximately 6 mo of age. There was a period of 2 weeks between the end of the primary infection study and the initiation of a secondary infection during which all lambs were fed ad libitum. At the end of this period, S and B lambs had mean BW of 50.2 ± 0.75 kg and 37.6 ± 1.07 kg, respectively. To obtain a good estimate of the effects of re-infection on ADFI and PLC, 12 lambs of each breed were re-infected (treatment INF2) with the same parasite, dose and infection regime as described in experiment I and were fed ad libitum. Non-re-infected control lambs received a "sham" infection at the same time. Eight control lambs of each breed were fed ad libitum (treatment $C2_{al}$). The remaining control lambs in each breed (n = 4) were fed at 90% of ad libitum (treatment $C2_{90}$) to account for effects of variation in ADFI *per se* on PLC. Treatments were balanced for breed, sex and initial weight as described previously. Experiment II lasted for 12 weeks and the experimental facilities and diets and infection details were identical to those used in experiment I. The design of experiment II and the timing of the procedures are set out in Table 3.1.

Table 3.1. Summary of experimental design and timing of treatments for Experiment I and II

Item	Number of lambs[1]	Treatments		Timing of experimental treatments[3]
		Food allowance	Infection[2]	
Experiment I (Primary infection)				
INF	48	Ad libitum	+	Wk 0 to 12
C_{al}	24	Ad libitum	-	Wk 0 to 12
C_{90}	8	90% of ad libitum	-	Wk 2 to 12
C_{80}	8	80% of ad libitum	-	Wk 2 to 12
C_{70}	8	70% of ad libitum	-	Wk 2 to 12
Experiment II[4] (Secondary infection)				
INF2	24	Ad libitum	+	Wk 0 to 12
$C2_{al}$	16	Ad libitum	-	Wk 0 to 12
$C2_{90}$	8	90% of ad libitum	-	Wk 2 to 12

[1] Half of the lambs in each treatment were Suffolk × Greyface lambs and half were Scottish Blackface lambs.

[2] All lambs were given 10 mL of water orally, 3 times/week for 12 weeks, which did (+) or did not (-) contain 7,000 *T. circumcincta* larvae (i.e., 21,000 larvae/week). At the end of each infection period (week 12) the lambs were de-wormed with a combination of levamisole at a rate of 0.2 mL/kg BW and fenbendazole at a rate of 7.5 mg/kg BW.

[3] Lambs allocated to the restrictedly fed treatments were fed ad libitum for the first 2 weeks of the experiment.

[4] Experiment II (secondary infection) started 2 weeks after the end of Experiment I. All lambs included in Experiment II were allocated from the INF treatment in Experiment I.

3.3.3. Sample Collection and Measurements.

In both experiments the measurements and procedures were carried out over similar time-scales using identical protocols.

Bodyweight and food intake measurements. All lambs were weighed once weekly from arrival in the animal house until the end of each experiment. Amounts of distributed food (as-fed), were recorded daily and any accumulated residues were weighed twice weekly (Monday and Thursday). Voluntary ADFI was calculated from the weekly amounts of food offered and weekly refusals.

Fecal egg counts. For the fecal worm egg count (FEC) determination, a 5 to 10 g fecal sample was taken from the rectum of all the lambs at weekly intervals from day –10 onwards during the entire experimental period. The individual fecal samples were processed immediately. The number of eggs per gram of fresh feces (epg) was determined using a modified flotation technique (Christie and Jackson, 1982), in which polyallomer centrifuge tubes (Beckman) were used to separate the egg bearing layer after flotation in saturated sodium chloride solution.

Blood samples. Weekly blood samples of around 8 mL were taken from the jugular vein into heparinized Vacutainers (Becton Dickinson, Oxford, UK) from housing until the end of each experiment. Blood samples were centrifuged for 15 min at 2,500 × *g*, and the plasma was separated and then stored at – 20°C pending analysis for leptin.

3.3.4. Leptin Radioimmunoassay (RIA)

A ruminant leptin RIA, developed at the Agri-Food and Biosciences Institute, Belfast, was used to determine PLC. This assay is a double-antibody, ovine-specific RIA

developed using purified recombinant ovine leptin (a gift from Prof. A. Gertler of The Hebrew University of Jerusalem, Israel). This leptin was used for antibody generation in guinea-pigs and also for in-house production of radio-iodinated leptin (label).

Preparation of radio-iodinated leptin. Radio-iodinated leptin was prepared by iodination with sodium ^{125}I-iodide (Amersham, Bucks, UK) using Iodogen coated tubes (Perbio Science, Northumberland, UK). To an Iodogen coated tube, 5 μL (500 μCi) of sodium ^{125}I iodide solution and 70 μL of 0.1M tris-Cl buffer pH 6.8 containing 3 μg of recombinant ovine leptin were added in rapid succession. The contents of the tubes were gently mixed and the reaction was allowed to proceed for 3 min at room temperature with occasional gentle mixing until termination by addition of 500 μL phosphate buffered saline (PBS) with a pH of 6.8. The contents were then immediately transferred to a clean polypropylene tube. After 30 min, the diluted reaction mixture was applied to a pre-calibrated 25mm × 500mm glass chromatographic column containing Sephadex G-75 (Amersham Pharmacia Biochem, Bucks, UK) equilibrated in 0.05M PBS, pH 6.8, containing 0.1% RIA grade bovine serum albumin (BSA; Sigma Chemical Co., Poole, Dorset, UK). The column was eluted using the same buffer and collecting 2 mL fractions. Aliquots of 5 μL of all fractions were counted for radioactivity in a Cobra II gamma counter (Packard Canberra Ltd., Berks, UK) and an overnight binding check (of labeled leptin to antiserum) was made on alternate fractions across the predicted leptin peak. Fractions with satisfactory binding activity were pooled to provide a single stock batch of label that was frozen in appropriately-sized aliquots.

Ovine RIA protocol and validation. The assay standard used in experiment I was pure recombinant ovine leptin (DSL Ltd., London, UK). This was dissolved in 1 mL de-ionised water and stored as 20 x 50 μL aliquots (10 ng/μL stock solution) at –75°C. Stock

aliquots were serially diluted with assay buffer to provide standards containing 50, 25, 12.5, 6.25, 2.125, 1.56, 0.78, 0.395, 0.1975 and 0.098 ng leptin/mL. Assays were conducted in polystyrene LP4 tubes (Thermoquest Ltd., Hants, UK) containing 100 μL of sample (in duplicate), 100 μL of assay buffer (0.05M PBS containing 5 g/L bovine serum albumin, 0.5 g/L Triton X-100 and 0.025M EDTA disodium salt with addition of 0.2 g/L sodium azide as a preservative and, if necessary, pH adjusted to 7.4 with 0.01 M sodium hydroxide) and 100 μL of antibody (at a final tube dilution of 1:160,000). Standard curve tubes (containing assay standard instead of sample) were prepared in triplicate. Assay tubes were incubated at 4°C for 20 h before addition of 100 μL of ^{125}I-leptin (14,000 to 16,000 counts per min) in assay buffer. The tubes were incubated for a further 48 h and then bound and free ligand were separated by adding 100 μL of cellulose-bound anti-guinea-pig IgG (Sac-Cel; IDS, Washington Tyne & Wear, UK). After gentle mixing and resting for 30 min at ambient temperature, 1 mL deionised water was added to all tubes (except 'total counts' tubes). Tubes were centrifuged (1,900 × *g*; 4°C; 20 min) and the supernatant above each pellet was aspirated by a vacuum pump via a trap. The residual pellets were counted for a minimum of 2 min in the gamma counter. Plasma samples were analysed in a series of 7 and 2 assays for experiments I and II respectively and assays were balanced internally for breed and treatment. The leptin used for iodination ('Gertler' ovine leptin) was used as the standard in experiment II.

Leptin RIA characteristics. Because the standards used in the 2 series of assays differed, the leptin results in the 2 experiments are not directly comparable. In experiment I, the assay coefficient of variation (CV) calculated from 6 replicates of an ovine plasma control sample with a mean pre-determined leptin concentration of 12.1 ± 0.64 ng/mL was 10.2% (range 5.9 to 14.2%) for intra-assay CV and 12.9% for inter-assay CV. In experiment II the mean intra-assay CV calculated from 5 replicates of an ovine plasma control samples

with a mean pre-determined leptin concentration of 5.5 ± 0.23 ng/mL, was 12.9% (range 11.6% to 14.2%). Inter-assay CV was 12.7%.

3.3.5. Statistical Analysis

Body weight, ADFI, ADFI relative to BW (RADFI, $g \cdot d^{-1} \cdot kg^{-1}$) and plasma leptin data were analyzed by ANOVA using the MIXED procedure of SAS (SAS 9.1.3; SAS Institute Inc., Cary, NC, USA). The statistical model for ADFI, BW and RADFI data analysis contained the fixed effects of breed, infection, sex, time, and interactions, with comparisons based on the data obtained from the ad libitum fed animals only. Additionally, plasma leptin analysis included the observations of the restrictedly fed animals. Comparison of actual plasma leptin levels across treatments was made by a model that included the main effects of breed, treatment (see Table 3.1.), sex, time, and their interactions (model 1). However, the effect of infection on PLC was assessed through a similar model that contained the RADFI of the lambs as a co-variable in addition to the main effects of breed, sex, infection, time, and their interactions (model 2). All models for leptin included an assay effect to take into account the between-assay variation. In every statistical model the random effect was animal nested within breed. Sex and its interactions did not affect any of the variables analyzed (in all cases $P > 0.05$) and were therefore ignored in the presentation of results. Data are reported as least square means and their SEM and differences tested by a t-test. Body weight gain (kg/week) for each animal was calculated by linear regression and data were analysed by ANOVA (General Linear Model) with the fixed effects of breed and infection. Effects of breed and infection were analysed on the basis of data of ad libitum fed animals only and data are reported as least squared means and their SEM. Prior to statistical analysis of FEC, data were log-transformed according to $\log_{10}(x+1)$, in order to normalise residuals. Log-transformed FEC data were analysed by repeated measures ANOVA (GenStat Release 7.2 Lawes Agricultural Trust, Rothamsted Experimental Station). Fecal egg counts

data are reported as back-transformed means according to (Johnson et al., 1998) as 10 to the power of ($\mu + 0.5 \times \sigma^2$), with 95% confidence intervals (CI).

3.4. Results

3.4.1. Experiment I (Primary Infection)

Faecal egg counts. Fecal samples taken on day -10 from all lambs had a mean epg of 42 (95% CI was 31 to 58) and 18 (95% CI was 9 to 35) for all S and B lambs, respectively. Following the unexpected finding that lambs were not parasite-free, all animals were immediately treated with the anthelmintics fenbendazole and levamisole (same dosage as mentioned above), before being infected according to the experimental protocol. Mean back-transformed FEC for the infected groups are shown in Figure 3.1a. With the exception of day -10, non-infected lambs (sham infected) had zero FEC throughout the experiment.

In both breeds, FEC reached a maximum value during the third week of infection with mean epg of 113 (95% CI was 81 to 158) and 73 (95% CI was 51 to 102) for S and B lambs, respectively. From the third week of infection FEC declined gradually to zero. There was a breed by time interaction ($P = 0.02$) because lambs of the S breed had higher mean FEC than B lambs in the middle, but not at the beginning or the end, of the experimental period (Figure 3.1a).

Food intake. The mean ADFI for S and B lambs in all treatments are shown in Figures 3.2a and 3.2b, respectively. Absolute ADFI was affected by breed ($P < 0.001$; Table 3.2) and was 2.19 ± 0.066 kg·d^{-1} and 1.49 ± 0.066 kg·d^{-1} in C_{al} lambs of S and B breed, respectively, but RADFI was not ($P = 0.99$; Table 3.2). However, there was a breed by time interaction for ADFI ($P < 0.001$) and RADFI ($P = 0.01$), indicating that the rate of the increase in ADFI and RADFI was higher in S lambs compared to B lambs (Table 3.2).

Dosing with parasites caused a significant reduction in ADFI in S lambs but not in B lambs as indicated by the interaction between infection and breed ($P = 0.03$; Table 3.2). The difference in ADFI between INF and C_{al} S lambs was around 12% and was persistent throughout the course of the 12-week parasitic challenge (Figure 3.3a). Similarly, RADFI was decreased by infection in S lambs but not in B lambs as indicated by the interaction between infection and breed ($P = 0.03$; Table 3.2).

Body weight and body weight gain. The average weekly BW for S and B lambs is shown in Figures 3.2c and 3.2d, respectively. Ad libitum fed lambs of the B breed had approximately 16% lower BW gain compared to S lambs ($P < 0.001$; Table 3.2). Infection affected the BW gain of lambs ($P = 0.01$, Table 3.2), with a reduction in BW gain of 15% and 7% in S and B lambs, respectively, but the interaction between breed and infection was not significant ($P = 0.15$, Table 3.2). All levels of food restriction resulted in lower BW gain in both breeds ($P < 0.001$; Table 3.2).

Plasma Leptin Concentrations. Figures 3.2e and 3.2f show the mean PLC during experiment I of S and B lambs, respectively. A direct comparison of actual PLC across treatments, without taking into account the RADFI of the lambs in each treatment (Model 1), indicated no differences in PLC between INF and C_{al} lambs in both breeds ($P = 0.53$; Table 3.2). Restrictedly fed lambs had lower PLC compared to the ad libitum fed lambs in both breeds ($P < 0.05$; Table 3.2). However, when the highly significant effect of co-variable RADFI on PLC was taken into account (Model 2), there was a significant positive effect of infection ($P = 0.02$; Table 3.2) on PLC. Both models showed that PLC was not affected by breed and increased with time ($P < 0.001$; Table 3.2).

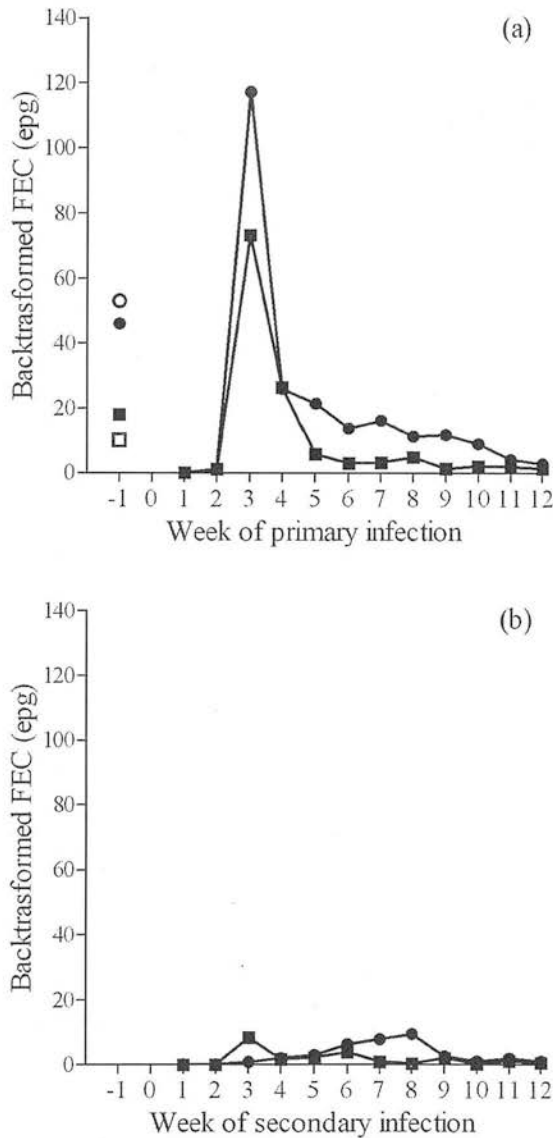

Figure 3.1. Mean weekly back-transformed log10 fecal egg counts (FEC) in number of eggs per gram of fresh feces (epg) of Suffolk × Greyface (S; ●,○) and Scottish Blackface (B; ■,□) lambs during experiment I (a; primary infection) and experiment II (b; secondary infection). In panel (a), open symbols (○,□) refer to FEC of 24 control S (○) and 24 B (□) lambs measured 1 week before the beginning of the experiment. In both panels, closed symbols (●,■) refer to FEC of infected S (●) and infected B (■) lambs. During both experiments infected lambs received orally 21,000 third-stage-larvae (L3) of *Teladorsagia circumcincta* per week (1 dose of 7,000 L3 3 times/week for 12 weeks). Fecal egg counts from the non-infected lambs during both experiments were zero, and are not shown.

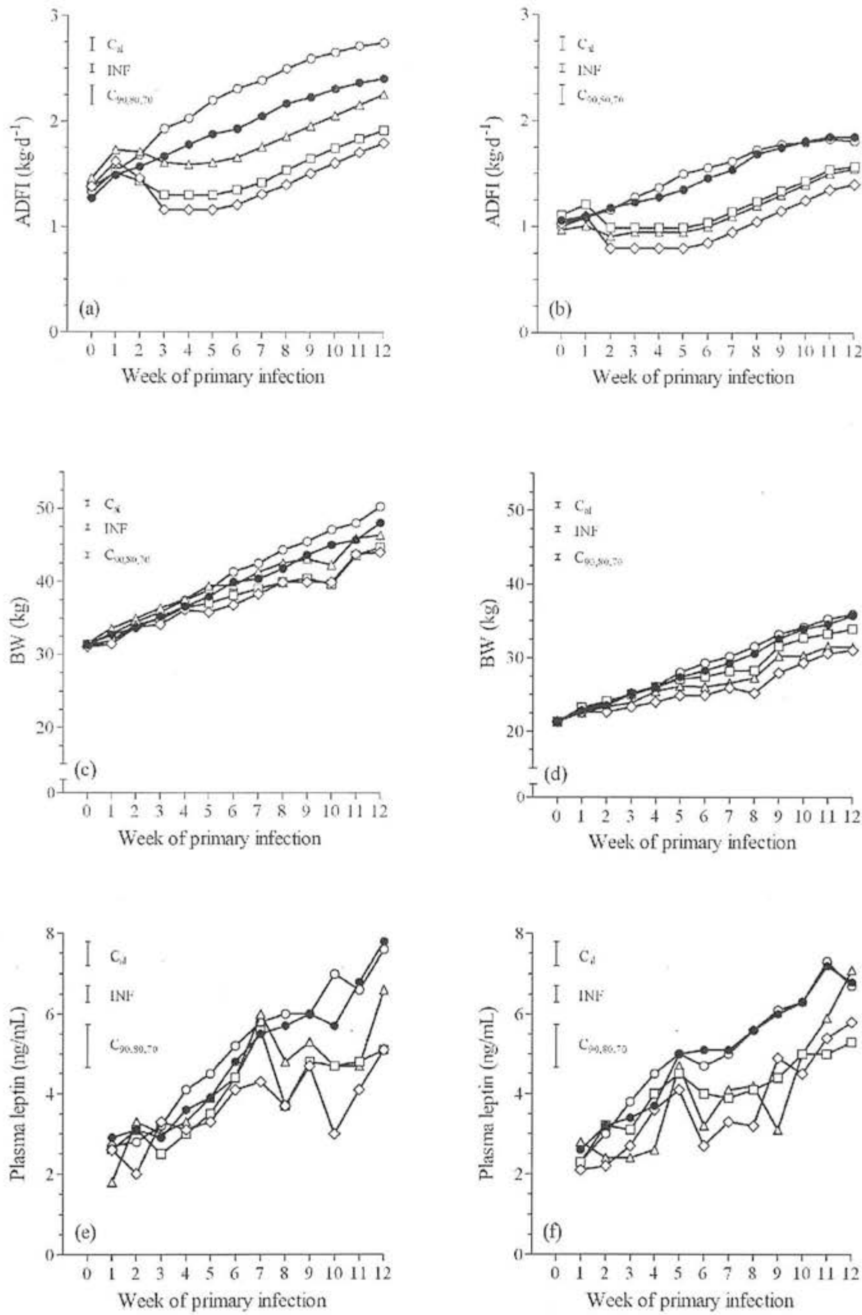

Figure 3.2. The (a and b) ADFI, (c and d) BW, and (e and f) plasma leptin concentrations of non-infected lambs fed ad libitum (Con; —○—), restricted-fed at 90% (C_{90}; —△—), 80% (C_{80}; —□—) and 70% (C_{70}; —◇—) of ad libitum and infected and fed ad libitum (INF; —●—) Suffolk × Greyface (panels a, c, and e) and Scottish Blackface (panels b, d, and f) lambs during experiment I (primary infection). The SEM in each treatment group are shown by vertical bars. The SEM for ad libitum and restricted-fed treatments are based on error mean squares pooled over ad libitum and restricted-fed animals, respectively. The SEM of the leptin data are based on error mean squares pooled over all treatments.

Table 3.2. Least-square means of ADFI, ADFI as a proportion of BW (RADFI), BW gain, and plasma leptin concentrations (PLC) of Suffolk × Greyface and Scottish Blackface lambs during Experiment I

	Suffolk × Greyface					Scottish Blackface					SEM[2]			*P*-value[3]						
Item[1]	INF	C_{al}	C_{90}	C_{80}	C_{70}	INF	C_{al}	C_{90}	C_{80}	C_{70}	INF	C_{al}	CR[4]	B	I	T	FR	B×I	B×T	RADFI
ADFI, kg/d	01.93	02.19	01.82	01.53	01.43	01.47	01.49	01.16	01.21	01.04	0.046	0.066	0.061	< 0.001	0.01	< 0.001		0.03	< 0.001	
RADFI, $g \cdot d^{-1} \cdot kg^{-1}$	49.3	54.4	45.8	39.8	37.9	51.8	52.0	42.2	42.3	39.3	0.92	1.31	1.32	0.99	0.02	< 0.001		0.03	0.01	
BW gain, kg/week	01.35	01.59	01.16	00.97	00.97	01.20	01.27	00.89	00.99	00.75	0.048	0.069	0.11	< 0.001	0.01		< 0.001	0.15		
Plasma leptin[5], ng/mL	05.3	05.1	02.4	02.9	02.5	05.0	04.7	04.0	02.6	02.1	0.28	0.40	0.69	0.41	0.53	< 0.001	≤ 0.05[6]	0.79	0.002	
	INF			NINF		INF			NINF		INF		NINF							
Plasma leptin[7], ng/mL	5.2			4.4		4.9			4.2			0.28		0.31	0.02	< 0.001		0.78	< 0.001	< 0.001

[1] INF = Lambs fed ad libitum and infected with 21,000 third-stage-larvae (L_3) of *T. circumcincta* per week (1 dose of 7,000 L_3 3 times a week for 12 weeks; n = 24 per breed); C_{al} = Non-infected, fed ad libitum lambs (n = 12 per breed); C_{90} = Lambs non-infected and fed at 90% of ad libitum (n = 4 per breed); C_{80} = Lambs non-infected and fed at 80% of ad libitum (n = 4 per breed); C_{70} = Lambs non-infected and fed at 70% of ad libitum (n = 4 per breed).

[2] Standard errors for ad libitum and restricted fed treatments are based on error mean squares pooled over ad libitum and restricted fed animals respectively.

[3] B = breed; I = parasite infection; T = time; FR = food restriction; RADFI = relative average daily feed intake ($g \cdot kg^{-1} . d^{-1}$). The *P*-values of the effects of B, I, and their interaction were calculated based on error mean squares of the ad libitum fed animals only. The *P*-values of the effect of FR were calculated based on error mean squares pooled over ad libitum and restrictedly fed animals.

[4] CR = Standard error of the mean of the restrictedly fed lambs (C_{90}, C_{80} and C_{70})

[5] Leptin LS means were calculated based on a statistical model where each treatment was included as a fixed factor. Standard errors are based on error mean squares pooled over all treatments.

[6] The level of significance of each restrictedly fed treatment was: C_{90}, $P = 0.04$; C_{80}, $P = 0.05$; C_{70} $P = 0.008$.

[7] Leptin LS means were calculated based on model in which RADFI was included as co-variable. NINF = Non-infected lambs (n = 24 per breed)

3.4.2. Experiment II (Secondary Infection)

Faecal egg counts. Mean weekly FEC remained very low and did not differ between the two breeds ($P = 0.9$; Figure 3.1b).

Food intake. The mean ADFI for S and B lambs in all treatments are shown in Figures 3.3a and 3.3b, respectively. Absolute ADFI was affected by breed with 2.75 ± 0.087 $kg \cdot d^{-1}$ and 1.99 ± 0.087 $kg \cdot d^{-1}$ for the C_{al} lambs of S and B breed, respectively ($P < 0.001$; Table 3.3). During the course of the secondary parasitic infection, the pattern of ADFI of INF and C_{al} lambs in the two breeds was similar to that observed during the primary infection. The interaction between breed and infection tended to be significant ($P = 0.08$; Table 3.3) and this was due to the difference of around 12% in ADFI between INF and C_{al} S lambs but not B lambs (Figure 3.3).

Body weight and body weight gain. The average weekly BW in S and B lambs is shown in Figures 3.3c and 3.3d, respectively. The two breeds differed in BW gain with S lambs gaining 36% more weight than B lambs ($P = 0.005$; Table 3.3). Secondary infection with T. *circumcincta* resulted in a significant reduction in BWG in S lambs but not in B lambs, as indicated by the significant interaction between breed and infection ($P = 0.001$; Table 3.3).

Plasma Leptin Concentrations. Figures 3.3e and 3.3f show the mean PLC in S and B lambs during experiment II. The results of the analysis of Model 1 showed no differences in PLC between restrictedly fed and C_{al} lambs (Table 3.3). Plasma leptin concentrations tended to be higher in INF lambs than in C_{al} lambs ($P = 0.07$; Table 3.3). However, the effect of co-variable RADFI on PLC was highly significant and when this was taken into account (Model 2), infection had a positive effect on PLC ($P = 0.004$; Table 3.3). Both models showed that PLC was not affected by breed and increased with time ($P < 0.001$; Table 3.3).

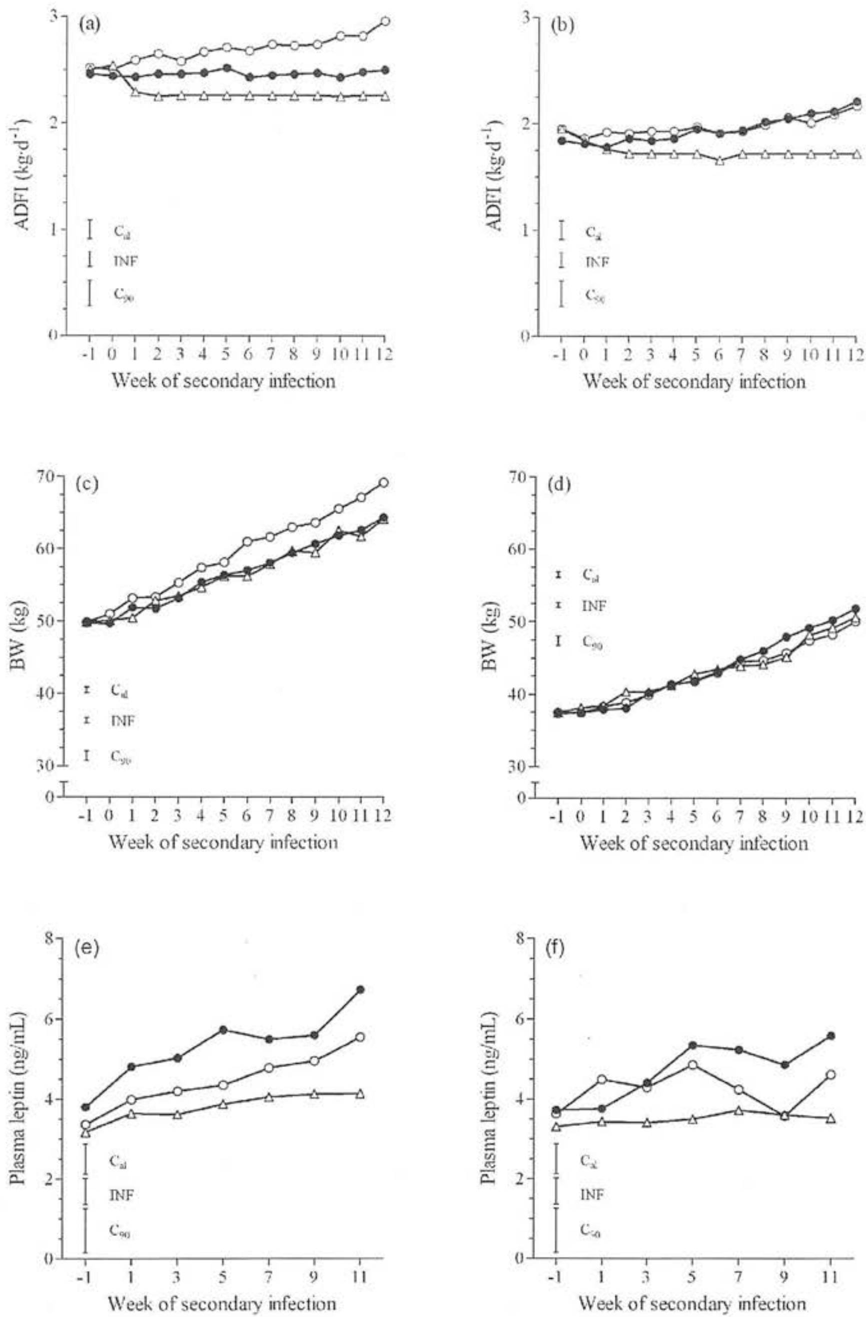

Figure 3.3. The (a and b) ADFI, (c and d) BW, and (e and f) plasma leptin concentrations of non-infected lambs fed ad libitum (Con; —○—), restricted-fed at 90% (C_{90}; —△—) and fed ad libitum (INF; —●—) Suffolk × Greyface (panels a, c, and e) and Scottish Blackface (panels b, d, and f) lambs during experiment II (secondary infection). The SEM in each treatment group are shown by vertical bars. The SEM for ad libitum and restricted-fed treatments are based on error mean squares pooled over ad libitum and restricted-fed animals, respectively. The SEM of the leptin data are based on error mean squares pooled over all treatments.

Table 3.3. Least-square means of ADFI, ADFI as a proportion of BW (RADFI), BW gain, and plasma leptin concentrations of Suffolk × Greyface and Scottish Blackface lambs during Experiment II

	Suffolk × Greyface			Scottish Blackface			SEM[2]			*P*-value[3]						
Item[1]	INF	C_{al}	C_{90}	INF	C_{al}	C_{90}	INF	C_{al}	C_{90}	B	I	T	FR	B×I	B×T	RADFI
ADFI, kg/d	02.46	02.75	02.27	01.99	01.99	01.71	0.076	0.087	0.13	< 0.001	0.11	< 0.001		0.08	0.88	
RADFI, $g \cdot d^{-1} \cdot kg^{-1}$	42.8	45.8	41.2	44.9	46.9	40.8	1.25	1.40	2.33	0.40	0.12	< 0.001		0.99	0.81	
BW gain, kg/week	01.15	01.46	01.12	01.19	00.94	00.96	0.074	0.084	0.087	0.005	0.70		0.09	0.001		
Plasma leptin[4], ng/mL	05.7	04.3	02.6	04.7	04.1	02.6	0.45	0.53	0.78	0.16	0.07	< 0.001	0.10	0.42	0.57	
	INF	NINF		INF	NINF		INF	NINF								
Plasma leptin[5], ng/mL	5.8	4.1		4.6	2.6			0.44		0.08	0.004	< 0.001		0.50	0.22	< 0.001

[1] INF = Lambs fed ad libitum and infected with 21,000 third-stage-larvae (L_3) of *T. circumcincta* per week (1 dose of 7,000 L_3 3 times/week for 12 weeks; n = 12 per breed); C_{al} = Non-infected, fed ad libitum lambs (n = 8 per breed); C_{90} = Lambs non-infected and fed at 90% of ad libitum (n = 4 per breed).

[2] SEM for ad libitum and restricted fed treatments are based on error mean squares pooled over ad libitum and restricted fed animals, respectively.

[3] B = breed; I = parasite infection; T = time; FR = food restriction; RADFI = relative ADFI ($g \cdot d^{-1} \cdot kg^{-1}$). The *P*-values of the effects of B, I, and their interaction were calculated based on error mean squares of the ad libitum fed animals only. The *P*-values of the effect of FR were calculated based on error mean squares pooled over ad libitum and restrictedly fed animals.

[4] Calculated based on a statistical model where each treatment was included as a fixed factor. SEM are based on error mean squares pooled over all treatments.

[5] Calculated based on model in which RADFI was included as co-variable. NINF = Non-infected lambs (n = 12 per breed).

3.5. Discussion

The findings of this study relate to the differences in response to infection between the lambs of the two breeds, the effects of infection on ADFI and the effect of infection on the relationship between PLC and ADFI in lambs.

3.5.1. Breed effects

The breeds used in the present study are known to vary in their intrinsic capacity for growth and mature size in a common environment (Emmans and Friggens, 1995; Lewis et al., 2004). The performance of the ad libitum fed S lambs was indeed superior to that of the ad libitum fed B lambs in both experiments I ($P < 0.001$) and II ($P = 0.005$), as expected.

Previous studies have reported that sheep selected more intensively for high productivity, such as fast growth or high fleece weight, are more susceptible to parasitic infection, as evidenced by high FEC and worm burdens, than animals selected less intensively (Amarante et al., 2004; McEwan et al., 1992; Miller et al., 1998). According to a recently developed framework, susceptible animals can be expected to show a larger depression in intake that lasts longer than the anorexia in less susceptible animals (Sandberg et al., 2006). This study is the first to investigate the effect of breeds of different production potential on the degree of anorexia in sheep. The first aim of this study was to test the hypothesis that variation in response to infection as measured by FEC would be associated with variation in the degree of anorexia following parasite infection. In addition, aim of the present study was to test whether such an infection would result in elevated leptin levels and whether breed differences in anorexia would be positively correlated with breed differences in PLC.

The results obtained are consistent with these expectations and seem, at least at first sight, to support the hypotheses. Primary parasite infection resulted in a higher FEC in S than in B lambs. In both breeds, animals started to show moderate levels of eggs in their faeces by the second week of dosing and rose to a maximum value during the third week of infection (Figure 3.1a). From the fourth week of infection and onwards FEC gradually declined to zero which suggests that development of immunity had started to occur. This pattern of FEC is consistent with experimental infections with *T. circumcincta* (Coop et al., 1977; 1982) where the highest value is observed within 3 to 4 weeks post infection. The absolute FEC values were relatively low compared to previous studies with similar doses of infection in parasite naïve lambs (Coop et al., 1982; Symons et al., 1981) but infection level was sufficient to affect ADFI. Although precautions had been taken to avoid exposure to parasites, FEC measured at housing, although low, were not zero (Figure 3.1a). Hence, lambs were not entirely parasite naïve prior to the start of the experiment. Because the pasture was newly established and had never been grazed before, it seems likely that the anthelmintic treatment applied to the ewes had not been completely effective. Consequently, it is likely that the worm eggs excreted by the ewes were the source of infection to their lambs, resulting in the low FEC observed 10 days prior to the start of the primary infection. During the course of the secondary infection, FEC remained very low and no differences were noted between the breeds (Figure 3.1b). Since there is evidence that animals can lose some of their acquired immunity when their exposure to pathogens is discontinued (Barger, 1988; Jackson et al., 2004) these findings suggest that a period of 2 weeks between a primary and a secondary infection will not result in a significant loss of immunity in these breeds.

There were significant breed effects on anorexia, as hypothesized. Infected B lambs did not differ in ADFI from non-infected lambs during either primary or secondary infection. This means that, with the exception of a very small increase in FEC during primary infection, B lambs did not respond to infection at all. This virtual absence of effects of

infection on B lambs was unexpected. Much more pronounced responses, in terms of FEC and ADFI, to infection using the same model have been amply demonstrated in many genotypes of lambs (Coop et al., 1977; Sykes and Coop, 1977; Symons et al., 1981). In their review, Coop and Holmes (1996) characterized Scottish Blackface as a relatively parasite resistant breed based on FEC data obtained with *H. contortus* infection. However, to our knowledge, effects of infection with *T. circumcincta* on ADFI of B lambs have not been investigated before and, therefore, no comparisons with the present data are available.

The low FEC and the absence of anorexia in B lambs could have been related to food quality. As judged from animal growth rates, the quality of the food allowed for good performance. Fecal egg counts are generally higher in lambs with access to poor rather than high quality food (Van Houtert and Sykes, 1996). According to a nutrient partitioning framework developed by Coop and Kyriazakis (1999), nutritional limitation can affect lambs of higher production potential more than animals of lower production potential. Higher growth rates of B and S crossbreds fed high quality food have been observed in our institute before (see e.g. Lewis et al., 2004), suggesting that the food used in the present experiments was limiting and this may have affected the two genotypes differently. In addition, lambs were not completely parasite naïve before the experiment started, as discussed earlier, and previous exposure to parasites could have affected the response of B lambs differently from S lambs in the first experiment. At the start of experiment II however, lambs of both breeds had been exposed to *T. circumcincta* for 12 weeks. Differences in previous exposure cannot, then, explain the breed effects on anorexia. It can be concluded, therefore, that in terms of FEC and anorexia, S lambs were affected more by nematode infections than B lambs when both breeds have access to a good quality food.

3.5.2. Effects of (re-)infection on food intake

During the course of primary infection, a significant reduction in ADFI (anorexia) of about 13% was observed from the second week of infection in parasitized S lambs. This depression of intake was persistent throughout the course of the 12-week parasitic challenge (Figure 3.3a), which is a common observation in crossbred lambs continuously infected with *T. circumcincta* (Coop et al., 1977; Sykes and Coop, 1977; Symons et al., 1981). This long-lasting depression of appetite is very likely to be directly related to the host's immune response to gastrointestinal parasites (Greer et al., 2005) rather than the presence of parasites per se (Coop et al., 1977; Sykes and Coop, 1977).

Ruminants are frequently exposed to infective forms of nematode parasites, but there is very little information on whether re-exposure of previously infected animals results in renewed reduction in ADFI. A recent study has shown that re-infection did not result in anorexia in previously infected non-pregnant or non-lactating ewes (Greer et al., 2005). However, the present study showed that re-infection with *T. circumcincta* resulted in a reduction in ADFI of S lambs that was similar to the reduction observed during primary infection in the same breed (around 12%). The interaction between breed and infection was significant during primary infection but only tended to be significant during re-infection, which is likely related to the fewer number of lambs included in experiment II. This suggest that re-exposure to nematode infection can cause anorexia in lambs, as has been observed previously in other species (Houdijk et al., 2003; Mercer et al., 2000). In the current study, anthelmintic treatment was followed by a parasite-free period of 2 weeks before re-infection started, which is a similar design to that reported in the ewe study (Greer et al., 2005). The difference in findings with ewes and lambs are, therefore, not the result of differences in the length of the parasite-free period. Because young animals have been observed to be more susceptible to infection than adult sheep, the re-occurrence of anorexia in lambs, but not in

ewes, may be a result of the difference in age and thus in susceptibility to infection (Smith et al., 1985).

An alternative interpretation of these data is that de-worming resulted in a recovery of ADFI in the control animals while re-exposure to parasites prevented recovery in the re-infection treatment. This view is consistent with the finding that frequent anthelmintic treatment did not remove the depressant effect of a continuing *T. circumcincta* infection on voluntary ADFI of lambs (Coop et al., 1982). Although the RADFI of infected lambs during re-infection was not significantly lower than that of control animals, this is a common phenomenon observed in parasitized lambs as discussed by Sykes (1982). However, effects of re-infection after longer parasite-free periods on subsequent ADFI of sheep remain to be investigated.

3.5.3. Effects of (re-)infection on PLC in relation to food intake

The relationship between PLC and ADFI during infection is complicated. Previous studies show that a reduction in ADFI as a result of restricted feeding in healthy animals, including sheep, results in a reduction in PLC (Blache et al., 2000; Delavaud et al., 2000; Marie et al., 2001; Morrison et al., 2001). Nematode infection was, therefore, expected to be associated with a decrease in PLC. Nevertheless, the literature shows that infection and the subsequent immune response are associated with an increase in PLC in several infectious diseases, including parasitism, in murine animal models (Barbier et al., 1998; Faggioni et al., 2001; Fantuzzi and Faggioni, 2000; Grunfeld et al., 1996; Mercer et al., 2000). Then PLC in infected animals showing anorexia is likely to result from both a stimulating effect (by infection) and a suppressive effect (from the reduction in ADFI). Such opposing effects on PLC have been suggested for endotoxemia in pigs (Barb et al., 1998) and it has been proposed that the same effects could be relevant during infections in ruminants (Kulcsar et al., 2005). The current study therefore, tested the hypothesis that nematode infection will

result in elevated PLC in lambs with similar ADFI to non-infected lambs. For that reason, restrictedly fed control lambs were included in the experiment to be able to quantify effects of ADFI on PLC in the absence of infection. The reduction in ADFI of restrictedly fed control lambs of both breeds resulted in systematic decreases in PLC compared to ad libitum fed control lambs in both experiments (Figures 3.2e, 3.2f and 3.3e, 3.3f), which is consistent with previous observations (Delavaud et al., 2002; Marie et al., 2001; Morrison et al., 2001).

The effects of nematode infection on PLC in ruminants have not been studied extensively. Data obtained in a recent study (Fox et al., 2006) suggested that infection with *T. circumcincta* results in elevation of PLC during day 5 to 13 after infection. However, these results are difficult to interpret because of the absence of any non-infected control lambs in that experiment. In contrast, the present study allowed a direct comparison of PLC between infected and non-infected ad libitum fed lambs. The results herein show that during the course of the primary parasitic infection, actual PLC, as analyzed by Model 1, did not differ significantly between infected and non-infected ad libitum fed lambs. Although PLC tended to be higher in infected lambs, especially in the S breed (Figure 3.3e) during re-infection, the effect was not statistically significant. These findings are in contrast to the acute increase in PLC that has been observed in earlier studies during nematode infection (Mercer et al., 2000; Roberts et al., 1999), acute intestinal inflammation (Barbier et al., 1998) and other disease models in rodents (Faggioni et al., 1997; Grunfeld et al., 1996; Sarraf et al., 1997). They are however, consistent with the lack of effect on PLC of acute endotoxemia in sheep (Soliman et al., 2001) and cows (Soliman et al., 2002) and salmonella infections in pigs (Jenkins et al., 2004). Gastrointestinal nematode infection does, therefore, not seem to result in an acute increase of PLC in ad libitum fed sheep.

In restrictedly fed sheep, infection with a combination of *T. circumcincta* and *T. colubriformis* did also not result in an increased in PLC (Liu et al., 2007). The present study

is the first to allow a comparison of PLC of ad libitum fed infected with non-infected lambs with similar levels of RADFI. Analysis of the PLC data with Model 2, where RADFI was included as a co-variable, showed that during primary infection, infected lambs had higher PLC than non-infected lambs with similar RADFI. This effect of infection on PLC was also observed during secondary infection (Table 3.3). These results are in agreement with the first hypothesis that was set out to test, i.e. that nematode infection can have a positive effect on plasma leptin levels in lambs when the effect of infection on RADFI is taken into account. Therefore, the current study provides some evidence for the suggestion of Kulcsar et al. (2005) that two opposing mechanisms (stimulation by inflammation and inhibition by reduced ADFI/energy metabolism) are likely to affect PLC during infection in sheep. It is, therefore, possible that leptin is involved in the immune response to nematode infection in lambs. Although such a role for leptin has also been suggested in one other recent study with parasitized periparturient ewes (Valderrabano et al., 2006), the importance of this role in sheep remains to be established.

The literature also shows that in ad libitum fed healthy animals, an increase in plasma leptin level is associated with a decrease in voluntary ADFI and leptin administration promotes anorexia (Henry et al., 1999; Morrison et al., 2001). If an increase in plasma leptin level depresses ADFI in lambs, this mechanism could play an important role in the infection-related anorexia that is observed in many disease models. Indeed, such a role for leptin has been proposed in a number of species (Grunfeld et al., 1996; Mercer et al., 2000; Sarraf et al., 1997). However, other studies have suggested that leptin itself is not responsible alone for the occurrence of anorexia during infection (Faggioni et al., 1997). For example the parasite-induced anorexia in rats was suggested to be related to the cytokine interleukin 6, which is released during the immune response and is a known mediator of anorexia in the acute-phase immune response, rather than to the elevated leptin concentrations observed during early infection (Roberts et al., 1999). In the present study, parasitic infection did not

result in an acute increase in PLC during both the primary and the secondary infection. In addition, although infected lambs had significantly higher PLC than non-infected lambs with similar ADFI in both breeds, anorexia was observed in lambs of the S breed only. These effects were observed during both the primary and the secondary infection. Therefore, these results show that, although leptin may be involved in the response of lambs to nematode infection, it is highly unlikely that leptin alone is responsible for the anorexia that is observed in parasitized lambs.

3.5.4. Conclusions

The data show that there were differences between B and S lambs in their response to nematode infection with *T. circumcincta*. Suffolk × Greyface lambs were more susceptible to infection than B lambs as evidenced by the larger increase in FEC. Suffolk × Greyface lambs also developed anorexia while B lambs did not. The data are, therefore, the first to show that infection with *T. circumcincta* depresses the ADFI of lambs of a susceptible breed more than that of lambs of a less susceptible breed to parasitic infection. In addition, the study demonstrated that susceptible lambs that are re-exposed to infection can show anorexia again. During both primary and secondary infection, infected lambs had higher PLC than non-infected lambs with similar ADFI. The results show that leptin may be involved in the response of lambs to infection but that it is unlikely that leptin alone is responsible for the anorexia that is observed following parasitic infection.

CHAPTER FOUR

Effects of breed and immune status on anorexia and plasma leptin concentrations of parasitized lambs

4.1. Abstract

Anorexia (i.e. reduction in voluntary food intake) is a major cause of the impaired production efficiency of the infected lambs. The degree of anorexia may be affected by the breed production potential, the immune status and the age of the lambs at the time of infection. Plasma leptin concentrations may be also affected by the nematode infection and it is possible that leptin has a role for the occurrence of anorexia in lambs. In this Chapter it is hypothesised that the degree of anorexia following infection i) will be greater in lambs selected intensively for growth than in unselected lambs ii) will be smaller in older than in young lambs iii) the degree of anorexia in re-infected lambs is affected by the length of the parasite free period and iv) nematode infection will increase the concentration of plasma leptin in lambs. These hypotheses were tested in three experiments two of which tested responses to a primary infection (experiments I and II) and one to a secondary infection (experiment III). In all experiments Suffolk × Greyface (S) and Scottish Blackface (B) lambs that are known to differ in their growth potential (S lambs are of higher growth potential than B lambs), were used. During the primary parasitic infections, lambs were either trickle infected with 7,000 infective *Teladorsagia circumcincta* larvae per day, 3 days per week, for a period of 10 (experiment I) or 7 weeks (experiment II) and fed *ad libitum*, or non-infected and fed either *ad libitum* or pair-fed to infected lambs. In experiment III, all lambs were fed *ad libitum* and were either non-infected or re-infected after a 4- or 8-week period after the end of the primary infection. The results showed that during the primary nematode infection lambs of the S breed were more susceptible than B lambs, as judged from the differences in faecal egg counts (FEC) and IgA response (experiment I). The breed differences were less pronounced in experiment II and III, as both breeds showed low FEC and broadly similar IgA response. The results showed that a primary infection is associated with anorexia in young lambs, but the degree of anorexia does not seem to be affected by the IgA response to

infection. Re infection of previously infected lambs did not result in renewed anorexia when lambs were re-infected after 4 or 8 weeks of the end of the primary infection. Finally the results showed that nematode (re)infection did not result in an increase of PLC and leptin itself is unlikely to be responsible for the anorexia of nematode infection in lambs.

4.2. Introduction

It has been well established that a primary nematode infection in lambs is associated with a reduction in food intake (i.e. anorexia) which can vary from 10% up to complete absence of food intake (Sykes, 1991; Coop and Sykes, 2001). Anorexia in turn, is a major cause of the impaired production efficiency of the infected lambs (Coop and Holmes, 1996; van Houtert and Sykes, 1996). Sandberg et al. (2006) postulated in a recently developed model that the degree (i.e. magnitude and duration) of the parasite-induced anorexia following a primary infection, may be higher in genotypes that are susceptible to infection than in more resistant genotypes. It has been concluded that selection for high production efficiency can have adverse effects on the ability of animals to deal with infection (Rauw et al., 1998). Breeds of sheep that have been selected more intensively for production efficiency (e.g. growth) are generally more susceptible to gastrointestinal infections than breeds that have been selected less intensively (McEwan et al., 1995; Baker, 1998; Bisset et al., 2001). The previous chapter provided some evidence that the degree of anorexia is likely to differ between breeds of different growth potentials. However, the breed differences in the degree of anorexia could have been affected by the immune status of the lambs prior to infection, as there is some evidence that may not have been entirely parasite naive. Moreover, young animals have been observed to be more susceptible to infection based on FEC than adults as evidenced by studies in sheep (Smith et al., 1985) and goats (Noordeen et al., 2001), but, it is unknown whether differences in the degree of anorexia exist between young and older animals following a primary nematode infection. Based on the available evidence it is hypothesised that in parasite naïve lambs (i) the degree of anorexia following a primary nematode infection will be greater in lambs that have been selected more intensively for growth potential than in lambs that have been selected less intensively and (ii) the degree

of anorexia following a primary nematode infection will be greater in young lambs (~3 months old) than in older lambs (~6 months of age).

A recent study with infected and concurrently immuno-suppressed lambs has shown that the parasite induced anorexia following a primary infection is not caused by the presence of parasites *per se* but is the direct consequence of acquisition of immunity (Greer et al., 2005). The same study showed that the expression of immunity following a secondary nematode infection is not associated with anorexia in previously infected adults non-reproducing ewes. However, infected animals can lose some of their acquired immunity when their exposure to pathogens is discontinued, (Barger, 1988; Jackson et al., 2004), although it is not known whether and when a complete loss of immunity occurs under parasite-free conditions in sheep. The experiments described in the previous chapter, provided some evidence that re-exposure to nematode parasites may result in renewed anorexia in a breed that is selected for growth production, however, the effect was not very strong. Therefore, there is some uncertainty as to whether re-infection of previously infected animals results in renewed anorexia or not and whether the degree of anorexia depends on the length of the parasite-free period. In this study it is hypothesized that the magnitude of anorexia will be greater in lambs that are re-infected after 8 weeks than in lambs that are re-infected after 4 weeks of the end of the primary infection, because of differences in host immune status at the start of re-infection.

Recently, an important role in the regulation of immune responses been ascribed to the hormone leptin (Lord et al., 1998; Fantuzzi and Faggioni, 2000) that is primarily recognised for its actions in regulating food intake and energy expenditure (Friedman and Halaas, 1998). Increased plasma leptin concentrations (PLC) have been observed in many disease models including parasitism in mice and rats (Moshyedi et al., 1998; Gualillo et al., 2000; Mercer et al., 2001). A role for leptin in the anorexia of infection has also been

proposed (Grunfeld et al., 1996; Barbier et al., 1998). In ruminants, the effects of nematode infection on PLC have not been studied extensively. A possible implication of leptin in the immune response of nematode infected sheep has been studied previously (Valderabano et al., 2006; Fox et al., 2006). However, these studies did not facilitate a comparison in PLC between infected and non-infected sheep. The previous chapter provided evidence that PLC in infected lambs is elevated compared to non-infected controls, when PLC data are corrected for the level of food intake. This was achieved by including food intake data of non-infected restrictedly fed lambs in the statistical model. However, in the previous chapter the level of food restriction resulted in intakes that were lower than those of the infected animals showing anorexia. In the present study, correction for the level of food intake in order to study the effect of infection on PLC was achieved by the involvement of non-infected lambs that were pair-fed to infected lambs. Thus, it is hypothesised that nematode infected lambs will have increased PLC compared to non-infected controls with similar food intakes.

4.3. Materials and Methods

4.3.1. Animals, housing and experimental design

Three experiments took place at the Scottish Agricultural College experimental farm after approval of the experimental protocols by the Animal Experiments Committee (Research Programme Number: ED RP 3.00/P4), and under Home Office license for experimental infection and blood sampling (PPL 60/3004).

Experiment I. Forty-eight Suffolk × Greyface (S) and 48 Scottish Blackface (B) lambs were used in this experiment. Half of the lambs in each breed were castrated males and half females. All lambs were born and weaned indoors and prior to lambing all ewes were orally drenched with a combination of fenbendazole (Panacur 10 %; Hoechst Roussel Vet Ltd, Milton Keynes, Bucks., UK) at a rate of 0.2 mL/kg body weight (BW) and levamisole (Nilverm Gold, Schering-Plough, Welwyn Garden City, UK) at a rate of 7.5 mg/kg BW, to remove worm burdens and ensure that lambs would not become infected from contact with ewe-derived parasites. Male lambs were castrated soon after birth and lambs of both breeds were weaned at approximately 10 weeks of age. After weaning, lambs were transferred to a naturally illuminated and ventilated experimental shed. Prior to the start of the experiment (week 0), lambs acclimatized to the experimental food and the environment for a period of 3 weeks, during which all lambs were fed *ad libitum.* During the first week of the adaptation period lambs were housed as groups. Subsequently, they were allocated randomly to individual pens (measuring 2.0 by 1.5 m), in which they stayed until the end of the experiment (week 10). All pens contained a food trough that allowed measurement of individual food intake, and a water bowl that gave animals free and continuous access to water. Fresh food was supplied in 2 daily portions (early morning and late afternoon) and

any refusals were weighed back twice a week (on Mondays and Thursdays) which allowed intake calculations of ad libitum-fed animals for periods of four and three days, respectively.

Half of the lambs (24 of each breed) were infected for 10 weeks and fed *ad libitum* (treatment INF; for infection details see below). Half (12 of each breed) of the non-infected controls, were fed *ad libitum* to allow estimation of anorexia in INF lambs (treatment AL). To measure effects of food intake on plasma leptin concentrations (PLC), the remaining 12 control lambs in each breed were pair-fed with 12 lambs of the same breed and gender of the INF treatment (treatment PF). These PF lambs received the same amount of food per kg BW as consumed by their *ad libitum* fed infected counterpart in the previous three to four days.

Lambs were assigned randomly to the above treatments after blocking for breed, sex and BW in week -1, which guaranteed an equal number of males and females per breed of similar BW per treatment. The mean BW on day -7 was 31.8 ± 0.43 kg and 21.3 ± 0.43 for S and B lambs, respectively. All lambs during the experiment were fed the same type of food (grass pellets) with an average composition per kg as-fed of 948g DM, 166g CP and 479g NDF. The experiment lasted for 10 weeks.

Experiment II. The 48 parasite naïve control lambs (24 S and 24 B) from the non-infected treatments (AL and PF) of experiment I were used in this experiment. The experimental design was similar to that of the experiment I, with the difference that all treatments had an equal number of lambs. Four weeks prior to the start of the experiment the lambs were group housed and were given *ad libitum* access to food. One week prior to the start of the experiment the lambs were moved to individual pens and food intake was recorded. In this experiment 8 lambs of each breed were infected with *T. circumcincta*, (same dose and infection regime as described in experiment I) and were fed *ad libitum* (treatment INF2). The remaining non-infected lambs were allocated in one of two treatments

in which they were either fed *ad libitum* (treatment AL2, n = 8 per breed) or pair-fed (treatment PF2, n = 8 per breed) with 8 lambs of the same breed and gender of the INF2 treatment (as described in experiment I). The lambs were allocated randomly to the above treatments after blocking for breed, sex and BW, which guaranteed an equal number per breed of males and females of similar BW per treatment. The experiment lasted for 7 weeks during which all lambs were fed grass/straw pellets with an average composition per kg as-fed of 917g DM, 103g CP and 532g NDF. At the start of the experiment the lambs were approximately 7 months of age. The experimental facilities and infection details were identical to those used in experiment I.

Experiment III. The 48 lambs previously infected in Experiment I (24 S and 24 B) were used in this experiment. The lambs were allocated randomly into three treatments of 8 lambs per breed (i.e. treatments CON, R8 and R4) after blocking for breed, sex and BW, and housed as treatment groups. This allocation guaranteed an equal number of males and females of similar BW within both breeds per treatment. Trickle infection for lambs in the CON and R8 treatments stopped immediately after the end of experiment I and at the same time they were drenched with a combination of fenbendazole and levamisole (same dose as described above), to remove worm burdens. Lambs in the R4 treatment continued to receive the trickle infection for an additional 4 weeks after which they were drenched. Four weeks after the end of infection in the lambs of the R4 treatment, a secondary infection with *T. circumcincta* was applied to the lambs of both the R8 and R4 treatment and lasted for 12 weeks. This design resulted in lambs being re-infected either 4 weeks (R4) or 8 weeks (R8) after the primary infection was terminated. The dose and infection regime used in this experiment were the same as that of experiment I. The lambs were moved to individual pens one week prior to the start of the secondary infection and food intake was recorded. Throughout the experiment all lambs were fed *ad libitum* grass/straw pellets (same

composition as in Experiment II). The experimental facilities and infection details were identical to those used in experiment I and II.

4.3.2. Infection details

Infective larvae for all experiments were obtained from four wether Suffolk × Greyface sheep that were housed separately and were infected with an anthelmintic susceptible strain of the nematode *Teladorsagia circumcincta*, which was donated by the Moredun Research Institute (Edinburgh, UK). Larvae were harvested every 14 days from faeces using a standard Baerman procedure. Following harvesting, infective third-stage larvae (L_3) were stored at 4 °C in tap water and used within 2 weeks of collection.

Infected lambs in all experiments were dosed orally using a syringe with 7,000 L_3 larvae of the nematode *Teladorsagia circumcincta* suspended in 10 mL of water every Monday, Wednesday and Friday. Similar rates of infection have previously been shown to lead to establishment of a patent *T. circumcincta* worm burden in growing lambs (Coop et al., 1985). All non-infected (control) lambs were given a similar volume of water ("sham" infection) at the same time, thus undergoing the same amount of handling stress as the infected animals.

4.3.3. Sample Collection and Measurements

In all experiments, measurements and procedures were carried out over similar time-scales using identical protocols.

Lamb performance and intake. At arrival and during all the periods that lambs were housed individually, BW was recorded weekly and body condition was scored every fortnight by the same operator by lumbar palpation on a scale from 0 to 5 with 0.25

increments (Russel et al., 1969). Amounts of distributed fresh food were recorded daily and accumulated residues were weighed twice weekly (Monday and Thursday) to allow estimation of average daily food intake (ADFI). The refusals of the pelleted food were recorded on as fed basis, as similar refusal levels were obtained for the individual animals and there was no evidence of feed separation in the bins.

Faecal egg counts. For the fecal worm egg count (FEC) determination, a 5 to 10 g fecal sample was taken from the rectum of all the lambs at weekly intervals from d –10 onwards during the entire experimental period. The individual faecal samples were processed immediately. The number of eggs per gram of fresh faeces (epg) was determined using a modified flotation technique (Christie and Jackson, 1982), in which polyallomer centrifuge tubes (Beckman) were used to separate the egg bearing layer after flotation in saturated sodium chloride solution. The FEC were expressed as number of eggs per gram fresh faeces (epg), and the technique used can detect nematode eggs down to 1 epg.

Blood samples. Blood samples were taken weekly from the jugular vein of the lambs into heparinized vacutainers from housing (pre-infection) onwards. Each sample was centrifuged for 15 min at 2600×g immediately after collection and approximately 3 ml of plasma was obtained and dispensed in 3 aliquots of around 1 ml each. Two aliquots were stored at -20°C pending analysis for leptin and immunoglobulin A (IgA) concentrations , while the other one was analysed for pepsinogen within 6h. In all experiments, plasma IgA, leptin and pepsinogen concentrations were determined in 6 lambs (3 males and 3 females) of each treatment in each breed that were selected randomly. Plasma pepsinogen concentration was determined by the modified method of Paynter (1992) and expressed in iU (international Units). IgA concentration was determined against somatic L3 antigens of *T. circumcincta* as described previously by Sinski et al., (1995).

Leptin Radioimmunoassay (RIA). Plasma leptin concentration (PLC) in the samples obtained from the three experiments was determined by a modification of the ruminant-specific leptin RIA described previously in Chapter 3. In the modified RIA, instead of the use of Sac-Cel (cellulose-bound anti-guinea-pig IgG), the separation of bound and unbound labelled leptin was achieved in a longer, two-step, process using soluble second antibody followed by a general protein precipitant (polyethylene glycol or PEG) as follows: After the 44-48h incubation of label, to each tube (except the total counts check tubes) was added 100μl of normal guinea pig serum (diluted 1:100 in assay buffer). After a brief vortex mix, 100μl of a 1:16 dilution of second antibody (goat anti-guinea pig IgG; IDS Ltd., Boldon, Tyne and Wear, Northumberland, UK) in assay buffer was added and all tubes again vortex mixed before returning to the fridge. Next day, 100μl of whole horse serum was added to all standard tubes as well as 'zero-binding' check tubes and 'non-specific binding' check tubes. After brief vortexing, 700μl of 10% PEG was added to all tubes (except total counts tubes) and all tubes were vortex mixed and returned to the fridge for a further 2 hours prior to centrifugation at 20 minutes at 3000 rpm (1900 x g) and at 4°C. After centrifugation, supernatant containing unbound ^{125}I-leptin was decanted from each tube and drained pellets were counted in a Cobra II gamma counter (Packard Canberra Ltd., Berks, UK). Ten replicates of mixed sera from a fat (BCS >3.5) sheep or a lean sheep (2.0 < BCS > 3.5) were included in each assay. The mean leptin concentration of this mixed serum control was ± 2.91 ng/ml. The mean intra-assay coefficient of variation (CV) of the three assays was 4.62 % while the inter-assay CV was 5.11 %.

4.3.4. Statistical Analysis

Similar statistical models were used for the analysis of the data obtained from the three experiments. ADFI, relative ADFI to BW (RADFI, $g \cdot d^{-1} \cdot kg^{-1}$), BW, BCS, and IgA data were analysed by repeated measures ANOVA with an auto-regressive correlation structure for residual errors over time, using the MIXED procedure of SAS (SAS 9.1.3; SAS Institute

Inc., Cary, NC, USA). All statistical models contained the fixed effects of breed, infection, sex, time, and their interactions.

Plasma leptin were analysed also by ANOVA using the MIXED procedure of SAS for repeated measurements. Comparison of actual plasma leptin levels between treatments was made by a model that included the main effects of breed, infection, sex, time, and their interactions (model 1). Leptin data were also analyzed by a similar model that contained either the BCS measurements or the RADFI of the lambs as a covariable in addition to the main effects of breed, infection, time, and their interactions (model 2). The relationships between PLC and BCS, and between PLC and RADFI were tested by comparing the respective covariable coefficient with its associated standard error. All models for leptin included an assay effect to take into account the between-assay variation. In every statistical model the random effect was animal nested within breed by treatment.

Individual bodyweight gain (BWG) of all lambs (g/day) was estimated by linear regression (least square method) of BW on time and data were analysed by ANOVA (General Linear Model) with the fixed effects of breed and infection. Effects of breed and infection were analysed on the basis of data of ad libitum fed animals only.

Log-transformed, according to $\log_{10}(x+1)$, pepsinogen and FEC data were analysed by repeated measures ANOVA (GenStat Release 7.2 Lawes Agricultural Trust, Rothamsted Experimental Station). Pepsinogen and FEC data are reported as back-transformed means according to Johnson et al., (1998) as 10 to the power of $(\mu+0.5\times\sigma^2)$, with 95% confidence intervals (CI). All other data are reported as least square means and their SEM and differences tested by a t-test. Sex and its interactions did not affect significantly any of the variables analysed and is therefore ignored in the presentation of results.

4.4. Results

4.4.1. Experiment I (Primary infection)

Faecal egg counts. Faecal samples taken on week -1 showed that all lambs were parasite naïve prior to the start of the primary trickle infection, as all lambs had zero FEC. Following the start of the nematode infection, FEC reached a maximum value during the third week of infection with mean epg of 154 (95% CI was 100 to 184) and 59 (95% CI was 41 to 106) for S and B lambs, respectively.

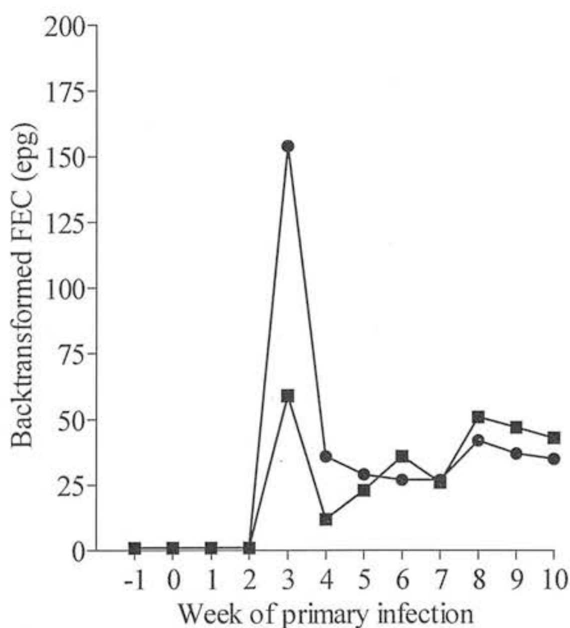

Figure 4.1. Mean weekly backtransformed $\log_{10}$ fecal egg counts (FEC) in number of eggs per gram of fresh feces (epg) of Suffolk × Greyface (S; ●) and Scottish Blackface (B; ■) lambs during experiment I (primary infection).

From the third week of infection FEC declined and remained relatively low until the end of the experiment, in both breeds. There was a breed by time interaction ($P = 0.001$) because lambs of the S breed had back-transformed higher mean FEC than B lambs in the

middle, but not at the beginning or the end, of the experimental period (Figure 4.1). All non-infected lambs (sham infected) had zero FEC throughout the experiment (data not shown).

Food intake. The ADFI for S and B lambs in INF and AL treatments are shown in Figure 4.2a. ADFI was affected by breed ($P < 0.001$; Table 4.1) and was 2.19 ± 0.045 kg/d and 1.53 ± 0.045 kg/d in AL lambs of S and B breed, respectively, but RADFI did not differ statistically between breeds ($P = 0.27$; Table 4.1). There was a breed by time interaction for ADFI ($P = 0.001$) and RADFI ($P < 0.001$; Table 4.1), indicating that the rate of the increase in ADFI and RADFI was higher in S lambs compared to B lambs.

Nematode infection caused a significant reduction in ADFI in lambs ($P = 0.02$) and the interaction between infection and breed was not significant ($P = 0.71$; Table 4.1). RADFI was also decreased by infection in both breeds ($P = 0.025$; Table 4.1) and the interaction between infection and breed was not significant ($P = 0.42$; Table 4.1). In both breeds the RADFI of the PF lambs was similar to that of the INF lambs ($P = 0.1$; Table 4.1).

Body weight, body weight gain and body condition score. On average S lambs were heavier than B lambs throughout the experiment ($P<0.0001$; Figure 4.2b). The BWG of the S lambs was approximately 20% higher to that of the B lambs ($P < 0.001$; Table 4.1). Infection did not affect the BWG of lambs ($P = 0.41$; Table 4.1) and the interaction between breed and infection was not significant ($P = 0.84$; Table 4.1). In both breeds, PF and INF lambs did not differ in their BW gain ($P = 0.23$; Table 4.1). BCS in lambs remained almost constant throughout the experiment and was not affected by breed ($P = 0.97$) or by infection ($P = 0.89$). BCS of the PF lambs did not differ from that of INF lambs ($P = 0.31$). Least squares (LS) mean BCS for both breeds and all treatments was 3.00 ± 0.05.

Plasma pepsinogen and IgA. The average weekly back-transformed pepsinogen concentrations are shown in Figure 4.3a. The interaction between breed, infection and time was not statistically significant (P = 0.69). Plasma pepsinogen concentrations increased significantly with time in INF lambs but not in non-infected lambs as indicated by the interaction between infection and time ($P < 0.001$). In both breeds pepsinogen concentrations started to decline after the sixth week of infection. Plasma IgA concentrations against week of infection are shown in Figure 4.3b. There was a significant interaction between infection, breed and time ($P = 0.013$) caused by the increase with time in INF lambs but not AL lambs and the significantly greater increase with time in B than in S lambs.

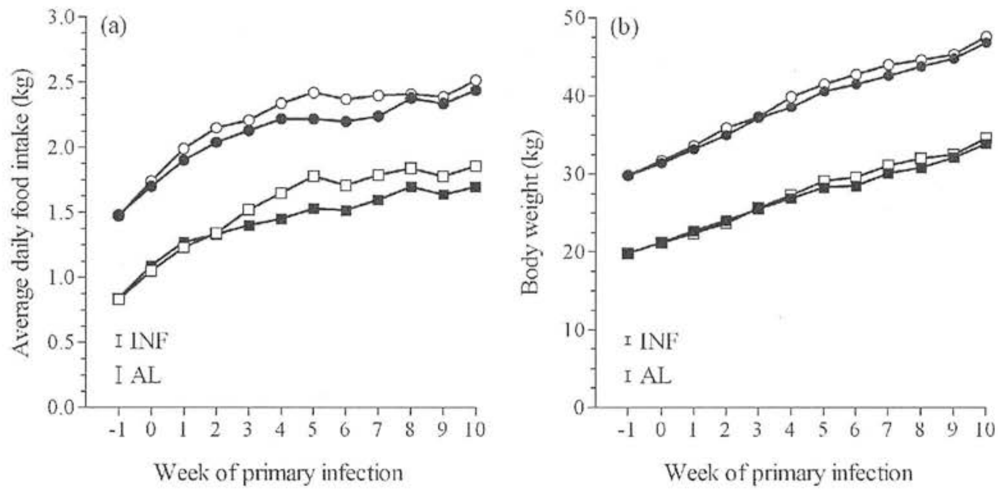

Figure 4.2. Average daily food intake (panel a) and body weight (panel b) of non-infected (AL; ○,□) and infected (INF; ●,■) fed *ad libitum* Suffolk × Greyface (○,●) and Scottish Blackface (□,■) lambs during the experiment I (primary infection). Standard errors of the means are shown by vertical bars and are based on error mean squares pooled over treatments.

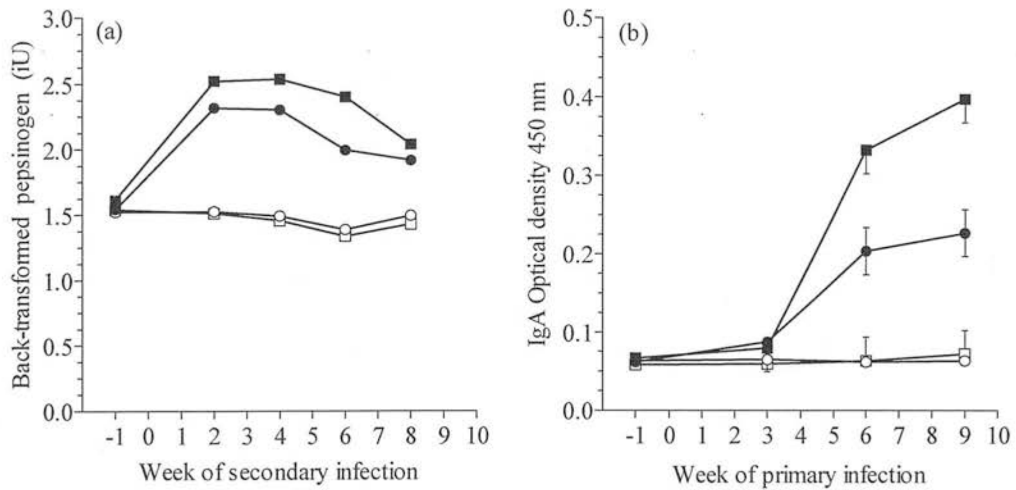

Figure 4.3. Mean weekly backtransformed $\log_{10}$ plasma pepsinogen (panel a) and IgA (panel b) of Suffolk × Greyface (○,●) and Scottish Blackface (□,■) lambs during the experiment I (primary infection). Infected lambs (●,■) were receiving orally 21,000 third-stage-larvae (L_3) of *T. circumcincta* per week (1 dose of 7,000 L_3 3 times a week for 12 weeks). Non-infected ad libitum fed lambs (○,□) were receiving at the same time a "sham" infection. Standard errors of the means are shown by vertical bars.

Plasma leptin concentration (PLC). Figure 4.4 shows the mean PLC of S and B lambs during the experiment. Analysis of the PLC data with models 1 and 2 showed that there were no significant effects of breed ($P > 0.17$) and infection ($P > 0.58$) on PLC or their interaction ($P > 0.41$). In addition model 2 showed that PLC was not affected significantly by the covariable BCS or RADFI ($P > 0.27$). All models showed that PLC was affected by time ($P < 0.001$), because of the gradual increase in PLC in all lambs towards the end of the experiment.

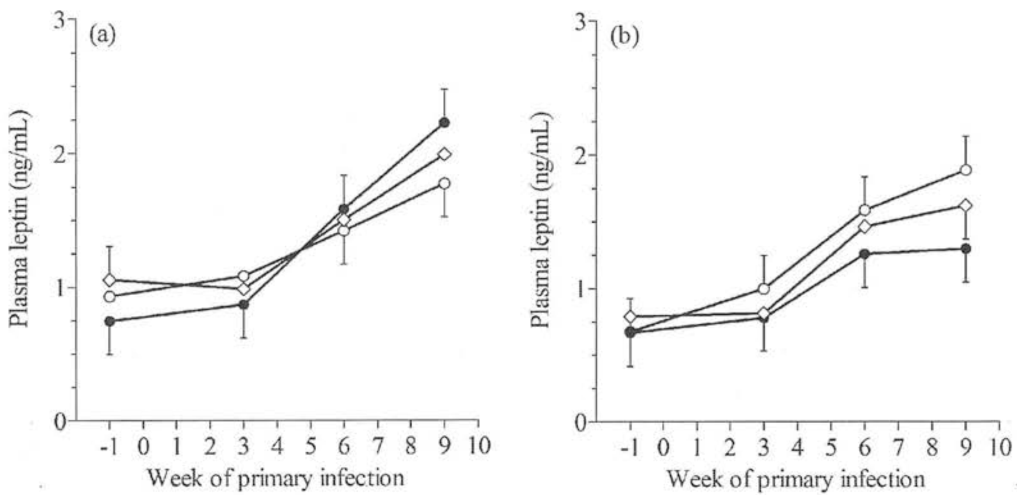

Figure 4.4. Mean weekly plasma leptin concentrations of Suffolk × Greyface (panel a) and Scottish Blackface (panel b) lambs during the experiment I (primary infection). Infected lambs (●) were receiving orally 21,000 third-stage-larvae (L_3) of *T. circumcincta* per week (1 dose of 7,000 L_3 3 times a week for 12 weeks). Non-infected *ad libitum* fed lambs (○) and non infected pair-fed lambs (◇) were receiving at the same time a "sham" infection. Standard errors of the means are shown by vertical bars.

Table 4.1. Least-square means of average daily food intake (ADFI), Relative ADFI to BW (RADFI), body weight gain (BWG) and plasma leptin concentrations (PLC) of the Suffolk × Greyface and Scottish Blackface lambs during experiment I (Primary Infection).

Experiment I	Suffolk × Greyface			Scottish Blackface			SEM[2]			*P*-value[3]						
Item[1]	INF	AL	PF	INF	AL	PF	INF	AL	PF	B	I	T	EPF	B×I	B×T	RADFI
ADFI (kg)	2.11	2.19	1.99	1.42	1.53	1.39	0.032	0.045	0.035	<0.001	<0.020	<0.001	0.009	0.71	<0.001	
RADFI (g)	54.70	56.10	52.20	52.9	55.7	52.3	0.800	1.100	1.100	<0.270	<0.025	<0.001	0.100	0.42	<0.001	
BWG (g)	220.00	227.00	204.00	184	173	162	8.900	12.600	12.900	<0.001	<0.410		0.230	0.84		
PLC[4] (ng/mL)	1.35	1.30	1.38	1.00	1.28	1.17	0.200	0.200	0.200	<0.370	<0.580	<0.001	0.630	0.41	<0.330	
	INF	NINF		INF	NINF		INF	NINF								
PLC[5] (ng/mL)	1.38	1.33		1.00	1.22		0.14	0.2		0.17	0.59	<0.001		0.43	0.21	0.34

[1] INF = Lambs fed ad libitum and infected with 21,000 third-stage-larvae (L_3) of *T. circumcincta* per week (1 dose of 7,000 L_3 3 times a week for 12 weeks; n = 24 per breed); AL = Non-infected, fed ad libitum lambs (n = 12 per breed); PF = Pair-fed lambs (n = 12 per breed) relatively to their body weight with 12 lambs of the same breed and gender of the INF treatment.

[2] Standard errors for ad libitum and restricted fed treatments are based on error mean squares pooled over ad libitum and restricted fed animals respectively.

[3] B = breed; I = parasite infection; T = time; EPF = effect of pair-feeding; RADFI = relative average daily feed intake (g/d). The *P*-values of the effects of B, I, T and their interaction were calculated based on error mean squares of the ad libitum fed animals only. *P*-values of the EPF were calculated based on error mean squares of the INF and PF animals only.

[4] Leptin LS means were calculated based on a statistical model where each treatment was included as a fixed factor. Standard errors are based on error mean squares pooled over all treatments.

[5] Leptin LS means were calculated based on model in which RADFI was included as co-variable. NINF = Non-infected lambs (n = 24 per breed)

4.4.2. Experiment II (Primary infection)

Faecal egg counts. Faecal samples taken one week prior to the start of the primary infection showed that all lambs were parasite naïve prior to the start of the experiment. Following the start of the nematode infection, FEC started to increase but remained very low (<10 epg) throughout the experiment and did not differ between breeds as indicated by the main effect of breed ($P = 0.31$) and the interaction between breed and time ($P = 0.21$). All non-infected lambs (sham infected) had zero FEC throughout the experiment.

Food intake. The ADFI for S lambs was significantly higher than that of the B lambs throughout the experiment ($P < 0.001$; Figure 4.5a). ADFI was not affected by infection ($P = 0.47$) and the interaction between breed, infection and time was not significant ($P = 0.9$). RADFI was not affected by infection ($P = 0.95$) or by breed ($P = 0.41$; Table 4.2). Pair-fed lambs tended to have lower RADFI compared to their counterparts ($P = 0.07$; Table 4.2).

Body weight, body weight gain and body condition score. On average S lambs were heavier than B lambs throughout the experiment ($P<0.001$; Figure 4.5b). The BWG of the S lambs was approximately 20% higher to that of the B lambs ($P < 0.027$; Table 4.2). Infection resulted in lower BW gain in the S lambs only, as shown by the interaction between breed and infection ($P = 0.04$; Table 4.2). In both breeds, PF2 and INF2 lambs did not differ in their BW gain ($P = 0.26$; Table 4.2).

BCS in lambs remained almost constant throughout the experiment and was not affected by breed ($P = 0.13$) or by infection ($P = 0.86$). BCS in PF2 lambs did not differ from that of INF2 lambs ($P = 0.37$). LS mean BCS for both breeds and all treatments was 3.1 ± 0.07 throughout the experiment.

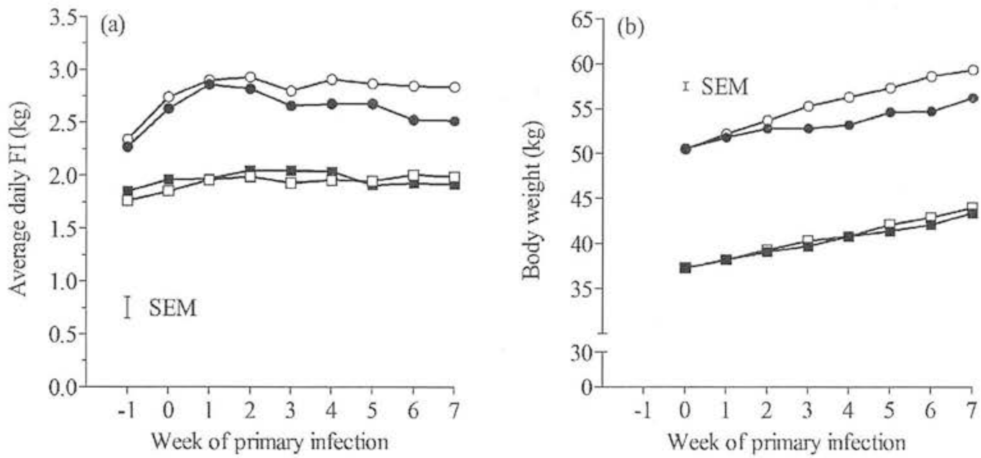

Figure 4.5. Average daily food intake (panel a) and body weight (panel b) of non-infected fed ad libitum (AL2; ○,□) and infected fed ad libitum (INF2; ●,■) Suffolk × Greyface (○,●) and Scottish Blackface (□,■) lambs during the experiment II (primary infection). Standard errors of the means are shown by vertical bars and are based on error mean squares pooled over treatments.

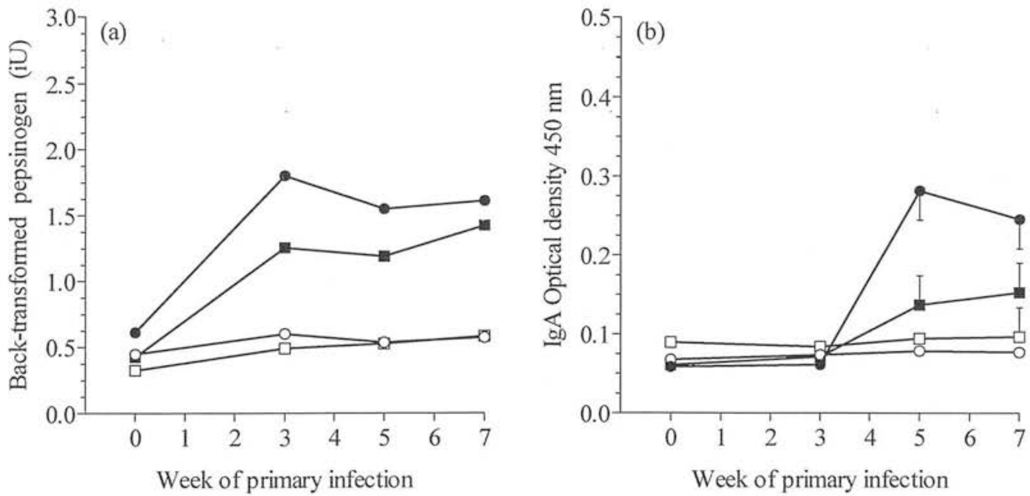

Figure 4.6. Mean weekly backtransformed $\log_{10}$ plasma pepsinogen (panel a) and IgA (panel b) of Suffolk × Greyface (○,●) and Scottish Blackface (□,■) lambs during the experiment II (primary infection). Infected lambs (●,■) were receiving orally 21,000 third-stage-larvae (L_3) of *T. circumcincta* per week (1 dose of 7,000 L_3 3 times a week for 12 weeks). Non-infected ad libitum fed lambs (○,□) were receiving at the same time a "sham" infection. Standard errors of the means are shown by vertical bars.

Plasma pepsinogen and IgA. The average weekly back-transformed pepsinogen concentrations are shown in Figure 4.6a. Plasma pepsinogen concentrations increased significantly with time in infected lambs but not in non-infected lambs as indicated by the interaction between infection and time ($P < 0.001$). The interaction between breed, infection and time was not statistically significant ($P = 0.33$).

Plasma IgA concentrations against week of infection are shown in Figure 4.6b. There was a statistically significant interaction between interaction between infection and time ($P < 0.0001$; Figure 4.6b) because plasma IgA increased in infected lambs over time. The interaction between breed, infection and time was not statistically significant ($P = 0.14$).

Plasma leptin concentration. Figure 4.7 shows the mean PLC of S and B lambs during the experiment. Analysis of the PLC data with models 1 and 2 showed that there were no significant effects of breed ($P > 0.5$) and infection ($P > 0.1$) on PLC or their interaction ($P > 0.1$). In addition model 2 showed that there was a statistically significant relationship between PLC and RADFI in both breeds, as evidenced by the covariable coefficient ($P < 0.001$). The estimated covariable coefficient showed that a difference in RADFI of 10 g was associated with a difference in PLC of 0.43 ng/ml (SE 0.131; $P = 0.001$). The model that included BCS as a covariable showed that there was no statistical evidence of a relationship between PLC and BCS ($P = 0.54$). All models showed that PLC was affected by time ($P<0.001$; Figure 4.7), because of the gradual increase in PLC in all lambs towards the end of the experiment and the interaction between time and breed or treatment was not significant ($P > 0.1$).

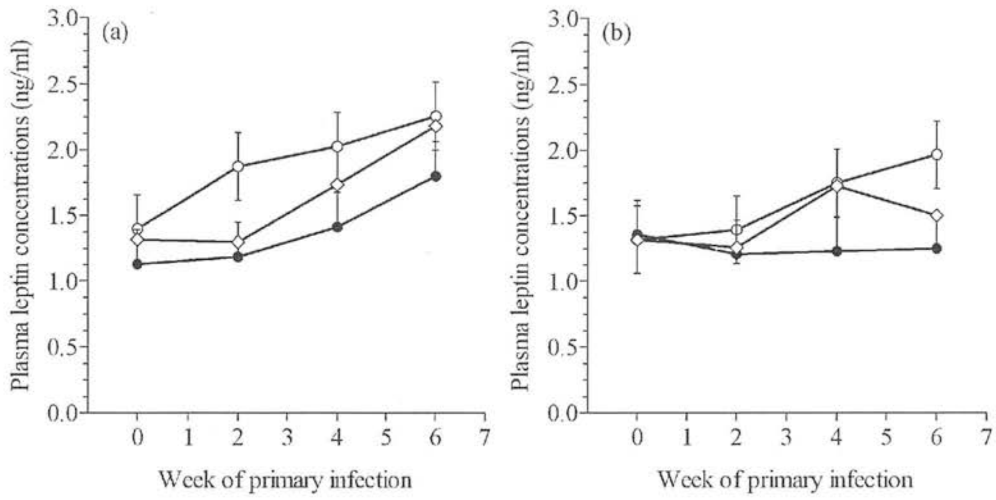

Figure 4.7. Mean weekly plasma leptin concentrations of Suffolk × Greyface (panel a) and Scottish Blackface (panel b) lambs during the experiment II (primary infection). Infected lambs (●) were receiving orally 21,000 third-stage-larvae (L_3) of *T. circumcincta* per week (1 dose of 7,000 L_3 3 times a week for 12 weeks). Non-infected *ad libitum* fed lambs (○) and non infected pair-fed lambs (◇) were receiving at the same time a "sham" infection.

Table 4.2. Least-square means of average daily food intake (ADFI), Relative ADFI to body weight (RADFI) BW, body weight gain (BWG) and plasma leptin concentrations (PLC) of the Suffolk × Greyface and Scottish Blackface lambs during experiment II (Primary Infection).

Experiment II	Suffolk × Greyface			Scottish Blackface			SEM[2]			*P*-value[3]						
Item[1]	INF2	AL2	PF2	INF2	AL2	PF2	INF2	AL2	PF2	B	I	T	EFR	B×I	B×T	RADFI
FI (kg/d)	2.66	2.82	2.46	1.97	1.94	1.83	0.09	0.09	0.07	<0.001	0.470	<0.001	0.034	0.28	0.002	
RADFI (g/d)	049.60	50.40	45.80	048.70	048.10	45.80	1.86	1.86	1.25	0.410	0.950	<0.001	0.070	0.72	0.007	
BWG (g/d)	121.00	191.00	143.00	131.00	146.00	140.00	13.30	13.30	13.30	0.027	0.004		0.260	0.04		
PLC[4] (ng/mL)	0.89	2.06	1.65	1.30	1.62	1.42	0.28	0.28	0.28	0.950	0.120		0.270	0.13	0.470	
	INF	NINF		INF	NINF		INF	NINF								
PLC[5] (ng/mL)	1.41	1.84		1.55	1.30		0.28	0.2		0.510	0.120	0.01		0.10	0.530	<0.001

[1] INF_2 = Lambs fed ad libitum and infected with 21,000 third-stage-larvae (L_3) of *T. circumcincta* per week (1 dose of 7,000 L_3 3 times a week for 12 weeks; n = 8 per breed); AL_2 = Non-infected, fed ad libitum lambs (n = 8 per breed); PF = Pair-fed lambs (n = 8 per breed) relatively to their body weight with 12 lambs of the same breed and gender of the INF_2 treatment.

[2] Standard errors for ad libitum and restricted fed treatments are based on error mean squares pooled over ad libitum and restricted fed animals respectively.

[3] B = breed; I = parasite infection; T = time; EPF = effect of pair-feeding; RADFI = relative average daily feed intake (g/d). The *P*-values of the effects of B, I, T and their interaction were calculated based on error mean squares of the ad libitum fed animals only. *P*-values of the EFR were calculated based on error mean squares of the INF and PF animals only.

[4] Leptin LS means were calculated based on a statistical model where each treatment was included as a fixed factor. Standard errors are based on error mean squares pooled over all treatments.

[5] Leptin LS means were calculated based on model in which RADFI was included as co-variable. NINF = Non-infected lambs (n = 8 per breed)

4.4.3. Experiment III (Secondary infection)

Faecal egg counts. During the course of the secondary infection mean weekly FEC in both breeds remained very low (at all time points FEC was below 5 eggs per g faeces). FEC did not differ between the two breeds (P = 0.9) and treatments (P = 0.5) and the interaction between breed, treatment and time was not significant (P= 0.16).

Food intake. The ADFI differed between the two breeds as S lambs had significantly higher intakes than B lambs throughout the experiment (P < 0.001; Figure 4.8a). Re-infection did not cause any reduction in ADFI in both R4 and R8 treatments (P > 0.4) and the interaction between breed, treatment and time was not significant (P = 0.1). RADFI was not affected either by treatment or by breed (P > 0.44).

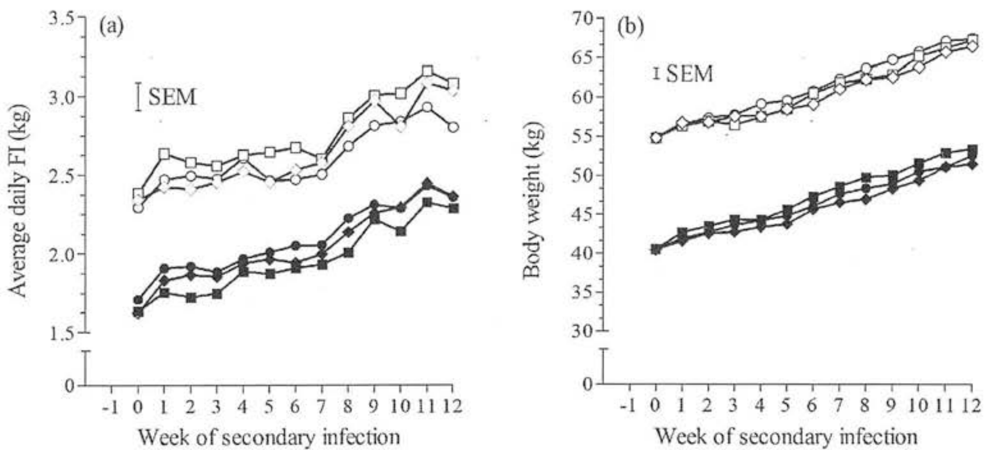

Figure 4.8. Average daily food intake (panel a) and body weight (panel b) of non-infected (CON; ○,●), re-infected after 8 weeks of the end of the primary infection (R8; ◇,◆) and re-infected after 4 weeks of the end of the primary infection (R4; □,■) fed ad libitum Suffolk × Greyface (○,◇,□) and Scottish Blackface (●,◆,■) lambs during the experiment III (secondary infection).

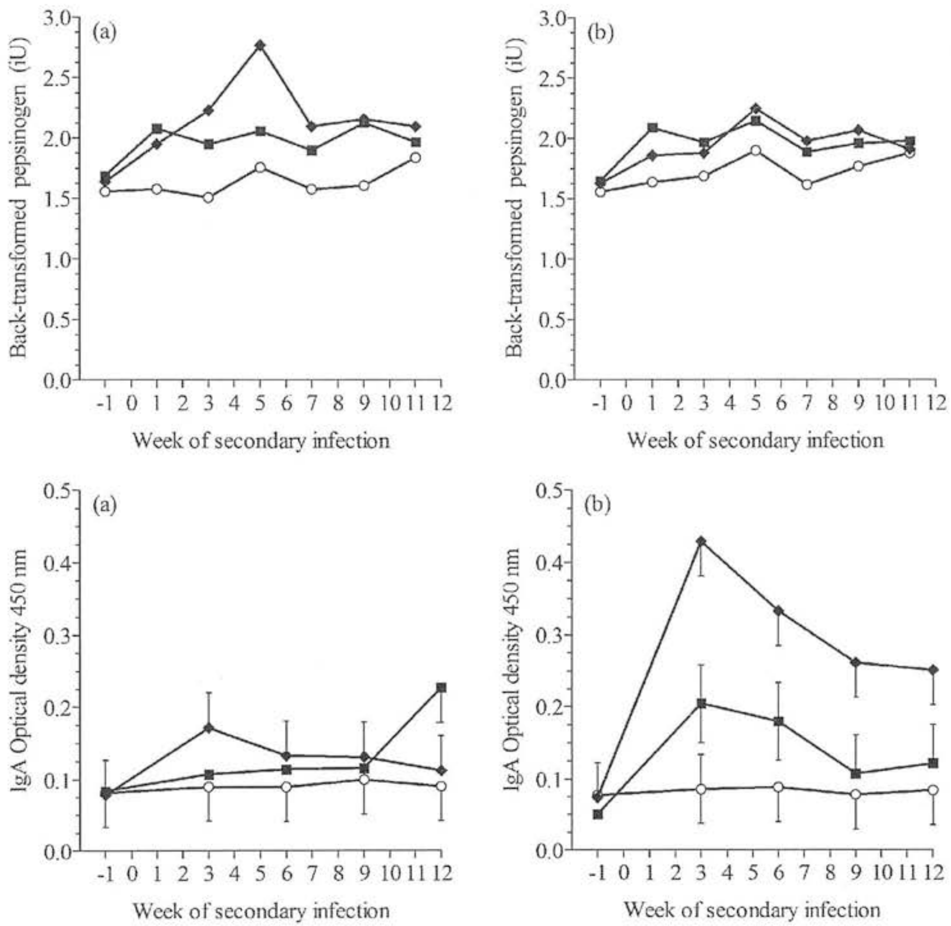

Figure 4.9. Mean weekly IgA and backtransformed $\log_{10}$ plasma pepsinogen concentrations of Suffolk × Greyface (panels a) and Scottish Blackface (panels b) lambs during the experiment III (secondary infection). Lambs were re-infected with 21,000 third-stage-larvae (L_3) of *T. circumcincta* per week (1 dose of 7,000 L_3 3 times a week for 12 weeks) either after 8 weeks of the end of the primary infection (R8; ◆) or after 4 weeks of the end of the primary infection (R4; ■). Non-infected *ad libitum* fed lambs (○)were receiving at the same time a "sham" infection.

Body weight, body weight gain and body condition score. During the course of the secondary infection S lambs were significantly heavier than B lambs ($P < 0.001$; Figure 4.8b). BW gain did not differ between the breeds ($P = 0.6$) and was not affected by treatment ($P > 0.18$). BCS was not affected by breed ($P = 0.63$) or by treatment ($P > 0.65$) and remained almost constant throughout the experiment (LS mean 3.01 ± 0.06).

Plasma pepsinogen and IgA. The average weekly back-transformed pepsinogen concentrations are shown in Figure 4.9. In both re-infection treatments plasma pepsinogen concentrations of the lambs were higher compared to non infected control lambs as shown by the main treatment effect ($P = 0.04$) and its interaction with time ($P = 0.01$). Plasma pepsinogen concentrations were not affected by breed ($P = 0.71$) and did not differ between the re-infection treatments ($P = 0.5$).

Plasma IgA concentrations increased significantly as a result of the re-infection but the increase was significant only for the B lambs of the R8 treatment as shown by a statistically significant interaction between breed, treatment and time ($P = 0.01$; Figure 4.9b).

Plasma leptin concentration. Analysis of PLC with model 1 (no covariables included) showed no differences between treatments ($P = 0.53$) and breeds ($P = 0.2$). When data were analyzed with model 2, in which, RADFI was included as a covariable a statistically significant positive relationship between PLC and RADFI was observed, as evidenced by the covariable coefficient ($P = 0.012$). The estimated covariable coefficient showed that a difference in RADFI of 10 g was associated with a difference in PLC of 0.72 ng/ml (SE 0.205; $P = 0.018$). According to this model PLC was not affected by infection ($P = 0.69$) or breed (P = 0.28). The model that included BCS as a covariable showed also that there was a significant statistical relationship between PLC and BCS ($P = 0.02$). The estimated covariable coefficient showed that a difference in BCS of 1 was associated with a

difference in PLC of 0.61 ng/ml (SE 0.265; $P = 0.02$). The effect of re-infection on PLC however, remained non significant ($P = 0.62$). All models showed that PLC was affected by time ($P < 0.001$), because of the gradual increase in PLC in all lambs towards the end of the experiment (Figure 4.10).

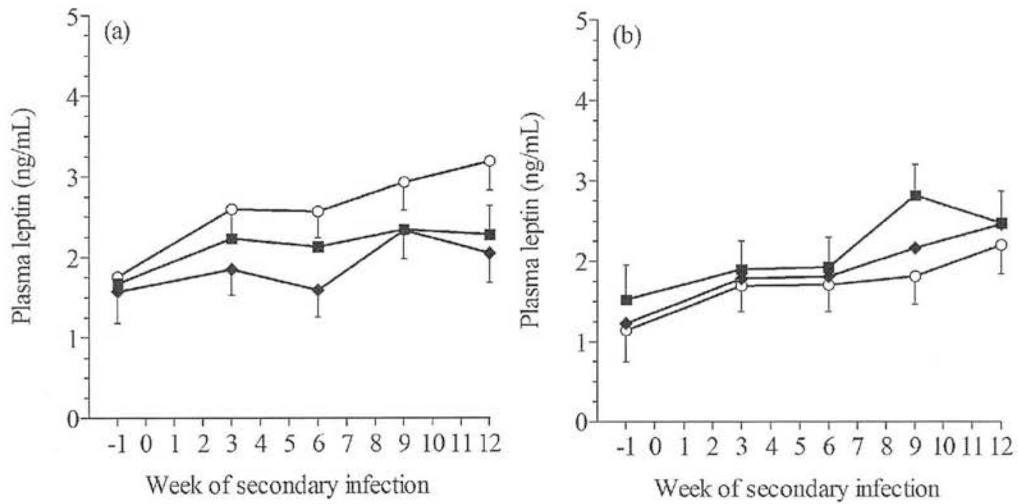

Figure 4.10. Mean weekly plasma leptin concentrations of Suffolk × Greyface (panel a) and Scottish Blackface (panel b) lambs during the experiment III (secondary infection). Lambs were re-infected with 21,000 third-stage-larvae (L_3) of *T. circumcincta* per week (1 dose of 7,000 L_3 3 times a week for 12 weeks) either after 8 weeks of the end of the primary infection (R8; ◆) or after 4 weeks of the end of the primary infection (R4;■). Non-infected *ad libitum* fed lambs (○)were receiving at the same time a "sham" infection.

4.5. Discussion

The present study examined the effects of a primary and a secondary nematode infection on ADFI, and the relationship between anorexia and PLC in two breeds of lambs that differed in their intrinsic capacity for growth.

4.5.1. Effects of breed and age on performance and response to infection

The present experiments compared S and B lambs in their immune response (i.e. FEC and IgA) to a primary and a secondary nematode infection. S lambs have been selected more intensively for growth than B lambs and the differences in performance between these breeds have been demonstrated in earlier studies (Emmans and Friggens, 1995; Lewis et al., 2004). The results of the present experiments show that the performance, in terms of BWG, of the ad *libitum* fed S lambs was indeed superior to that of the *ad libitum* fed B lambs in both experiments I ($P < 0.001$) and II ($P = 0.005$), as expected. The breed differences in BWG between the two breeds seem, however, to diminish as lambs grow heavier and approach maturity (Experiment III).

The lambs used in experiment I and II had not been exposed to infective larvae before and, therefore, were considered parasite naïve prior to the start of the experiments. In support of this assumption are both the FEC and IgA results that refer to the period before the start of the primary trickle infection with the nematode *T. circumcincta*. During experiment I, infected animals started to show moderate levels of eggs in their faeces by the second week of dosing and these rose to a maximum value during the third week of infection (Figure 4.1). From the fourth week of infection, FEC sharply declined which suggests that development of immunity had started to occur, although FEC did not reach zero values at the end of the experiment in either breed. The FEC values observed in experiment I were not as

high as those reported in earlier studies (i.e. 450 epg or more around the third to fifth week of infection) that used similar doses of *T. circumcincta* infection in parasite naïve lambs (Symons et al., 1981; Coop et al., 1982). This is possibly related to the quality of the food used in experiment I, as improved nutrition can enhance the degree to which the animals can acquire immunity (Coop and Holmes, 1996; Coop and Sykes; 2001).

There was a breed effect on FEC, mainly because FEC were raised to higher values in S than in B lambs. This observation is in accordance with earlier studies that compared breeds of sheep that differ in their production level in terms of growth or fleece weight (McEwan et al., 1992; Miller et al., 1998; Amarante et al. 2004; Good et al., 2006). FEC can be considered as an indirect measure of immunity that has frequently been used to compare differences in resistance to infection between breeds (Bisset et al., 2001; Amarante et al., 2004; Good et al., 2006). The results of experiment I suggest, therefore, that S lambs are more susceptible to infection than B lambs, which is a finding that concurs with the results of the primary infection experiment, as described in Chapter 3.

In both experiments I and II of the current study, primary infection resulted in a significant increase in IgA and the effect was more pronounced in B that in S lambs, during experiment I. In experiment III a rapid increase in IgA was observed also in B lambs but only in the R8 treatment. IgA response to infection is regarded to be the most important manifestation of immunity in growing lambs as it affects worm growth and causes a reduction in worm fecundity (Stear et al., 1995; Strain and Stear 1999). Immunity to nematode infections in sheep, associated with an IgA response, has been shown to have a significant nutritional cost for the host (Greer et al., 2005). Therefore, the differences in immune response to infection between S and B lambs as shown both by IgA and FEC results, could well be mediated by differences in the host's prioritization of nutrients towards its various physiological functions, as suggested by Colditz (2003, 2008).

The breed differences in FEC were not apparent either in experiment III (re-infection) or experiment II (primary infection of 6-month lambs) during which there was hardly any effect of (re)infection on FEC. The very low FEC that was observed during the primary infection in experiment II, in contrast to that of experiment I, suggests that the susceptibility to *T. circumcincta* infection is age-related. A similar pattern of age related susceptibility occurs in many other nematode infections of tropical sheep (Urquhart et al., 1996) and cattle in both temperate (Urquhart et al., 1996) and tropical regions (Stear et al., 1990). The difference in response to infection between the 4-month and the 6-month old lambs, as evidenced by FEC in the current experiments (I and II), was not reflected by differences in IgA concentrations. The timing and the size of the IgA response of the lambs was broadly similar in experiments I and II. A remarkable difference in IgA response to infection was reported earlier by Smith et al. (1985) for 4½- and 10-months old lambs, with younger lambs showing significantly poorer IgA response. Therefore, the finding that the IgA response was similar between the 3- and the 6-month old lambs in the present study could be related to the fact that the lambs differed only three months in age.

With regards to experiment III, FEC did not differ between R8 and R4 treatments and it could be concluded that a loss of immunity did not occur within the 8-week parasite free period. However, if a loss of immunity had occurred, it is very unlikely that such a result would have been detected, because it would probably have been wiped out by the strong effect of age, as it is demonstrated by experiment II. This is likely because re-infected lambs were already 7 months old at the start of re-infection. In support of this view are the results of Kimambo et al., (1998) which report that after a prolonged (6 months) parasite-free period no worm eggs were detected in the faeces of nematode re-infected sheep of 21 months old. The IgA response to re-infection, at least within the R8 lambs, suggests a difference in immune response between S and B lambs. The results also support the view that previously infected lambs have better ability to mount an immune response than previously naïve lambs

following infection, as shown by their earlier IgA response to nematode challenge (Figure 4.9b. *vs.*4.3b and 4.6b).

Plasma pepsinogen concentrations were affected by infection in all experiments, but they were not affected by breed, age or immune status of the lambs. An increase in plasma pepsinogen concentration is a reflection of the abomasal epithelium damage, which is both necessary and sufficient to allow pepsinogen to diffuse into the lymph and subsequently into the bloodstream (Baker et al., 1993; Stear et al., 1999). The damage in the abomasal epithelium can be a direct consequence of the established adult worms on the epithelium (Berghen et al., 1993) and/or a hypersensitivity reaction as part of the developing immune response that involves hyperplasia of mucosal mast cells (Yakoob et al., 1983; Smith et al.,. 1984; Huntley et al., 1987). Increased plasma pepsinogen concentrations have been frequently observed in immune animals that can mount effective immune response to ingested larvae (Barger 1982; Yakoob et al., 1983; Stear et al., 1994; Lawton et al., 1996; Stear et al., 1999; Stear et al., 2001). Therefore the increase in pepsinogen concentration that was not accompanied with an increase in FEC, at least in experiments II and III, is likely the result of immune response on gastro-intestinal integrity, rather than a direct consequence of the abomasal damage caused by the action of parasites *per se.*

4.5.2. Effects of (re-)infection on food intake and performance

During the course of the primary infection in experiment I, a significant reduction in food intake was observed from the third week of infection and onwards in parasitized lambs in both breeds. The reduction in ADFI in both breeds was around 6%, which is relatively small compared to depressions of appetite of around 12% or more that earlier studies report in infected lambs with *T. circumcincta* (Coop et al., 1977; Sykes and Coop, 1977; Symons et al., 1981). However, this finding is interesting in view of the results of the previous experiment (described in Chapter 3), where the same dose of *T. circumcincta* resulted in a

well established anorexia (of around 12%), at least in infected Suffolk lambs. The lambs in the previous experiment (experiment I; Chapter 3) were fed a similar type of food (grass pellets), which had a protein content of 186g kg per fresh food, while the lambs in the current experiment were fed on grass pellets with a slightly less protein content (i.e.166 g per kg fresh food). It is therefore unlikely that the dietary protein content of the food could have contributed in the differences in the degree of anorexia as shown in the two experiments. Although the effects of dietary protein on anorexia have not been studied extensively, relevant to this suggestion is the observation that the degree of anorexia in lambs infected with *T. colubriformis* and fed either a high (206 g/kg DM) or a low (86 g/kg DM) protein diet, did not differ significantly between the nutritional treatments (Kyriazakis et al., 1996). Van Dam et al., (1997) also report that the protein content of the food does not affect the degree of anorexia in goats following trypanosome infection.

The results of current experiments support the view that anorexia is associated with the acquisition phase of the developing immune response in growing lambs (Coop et al., 1977; Sykes and Coop, 1977; Symons et al., 1981; Greer et al., 2005) as, both breeds showed anorexia during primary infection (experiment I) but not during the secondary infection (experiment III). However, the finding that the 6-month old parasite naïve lambs of experiment II did not show significant reduction in ADFI following infection was unexpected, and there is not a clear explanation for this result. It seems however, that the occurrence of anorexia is not related to humoral immune responses, as it is evidenced by the IgA results, previously suggested by Greer et al., (2005). In all experiments infection resulted in a stimulation of IgA response, but anorexia observed only during the primary infection in the 4-month old lambs (experiment I). In addition, although there was a stronger IgA response to infection by B lambs in experiment I, as shown by the interaction between breed, infection and time, the reduction in intake did not differ significantly between the breeds (Table 4.1). Recently, a study with infected and concurrently immuno-suppressed

lambs provided evidence that the parasite-induced anorexia of sheep is a direct consequence of the immune system activation (Greer et al., 2005). Recent studies (Pernthaner et al., 2005, 2006) have also revealed novel evidence that an early immune response to nematode infection in sheep depends on pro-inflammatory cytokine production, as it has been suggested earlier for parasitised murine models (Roberts et al., 1999; Maizels and Yazdanbakhsh, 2003). Currently, it is not known whether pro-inflammatory cytokines contribute to the anorexia of the nematode infections in sheep. However, as the findings of the present experiments do not support a strong implication of the humoral responses that are associated with IgA production, it could be possible that cellular responses may be involved. It could be possible also that the pro-inflammatory type reaction evolves more quickly to a specific type immune reaction in older animals and thus the extent of anorexia following infection is less pronounced. While immunity to parasites diminishes with time if challenge is withdrawn (Dineen and Wagland, 1966), in support of the view that acute type reaction evolves more quickly to a specific type immune reaction are the results of Kimambo et al., (1998) which found that after a prolonged (6 months) parasite-free period infection did not cause any inappetence in 21-montths old sheep.

4.5.3. Effect of (re)infection on PLC in relation to food intake.

Although it appears that in a range of different species, the adipose-tissue derived hormone leptin is associated with immune reactivity, the role of leptin on the immune response against gastrointestinal nematode infections in sheep has been little explored. The aim of the current study was to test the hypothesis that a nematode challenge in lambs would result in elevated plasma leptin levels during the acquisition and/or development of immunity.

The results show that during the course of the primary infection (experiment I and II) but also the secondary infection (experiment III), actual PLC, as analyzed by Model 1,

did not differ significantly between infected and non-infected ad libitum fed lambs. These results concur with the earlier findings of the previous experiments in the present thesis (Chapter 3), as infection did not result in an acute increase in PLC. These results are also in agreement with the only available studies on the effect of nematode infection on PLC in *ad libitum* (Fox et al., 2006) and restrictedly fed (Liu et al., 2007) lambs. The findings are in contrast to the increase in PLC that has been observed in studies during nematode infection (Mercer et al., 2000; Roberts et al., 1999), acute intestinal inflammation (Barbier et al., 1998), and other disease models in rodents (Faggioni et al., 1997; Grunfeld et al., 1996; Sarraf et al., 1997). Gastrointestinal nematode infection does, therefore, not seem to result in an acute increase of PLC in *ad libitum* fed sheep.

It is well recognized that starvation results in reduced PLC in healthy animals including sheep (Blache et al., 2000; Delavaud et al., 2000; Adam et al., 2002; Altmann et al., 2005). Parasitised lambs are expected to show anorexia as a result of infection and therefore, this decrease in intake could have itself an effect on PLC in parasitised sheep. On the other hand, an increase in PLC or leptin administration reduces ADFI in a dose-dependent manner in many animal species, including ruminants (Barrachina et al., 1997; Wang et al., 1997; Bard et al., 1998; Henry et al., 1999; Morrison et al., 2001). Furthermore, PLC increases in many models of disease following infection (Mercer et al., 1999; Moshyedi et al., 1998; Barbier et al., 1998). Barb et al., (2001) suggested that leptin regulation in pigs during endotoxin challenge is under the control of a both stimulatory (i.e. by inflammatory mediators) and inhibitory (i.e. due to changes in energy metabolism) mechanism, and Kulcsar et al.,. (2005) have argued that this might be also the case during infections in sheep. The results of the previous Chapter (3) provided support of this suggestion, as PLC was significantly affected by infection, when leptin data were corrected for the level of food intake. However, in the present experiments, the effect of infection on PLC was not significant neither in the previously naïve (experiments I and II) nor in the previously

infected lambs (experiment III) when leptin data were corrected for the level of food intake or BCS (model 2). Because infection resulted in a relatively small anorexia in experiment I in both breeds, and no anorexia at all in the parasitised lambs in experiment III, the ADFI of the pair-fed lambs was not significantly reduced compared to the intake of their infected counterparts (Tables 4.1 and 4.2). It is therefore unlikely that the food intake data of the pair-fed lambs have provided significant correction for the effect of the level of food intake on PLC. The PLC results of the pair-fed lambs suggest that a reduction in ADFI of around 6% is not strong enough to result in significant and systematic lower PLC in lambs.

Therefore, whilst the hypothesis that gastrointestinal nematode infection results in an increase of PLC in lambs may not be rejected, the evidence from this study do not seem to provide support for it. The absence of any clear association between RADFI and changes in PLC in experiment I during which a reduction in intake was observed, suggests that leptin is not essential for the parasite induced anorexia in sheep.

4.5.4. Conclusions

The data show that breed differences in intrinsic capacity for growth are associated with susceptibility to infection as lambs of the S breed had higher FEC and weaker IgA response than lambs of the B breed. The susceptibility to infection seems to be age related as 7month old lambs had better response to a primary infection than 4-months old lambs as evidenced by the lower FEC, although IgA response appeared to be similar. The results showed that a primary infection is associated with anorexia in young lambs; however, the degree of anorexia was not affected by the IgA response to infection. Re infection of previously infected lambs did not result in renewed anorexia when lambs were re-infected after 4 or 8 weeks of the end of the primary infection. Finally the results show that nematode (re)infection did not result in an increase of PLC and leptin itself is unlikely to be responsible for the anorexia of nematode infection in lambs.

CHAPTER FIVE

Consequences of protein supplementation for anorexia, expression of immunity and plasma leptin concentrations in parasitised ewes of two breeds

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5.1 Abstract

The periparturient relaxation of immunity (PPRI) against parasites in ewes has a nutritional basis. This study investigated whether ewes experience a reduction in food intake (anorexia) during PPRI and if the magnitude of anorexia is affected by host production potential and dietary protein supplementation. The study also investigated whether nematode infection is linked to plasma leptin concentrations in periparturient ewes. The experiment was a 2×2×2 factorial design. Two breeds of twin-bearing / lactating ewes (Greyface cross; Scottish Blackface × Border Leicester), G (n=32) and Scottish Blackface, B (n=32) were used. Half of the ewes was trickle infected with 30,000 larvae of the abomasal parasite *Teladorsagia circumcincta* per week and the other half was not. During the experiment, all ewes had *ad libitum* access to a low protein diet that provided less protein than the recommended allowance. In addition, half of the ewes received a protein supplement that resulted in protein intakes that exceeded recommendations. Nematode infection resulted in a breakdown of immunity to parasites and a reduction in food intake in both breeds. The breeds differed in the extent of PPRI (G ewes having higher FEC than B ewes), but not in the magnitude of anorexia. Protein supplementation resulted in a reduction in FEC, but had no effect on the magnitude of anorexia. Plasma leptin concentrations changed significantly over time, but were not affected by protein supplementation or infection. It is concluded that infection with *T. circumcincta* in periparturient ewes results in anorexia that is not alleviated by protein supplementation and seems unrelated to plasma leptin concentrations.

5.2 Introduction

Anorexia, i.e. a reduction in voluntary food intake, is a prominent feature of many infections, including gastrointestinal parasitism. In sheep, the occurrence of anorexia after nematode infection has been investigated mainly in parasite-naïve lambs (Coop et al., 1982) and such studies have shown that food intake returns to normal when animals acquire a full immunity to the parasites (Kyriazakis et al., 1996). Reproductive ewes, however, can experience a breakdown of their acquired immunity to parasites during their periparturient period (from late pregnancy through to early lactation) and this is generally referred to as the "periparturient relaxation of immunity" (PPRI). The phenomenon manifests as an increase in faecal egg counts and worm burdens (Donald et al., 1982; Leyva et al., 1982; Barger, 1993). However, there is scant evidence whether or not ewes experience a reduction in food intake during PPRI. In addition it is unknown whether there are differences between breeds in the degree of anorexia during PPRI and whether these are linked to the production potential of the breeds.

Coop and Kyriazakis (1999) while developing a nutrient partitioning framework that accounts for nutrient allocation towards the various physiological functions of the host, have suggested that PPRI has a nutritional basis. A number of studies have shown that the extent of the PPRI can be reduced by an increased intake of metabolizable protein (MP) (Houdijk et al., 2000; 2001b). Whether enhanced protein nutrition affects the degree of anorexia in infected periparturient ewes is not known. In addition, it has been suggested that breeds that have been selected more intensively for production traits (e.g. growth) are more susceptible to gastrointestinal infections than breeds that have been selected less intensively (Bisset et al., 2001; Baker, 1998; Rauw et al., 1998). Whether or not such breed differences in production potential are reflected in the degree of anorexia following infection, is not known.

Maternal plasma leptin concentrations in non-infected ewes decline progressively during late pregnancy and early lactation (Ehrhardt et al., 2001) and it has been suggested that this reduction in leptin could have negative effects on immune function (Ingvartsen and Boisclair, 2001). Adipose tissue metabolism plays an important role in the regulation of immune responses (Fantuzzi, 2005) and increased plasma leptin concentrations as a result of infection or inflammation have been observed in many models of disease (Grunfeld et al., 1996; Barbier et al., 1998). Although a similar role for leptin has been suggested for nematode infected ewes by Valderrabano et al., (2006), this was not based on a comparison of leptin concentrations between infected and uninfected ewes.

The aim of the present study was to test the hypotheses that a) nematode infected ewes experience anorexia during the occurrence of PPRI and the magnitude of anorexia is greater in ewes selected more intensively for high productivity than in ewes that have been selected less intensively, b) the degree of anorexia can be reduced by dietary protein supplementation and c) nematode infection of periparturient ewes will result in increased plasma leptin concentrations (PLC) compared to non-infected periparturient ewes.

5.3. Materials and Methods

The Animal Experiments Committee of the Scottish Agricultural College approved the experimental protocol (AE ED 02/2005). The experiment was carried out under Home Office authority for experimental parasitic infection and repeated blood sampling (PPL 60/3004).

5.3.1. Animals, Housing and Husbandry

Sixty-four pregnant ewes, 32 Greyface crosses (Scottish Blackface × Border Leicester; G) and 32 Scottish Blackface (B), which were identified by scanning as bearing twins, were brought indoors 57 days (d_{-57}) before the realized mean parturition day (d_0). Upon housing, ewes were orally drenched with Ivermectin (Oramec, Merial, UK) and Levamisole (Nilverm Gold, Schering-Plough, Welwyn Garden City, UK) according to manufacturer's instructions, in order to remove residual worm burdens from previous exposure to parasites. The mean BW and BCS at d_{-57} were 73.1 ± 1.13 kg and 3.1 ± 0.04 and 53.2 ± 1.00 kg and 2.5 ± 0.04 for G and B ewes respectively. Ewes were housed in a naturally illuminated and ventilated shed in individual pens with solid floors until 5 weeks into lactation (d_{35}). The pens were 1.5 × 2.0 m and were bedded with a thick layer of saw dust that was topped up when required. Each pen was equipped with two feeding bins and ewes had free access to water from a bucket all day. Feeding bins were raised above floor level, a practice which prevented lambs from consuming any of the feed offered to the ewes.

5.3.2. Experimental Design

Ewes were assigned randomly to treatments on the basis of their BW and BCS measured on day_{-57}, and ensuring that an equal number (n=8) of similar ewes of each breed were allocated to each treatment. The experimental design was a 2×2×2 factorial, which

involved the two breeds of sheep, two levels of infection (infected and uninfected controls) and two feeding treatments (protein supplemented and unsupplemented).

5.3.3. Infection Treatments and Infection Details

The ewes were expected to have had previous exposure to gastrointestinal nematodes from field infections prior to housing. Following a 10-d adaptation period after housing, half of the ewes in each breed was trickle-infected (treatment: +) with the gastrointestinal nematode *Teladorsagia circumcincta* at a dose of 10,000 infective third-stage larvae, in 10 ml of water, each Monday, Wednesday and Friday until the end of the experiment. Similar rates of infection have previously been shown to lead to establishment of a patent *T. circumcincta* worm burden in periparturient ewes (Leyva et al., 1982; Houdijk et al., 2003). The larvae were incubated from eggs that were harvested from fresh faeces of infected whether donor sheep every 14 days. Non-infected ewes (treatment: −) were given a similar volume of water only (sham infection) at the same time, thus undergoing the same amount of handling as the infected ewes.

5.3.4. Feeding Treatments and Experimental Diets

From housing until d_{-28} all ewes were offered *ad libitum* hay as a sole diet in an effort to reduce body condition score (Tolkamp et al., 2007). During late pregnancy, i.e. from d_{-28} all ewes were fed the same low protein pelleted feed, *ad libitum,* until the end of the experiment. This diet was formulated to provide sufficient energy, minerals and vitamins but less than the estimated metabolisable protein (MP) requirements (Table 5.1) of the ewes. The feed provided an estimated 7g of MP per MJ metabolisable energy (ME), while the requirements for such twin bearing/lactating ewes are estimated to be 9g MP/MJ ME (AFRC, 1993; Houdijk et al., 2001b). For half of the ewes within each infection treatment this was the only food supplied (treatment: LP). The other half of the ewes in each infection

Table 5.1. Ingredients and chemical analysis of the experimental feeds*

Ingredients (g/kg fresh feed)	Basal Diet (Pelleted)	Soypass Supplement
Barley	290.0	-
Oatfeed	300.0	-
Citrus Pulp	300.0	-
50% Fat Premix	17.0	-
Molasses	50.0	-
Salt	8.0	-
Limestone flour	1.0	-
Calcined Magnesite	4.0	-
Dicalcium phosphate	10.0	-
Scotmin ewe/lamb	2.0	-
Urea	18.0	-
SoyPass® (xylose-treated soybean meal)	-	1000.0
Analyzed composition†		
Dry matter (DM)	879.0	794.0
Crude protein	135.0	514.0
Netural detergent fibre	269.0	223.0
Acid detergent fibre	189.0	56.8
Ether extract	33.3	10.8
Gross Energy	18.0	19.8
NCGD‡	734.0	827.0
Estimated energy and protein supply§		
Metabolizable protein (g/kg DM)	73.0	400.0
Metabolizable energy (MJ/kg DM)	10.5	12.5

*The diets were fed from d_{-28} to d_{35}, relative to parturition, d_0.

†Units are g/kg DM, except for dry mater (g/kg feed) and Gross Energy, MJ/kg DM.

‡ NCGD, Neutral detergentcellulase plus amylase and gamannase digestibility.

§According to Agricultural and Food Research Council, 1993.

treatment received, in a separate bin, an additional amount of a protein supplement (Soypass) which was calculated to increase the protein supply in the total feed to around 11g MP/MJ ME (treatment: HP). To supply sufficient MP, the amount of Soypass offered to the HP ewes was based on the intake of ewes in the study of Houdijk et al., (2001b). The amounts of Soyapass offered to the G ewes and the smaller B ewes were 330 and 250g/day during d_{-21} to d_7 and 450 and 340 g/day during d_7 to d_{35}, respectively.

5.3.5. Measurements

Ewe and Lamb Performance and Intake. Ewes were weighed at housing and then weekly throughout the study, as well as within 6 h after parturition. Lambs also were weighed within 6 h after birth and weekly thereafter. BCS estimates of ewes were first taken at housing and then weekly from d_{-42} onwards. BCS was measured by lumbar palpation on a scale from 0 to 5 with 0.25 increments (Russel et al., 1969) by the same operator. Ewe muscle and back-fat depths were measured by ultrasound scanning (Glasbey et al., 1996) from d_{-42} onwards.

The amounts of distributed food were calculated daily to achieve *ad libitum* intake. Refusals were weighed twice weekly (Monday and Thursday) for calculation of average daily food intake (ADFI) and averaged 15% of the amount of food offered, which is sufficient to measure *ad libitum* intake (Blaxter, 1989; Minson, 1990). Experimental foods were sampled while the daily allowances were being prepared and daily samples were bulked and analyzed for DM, CP, NDF and minerals. There were no refusals of the restrictedly fed soypass supplement through the experiment. The refusals of the pelleted food were recorded on as fed basis, as similar refusal levels were obtained for the individual animals and there was no evidence of feed separation in the bins.

Faecal Egg Counts. Faecal samples were taken twice weekly directly from the rectum, from day_{-57} onwards and analysed for faecal egg counts (FEC) according to a

modified flotation method (Christie et al., 1982). FEC was expressed as the number of eggs per gram (epg) of fresh faeces.

Plasma constituents. Blood samples were taken weekly from the jugular vein into heparinized vacutainers from day d_{-50} (pre-infection) onwards. The blood samples were centrifuged for 15 min at 2600×g, and the separated plasma stored at -20°C pending analysis for leptin, pepsinogen and albumin. Plasma pepsinogen was determined by the modified method of Paynter (1992) and expressed in iU (international Units). Plasma albumin as an indicator of host protein nutrition was determined by a spectrophotometric method using a commercial clinical test: IL Test™ Albumin (Instrumentation Laboratory SpA, Milano, Italy) and results are reported in g/l.

Leptin Radioimmunoassay (RIA). Plasma leptin concentration was determined by the use of a ruminant-specific leptin RIA as described previously in Chapter 3. This assay uses an anti-ovine leptin antiserum raised in guinea-pigs against recombinant ovine leptin (a gift from Prof A Gertler, The Hebrew University of Jerusalem) at a final assay dilution of 1:160,000. Pure recombinant ovine leptin (DSL Ltd., London, UK) was used as a standard at the following concentrations: 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.395, 0.1975 and 0.098 ng leptin/ml. Approximately 15000 cpm of radiolabeled leptin (^{125}I-leptin), prepared by iodination with sodium ^{125}I-iodide (Amersham, Bucks, UK), was added to each tube. The tubes were incubated for 48 h and then bound and free ligands were separated by addition of 100μl of cellulose-bound anti-guinea-pig IgG (Sac-Cel; IDS, Washington Tyne & Wear, UK). After centrifugation (3000 rpm; 4°C; 20 minutes) supernatant containing unbound ^{125}I-leptin was aspirated by vacuum pump via a trap and the residual, drained pellets were counted in a Cobra II gamma counter (Packard Canberra Ltd., Berks, UK). Six replicates of blood plasma from a fat (BCS >3.5) non-pregnant ewe and six from a lean (2.0 < BCS > 3.5)

non-pregnant ewe were used as high and low leptin controls throughout each assay. The mean plasma leptin concentrations for these high and low controls were 7.37 ± 0.64 ng/ml and 3.62 ± 0.36 ng/ml, respectively. The mean intra-assay coefficients of variation (CV) were 9.0% and 10.2.% while the inter-assay CVs were 14.5% and 17.5% for high and low controls respectively.

5.3.6. Statistical Analysis

The data obtained for each ewe during the periparturient period were synchronized to day relative to parturition (d_0). Mean lambing dates for G and B ewes were 20 April 2005 ± 1 day and 27 April 2005 ± 1 day, respectively. The average day relative to parturition associated with data obtained during the periparturient period was computed, and used to present the results.

ADFI, BW, BCS, muscle and back-fat depths were analysed by repeated measures ANOVA with an auto-regressive correlation structure for residual errors over time, using the MIXED procedure of SAS (SAS 9.1.3; SAS Institute Inc., Cary, NC, USA). The statistical models contained the fixed effects of breed, protein supplementation, infection, time, and their interactions. In every statistical model the random effect was animal nested within breed by treatment. Data are reported as least squares means and their standard error (SE) and their differences were tested by a t-test.

Because ADFI and BW changed dramatically from pregnancy to lactation, data obtained during lactation and pregnancy were analysed separately and initial ewe BW difference from the mean breed BW measured on d_{-57} was used as covariable. ADFI refers to the intake of fresh basal feed only and does not include the protein supplement. Achieved MP intake (MP_I) of the ewes was calculated on the basis of ADFI and the protein content of the foods. MP requirements (MP_R) for pregnant and lactating ewes were estimated on the

basis of maternal BW and milk yield according to recommendations of AFRC (1993). The ratio MP_I / MP_R of all ewes was subjected to statistical analysis in order to determine the degree of protein limitation or adequacy in the two breeds.

Plasma leptin data were analysed also by ANOVA using the MIXED procedure of SAS for repeated measurements. Comparison of actual plasma leptin levels between treatments was made by a model that included the main effects of breed, infection, protein supplementation, time, and their interactions (model 1). Leptin data were also analyzed by a similar model that contained either the back-fat measurements or the RADFI (Relative Food Intake: RADFI, $g \cdot kg^{-1} \cdot d^{-1}$) of the ewes as a covariable in addition to the main effects of breed, infection, protein supplementation, time, and their interactions (model 2). The relationships between PLC and back-fat depth, and between PLC and RADFI (Relative Food Intake: RADFI, $g \cdot kg^{-1} \cdot d^{-1}$) were tested by comparing the respective covariable coefficient with its associated standard error. All models for leptin included an assay effect to take into account the between-assay variation. In every statistical model the random effect was animal nested within breed by treatment.

Lamb BW gain (g/d) was calculated by linear regression and data were analysed by ANOVA (General Linear Model) with the fixed effects of breed and treatment and litter sex (i.e. ♀♀, ♀♂ and ♂♂ litters).

FEC and pepsinogen data were log-transformed according to $\log_{10}(x+1)$, in order to normalise residuals, prior to statistical analysis. Log-transformed data were analysed by repeated measures ANOVA (GenStat Release 7.2 Lawes Agricultural Trust, Rothamsted Experimental Station) as described above for the intake and performance measurements. FEC and pepsinogen data are reported as back-transformed means (according to 10^{α}, where $\alpha = \mu + 0.5 \times \sigma^2$) (Johnson et al., 1998) with 95% confidence intervals (CI, lower and upper

limit). FEC of non-infected ewes were all zero throughout the experiment and these were, therefore, not included in the statistical analysis.

5.4. Results

The balanced structure of the experiment (n=8 ewes per breed per treatment) was not maintained throughout the study. One ewe had still-born lambs (treatment LP+, B), three ewes failed to adapt to the experimental feeds (one treatment HP+, B; one treatment LP-, B; and one treatment LP-, G) and one ewe gave birth to triplets (treatment HP-, G). The data obtained from these animals were excluded from the statistical analysis and treated as missing values.

5.4.1. Faecal Egg Counts

Mean back-transformed FEC for infected ewes are shown in Figure 5.1. There was a significant breed effect on FEC (P<0.001) as a result of higher epg values in G than in B ewes. Protein supplementation resulted in a significant decrease (P=0.038) in FEC, but the

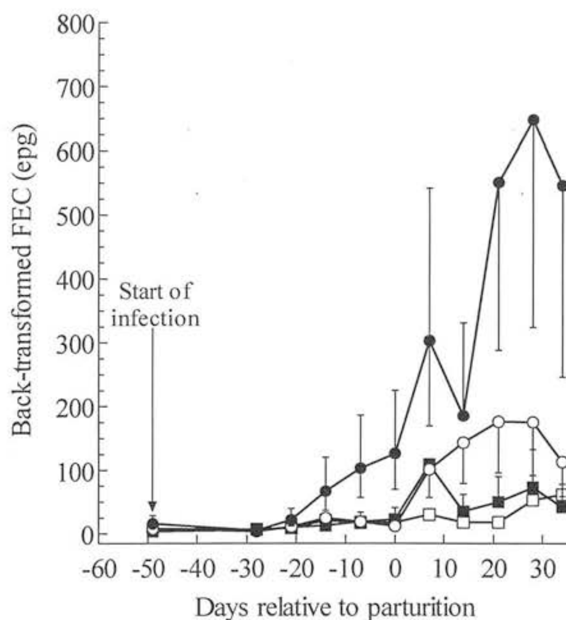

Figure 5.1. Faecal egg counts (FEC; epg, number of eggs per g fresh faeces) of Greyface cross (○,●) and Scottish Blackface (□,■) twin-bearing/lactating ewes, trickle infected with 30 000 third-stage infective larvae of *Teladorsagia circumcincta* per week and offered a protein supplement (○,□) or not (●, ■) during the periparturient period (d_{-28} to d_{33} of parturition (d_0)). The trickle infection started on d_{-47}. Values are back-transformed means with 95% CI depicted by vertical bars.

interaction between protein supplementation and breed was not significant (P=0.23). As expected, FEC changed significantly over time (P<0.0001). In all treatment groups, FEC were low until d_{-21} (<17 epg upper limit), increased with time to peak during early lactation and then tended to decrease towards the end of the experiment. Maximum FEC for both breeds were observed on the LP treatment, i.e. on d_7 for B ewes (119 epg; 95% CI 67, 213) and on d_{28} for G ewes (648 epg; 95% CI 325, 1294). Mainly as a result of these differences, the interaction of time with breed was significant (P=0.005).

5.4.2. Food intake

Figure 5.2 shows the observed mean ADFI of G and B ewes from d_{-24} until d_{31} of the experiment. Breeds did not differ in ADFI during late pregnancy (d_{-24} to d_0; P=0.53) or lactation (d_0 to d_{31}; P=0.85). ADFI in both breeds did not change with time during late pregnancy (P=0.09) but increased significantly during lactation in both breeds (P<0.001). Nematode infection resulted in a significant reduction in ADFI in both breeds during late pregnancy (reduction around 12%; P=0.026) and even more so during lactation (reduction around 22%; P=0.0036). Interactions between breed and infection were not significant in either period (P>0.5). Protein supplementation resulted in higher ADFI in G ewes but not in B ewes especially during lactation as indicated by the significant interaction between breed and protein supplementation (P=0.011; see also Figure 5.2 and Table 5.2). The interaction between protein supplementation and infection was not significant during either late pregnancy (P=0.58) or lactation (P=0.86), which showed that protein supplementation did not affect the extent of the observed anorexia.

Protein supplementation resulted in MP intakes that were higher than the estimated MP requirements, during both late pregnancy and lactation in both breeds (Table 5.2). Ewes that were fed the low protein basal diet only had a ratio of MP intake to MP requirements (MP_I:MP_R) below 1. This ratio was much lower for G ewes than for B ewes, which resulted

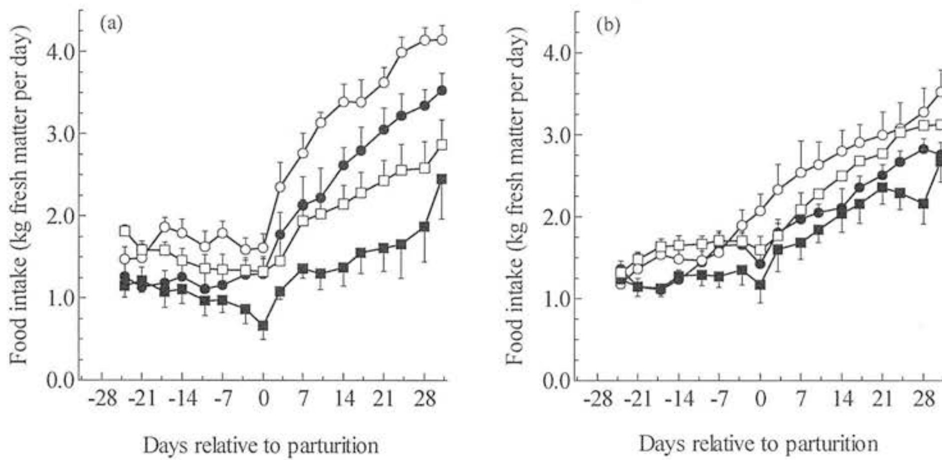

Figure 5.2. Group mean average daily food intake of Greyface cross (panel a) and Scottish Blackface (panel b) twin bearing / lactating ewes, trickle infected with 30,000 L_3 larvae of *T. circumcincta* per week (●,■) or non infected (○,□) and offered a protein supplement (HP; ○,●) or not (LP; □,■) during the periparturient period (d_{-28} to d_{33} of parturition (d_0)). The trickle infection started on d_{-47}. Standard errors are shown by vertical bars.

in a significant interaction between the effects of protein and breed during both late pregnancy and lactation (Table 5.2).

5.4.3. Ewe and Lamb Performance

During both periods, G ewes were heavier than B ewes (P<0.0001; see Figure 5.3). Infected ewes in both breeds had lower BW than non-infected ewes during both late pregnancy (P=0.003) and lactation (P=0.0002). A difference in ewe BW between HP and LP treatments was observed only in G ewes and this was reflected by a significant interaction between breed and protein supplementation during both late pregnancy (P=0.04) and lactation (P=0.01; see Figure 5.3). There was also a significant interaction between breed and time on ewe BW because B ewes gained more BW than G ewes, but this was apparent only during lactation (P=0.003). G ewes lost more BCS over time than B ewes did as indicated by a significant interaction between breed and time (P<0.0001). On average, infected ewes had lower BCS compared to non-infected ewes as shown by the main effect of infection (P=0.016; Table 5.3) but the infection by time interaction was not statistically

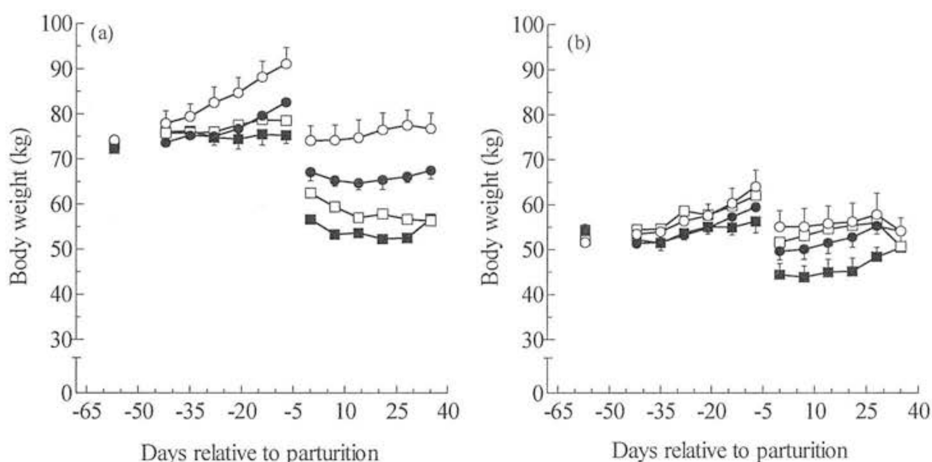

Figure 5.3. Group mean weekly BW of Greyface cross (a) and Scottish Blackface (b) twin bearing / lactating ewes, trickle infected with 30,000 L_3 larvae of *T. circumcincta* per week (●,■) or non infected (○,□) and offered a protein supplement (HP; ○,●) or not (LP; □,■) during the periparturient period (d_{-28} to d_{33} of parturition (d_0)). The trickle infection started on d_{-47}. Standard errors are shown by vertical bars.

significant (P=0.22). Unsupplemented ewes lost more BCS compared to protein supplemented ewes over time as shown by the interaction between protein supplementation and time (P=0.0003).

At lambing, mean whole litter BWs of G and B ewes were 10.6 ± 0.26) and 6.6 ± 0.26) kg (P<0.0001). Mean daily litter BW gains were 559 ± 29 g/d and 419 ± 27 g/d for G and B ewes respectively (P=0.001). Litter sex tended to affect litter birth weight with ♀♀, ♀♂ and ♂♂ -litter mean weights of 9.9 kg, 10.9 kg and 10.8 kg in G ewes and 5.9 kg, 7.0 kg and 6.9 kg in B ewes (± 0.4, P=0.07). Across treatments, HP ewes produced heavier litters than LP ewes (P=0.03) but there were no differences in birth weights between litters produced by infected and non infected ewes (P=0.3). There was a significant breed × protein supplementation interaction due to the faster growth of the G lambs from the HP treatment (P=0.006). Litters nursed by infected ewes tended to have lower whole litter BW gain (P=0.1) than litters nursed by non infected ewes but the interaction between breed and infection on whole litter weight gain was not significant (P=0.8).

Table 5.2. Achieved average daily fresh food intake from basal diet (kg) and total metabolisable protein (MP; g) intakes of twin bearing / lactating ewes that receive (HP) or did not receive (LP) a protein supplement and were infected (+) or non infected (-) with the nematode *Teladosargia circumcincta*†

Item	Greyface cross				Scottish Blackface					
	HP-	HP+	LP-	LP+	HP-	HP+	LP-	LP+	SEM§	Response
Pregnancy (Days -24 to 0)										
Intake, kg fresh	1.60	1.20	1.50	1.10	1.40	1.30	1.60	1.20	0.10	I***,
MP_I (MP intake, g)	211.00	181.00	94.00	67.00	169.00	165.00	102.00	77.00	7.80	P***, I***, B×P***
$MP_I : MP_R$‡	1.48	1.28	0.66	0.47	1.31	1.28	0.79	0.60	0.06	P***, I***, B×P*
Lactation (Days 0 to 35)										
Intake, kg fresh	3.10	2.30	2.10	1.70	2.70	2.10	2.40	1.80	0.25	P***, I***, B×P*
MP_I (MP intake, g)	339.00	290.00	142.00	104.00	281.00	243.00	153.00	114.00	16.40	P***, I***, B×P***
$MP_I : MP_R$‡	1.37	1.17	0.57	0.43	1.38	1.20	0.75	0.56	0.07	B*, P***, I***, B×P*

I, infection (+ v. -); P, protein supplementation (HP v. LP); B, breed (Greyface cross v. Scottish Blackface).

*P<0·05, ** P<0·01, *** P<0·001.

† Infection started on d_{-47} relative to parturition (d0) and animals were receiving oraly 30,000 L3 larvae per week. Non-infected animals were receiving only water ("sham" infected). Protein supplementation started on d_{-28}. The protein supplement was SoyPass® (xylose-treated soybean meal). For chemical analysis see Table 5.1.

‡Estimated MP requirements (MPR) for twin-bearing ewes based on maternal BW were 142g and 129g for Greyface cross and Scottish Blackface ewes, respectively. Estimated MP requirements for lactating ewes based on maternal BW and assuming milk yield of 3 and 2 kg/day were 248g and 203g for Greyface cross and Scottish Blackface ewes, respectively (according to Agricultural and Food Research Council, 1993).

§SEM are based on error mean squares pooled over treatment groups.

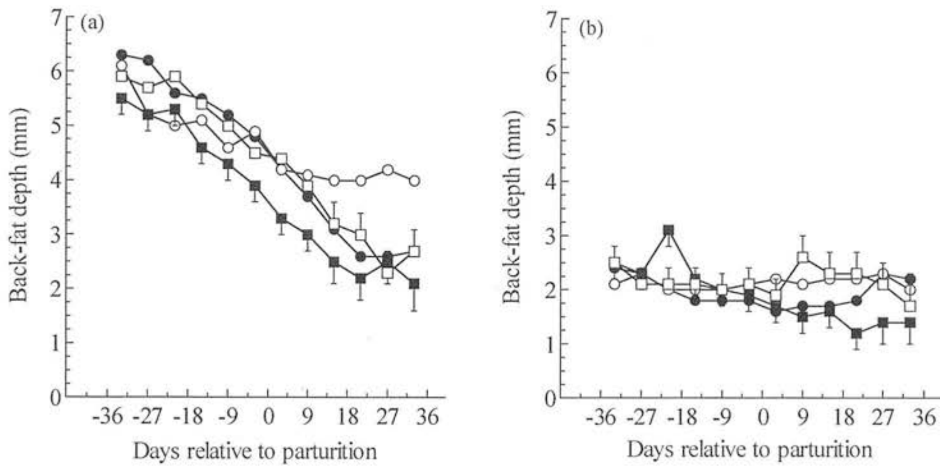

Figure 5.4. Group mean weekly back fat depth of Greyface cross (a) and Scottish Blackface (b) twin bearing / lactating ewes, trickle infected with 30,000 L_3 larvae of *T. circumcincta* per week (●,■) or non infected (○,□) and offered a protein supplement (HP; ○,●) or not (LP; □,■) during the periparturient period (d_{-28} to d_{33} of parturition (d_0)). Standard errors are shown by vertical bars. The trickle infection started on d_{-47}.

5.4.4. Back-fat and Muscle Depth

Mean back-fat depth was considerably higher in G ewes (4.3 ± 0.11 mm) than in B ewes (2.0, ± 0.11mm) during the periparturient period (P<0.0001) but because G ewes lost more BCS than B ewes the interaction between breed and time was significant (P<0.0001; Figure 5.4). Back-fat depth was affected positively by protein supplementation over time (P=0.04) and negatively by infection (P=0.05) in both breeds.

In ewes of both breeds, muscle depth decreased during late pregnancy (P<0.001). During lactation, muscle depth remained almost static in G ewes but increased in B ewes; that was reflected by a significant breed × time interaction (P<0.001). Infected ewes had significantly lower muscle depth compared to non infected ewes in both breeds (P=0.02; Table 5.3). Protein supplementation did not affect muscle depth in B ewes but it had a positive effect in G ewes as shown by a statistically significant interaction between breed and protein supplementation (P=0.03; Table 5.3).

Table 5.3. Average body condition score (BCS) and muscle depth (MD) of twin bearing / lactating ewes that receive (HP) or did not receive (LP) a protein supplement and were infected (+) or non infected (-) with the nematode *Teladosargia circumcincta*†.

Item	Greyface cross				Scottish Blackface				SE‡	Response
	HP-	HP+	LP-	LP+	HP-	HP+	LP-	LP+		
Body condition score (BCS)	2.9	2.7	2.7	2.5	2.4	2.4	2.4	2.2	0.08	B***, P***, I**,
Muscle depth (MD)	23.9	22.9	21.7	20.7	21.2	20.1	20.3	19.8	0.500	B***, P***, I**, B×P**

I, infection (+ v. -); P, protein supplementation (HP v. LP); B, breed (Greyface cross v. Scottish Blackface).

*P<0·05, ** P<0·01, *** P<0·001.

† Infection started on d_{-47} relative to parturition (d_0) and animals were receiving oraly 30,000 L3 larvae per week. Non-infected animals were receiving only water ("sham" infected). Protein supplementation started on d_{-28}. BCS and MD were measured weekly from d_{-42} to d_{30}. The protein supplement was SoyPass® (xylose-treated soybean meal). For chemical analysis see Table 5.1.

‡SEM are based on error mean squares pooled over treatment groups.

5.4.5. Plasma constituents

Plasma pepsinogen. Plasma pepsinogen concentrations were similar in non infected ewes of both breeds, were not affected by protein supplementation and did not change systematically with time (Figure 5.5). Infected ewes had higher plasma pepsinogen concentrations than non infected ewes and differences were significant from the first week of infection in both breeds (P<0.0001). Protein supplementation had no effect on pepsinogen concentrations in B ewes but it resulted in a decrease in plasma pepsinogen concentration in G ewes from parturition until the fourth week of lactation; this was reflected by significant breed × protein supplementation interaction (P=0.017).

Plasma albumin. Plasma albumin concentrations increased from late pregnancy to lactation in both breeds (P<0.0001; Figure 5.5). Ewes on the LP treatment had significantly lower plasma albumin concentrations compared to ewes on the HP treatment (P=0.0002) and

these differences were more pronounced during lactation. The breed by protein supplementation interaction was not significant (P=0.65). Infection resulted in significantly lower plasma albumin concentrations in both HP and LP treated ewes of both breeds (P=0.02).

Plasma leptin. Analysis of PLC with model 1 (no covariables included) showed no effect of infection (P=0.77) or of protein supplementation (P=0.52) but PLC was higher (P=0.005) in G (1.2 ± 0.11 ng/ml) than in B ewes (0.7 ± 0.11ng/ml). However, the breed difference in PLC disappeared (P=0.43) when data were analyzed with model 2, in which, back-fat depth was included as a covariable. This model showed that there was a statistically significant (P<0.001) positive relationship between PLC and back-fat depth, as evidenced by the covariable coefficient and the interaction between breed and back-fat depth was found to be statistically non-significant (P=0.22). The estimated covariable coefficient showed that a difference in back-fat depth of 1 mm was associated with a difference in PLC of 0.184 ± 0.028 ng/ml (P<0.001). According to this model PLC was also not affected by infection (P=0.87) or protein supplementation (P=0.54). The model that included RADFI as a covariable showed that there was no statistical evidence of a relationship between PLC and RADFI (P=0.27) but the breed effect remained significant (P=0.005). All models showed that PLC was affected by time (P<0.001), mainly because of the gradual increase in PLC after parturition.

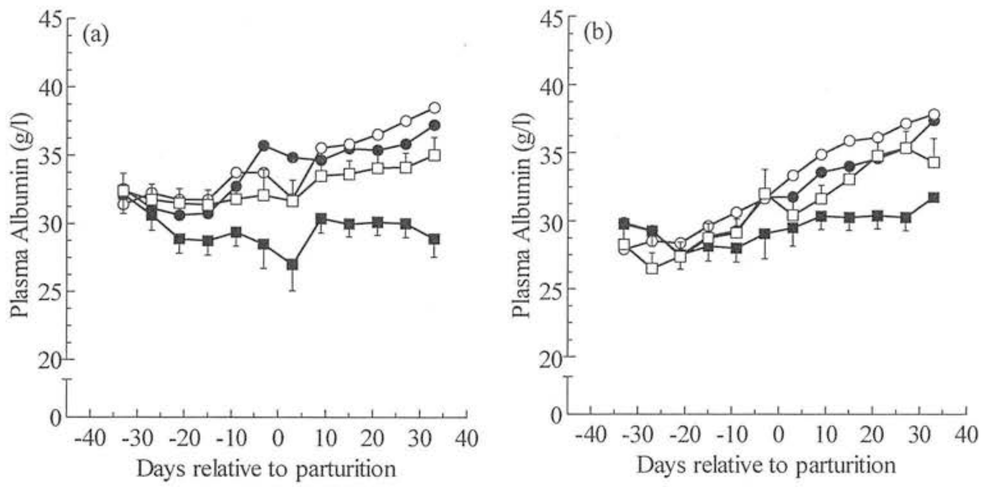

Figure 5.5. Plasma albumin concentrations of Greyface cross (panel a) and Scottish Blackface (panel b) twin bearing / lactating ewes, trickle infected with 30,000 L_3 larvae of *T. circumcincta* per week (●, ■) or non infected (○,□) and offered a protein supplement (○,●) or not (□,■) during the periparturient period (d_{-28} to d_{33} of parturition (d_0)). The trickle infection started on d_{-47}. Standard errors are shown by vertical bars.

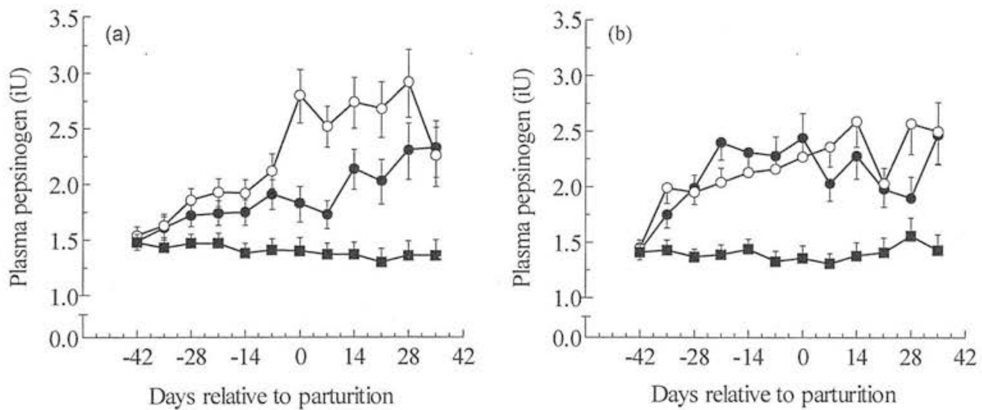

Figure 5.6. Back-transformed means of pepsinogen concentrations and 95% CI of Greyface cross (panel a) and Scottish Blackface (panel b) twin bearing / lactating ewes, trickle infected with 30,000 L_3 larvae of *T. circumcincta* per week (○,●) or non infected (■) and offered a protein supplement (●) or not (○) during the periparturient period (d_{-28} to d_{33} of parturition (d_0)). The trickle infection started on d_{-47}. CI are shown by vertical bars.

5.5. Discussion

A *T. circumcincta* challenge was imposed upon half of experimental periparturient ewes of two different breeds which did or did not receive a protein supplement to evaluate the effects of infection, breed and protein supplementation on parasite induced anorexia and PLC. The effects of breed and protein supplementation on PPRI will be considered before the discussion of the results in relation to the hypotheses developed in the introduction.

5.5.1. Effects of breed and protein supplementation on PPRI

Periparturient ewes displayed a loss of their acquired immunity to *T. circumcincta* infection as evidenced by an increase in FEC during late pregnancy and lactation. FEC as an indirect measure of immunity has proven to be an effective criterion with which to assess the extent of PPRI in ewes and it has frequently been used to compare differences in resistance to infection between breeds (Donald et al., 1982; Amarante et al., 1992; Barger, 1993; Woolaston and Baker, 1996; Bisset et al., 2001; Amarante et al., 2004; Good et al., 2006). In the LP treatment, infected G ewes had FEC which were more than 5 times higher than that of B ewes (Figure 5.1). In addition, the relaxation of immunity occurred earlier in G ewes compared to B ewes as indicated by the significant rise in FEC during late pregnancy (Figure 5.1). Significant differences in the extent and timing of PPRI between the two breeds suggest that B ewes were more resistant to *T. circumcincta* infection than G ewes under the same plane of nutrition.

In agreement with previous studies (Houdijk et al., 2000; 2001b), protein supplementation in G ewes limited the extent of PPRI during late pregnancy as evidenced by the significantly lower FEC during lactation (d_7 onwards; Figure 5.1). In B ewes, with the exception of the first week of lactation, the differences in FEC between HP and LP treatments were not as large as they were in G ewes (Figure 5.1). Protein supplementation

resulted also in lower pepsinogen levels, an indicator of mucosal damage by the parasite, in G ewes during lactation but it did not affect pepsinogen levels in B ewes (Figure 5.5).

The finding that the two breeds differed significantly in their resistance to infection could be due to the enhanced genetic resistance of the B breed *per se*. However, the nutrient partitioning framework of Coop and Kyriazakis (1999) suggests that the degree of nutrient scarcity can affect the degree of breakdown of immunity to parasites. Several studies with ewes (Donaldson et al., 1998; Houdijk et al., 2000; 2001b), goats (Chartier et al., 2000) and more recently with rats (Houdijk et al., 2005a,b) have shown that dietary MP scarcity exaggerates the extent of PPRI while an increased supply of, or a decreased demand for, MP reduces PPRI. Based on their own *ad libitum* food intake, unsupplemented G ewes achieved a lower proportion of their MP requirements than unsupplemented B ewes, as discussed below. This in itself could be sufficient explanation for the differences in PPRI observed between the two breeds. Whether B ewes would have displayed the same degree in PPRI as G ewes if they had been fed to the same degree of MP scarcity remains to be investigated.

5.5.2. Effects of breed and infection on food intake

Although the effects of gastrointestinal infection on sheep immune response has been studied extensively (Barger, 1993), our knowledge of the effects of nematode infection on food intake changes in periparturient ewes is limited. The only investigation of effects of nematode infection on ADFI in periparturient ewes is from Leyva et al., (1982) and their study reported a reduction in food intake. Nematode-infected barren ewes that maintain their acquired immunity to nematodes do not show a reduction in food intake (Greer et al., 2005). This suggests that in periparturient ewes the occurrence of anorexia is related with the relaxation of immunity.

The results show that infected ewes had significantly lower food intake than non-infected ewes during the periparturient period. However, the degree of anorexia was not

strongly associated with the differences in the extent of the PPRI between the high and the lower production potential breed. Therefore, the results of the present study do not support the hypothesis that anorexia is greater in ewes selected more intensively for high productivity than in ewes that have been selected less intensively. Leyva et al., (1982) reported a 16% reduction in food intake during lactation in parasitised Poll Dorset ewes fed on a good quality diet (145g C.P/kgDM), but they did not observe any reduction in food intake during late pregnancy. Our findings that infected ewes experienced a reduction in food intake during late pregnancy (around 12%) as well as during lactation (around 22%) show that anorexia in periparturient ewes can occur before any increase in FEC is observed.

Leyva et al., (1982) supported the view that the occurrence of anorexia in periparturient ewes is unlikely to be attributable to abomasal damage - as suggested by Sykes and Coop (1977) based on an experiment with parasitised lambs. Although the mechanisms underlying the parasite-induced anorexia in sheep remain unclear, a recent study has shown that the occurrence of anorexia in parasitised sheep is associated with the development (acquisition) of the immune response rather than the expression of immunity *per se* (Greer et al., 2005). Immune response was not depended on IgA production and was not accompanied with a reduction in food intake in non pregnant or non lactating ewes (Greer et al., 2005). Nevertheless, a significant increase in IgA levels is closely associated with the rise in FEC in periparturient ewes (Jeffcoate et al., 1992; Sykes et al., 2007) but also in infected parasite-naïve lambs (Stear et al., 1999). Greer et al., (2005) suggested that the physiological changes associated with the acquisition phase of the immune response, characterized by the stimulation of IgA production, are responsible for the loss in appetite in infected parasite-naïve lambs. Because mature animals restore their immune response following the PPRI, it is possible that the parasite-induced anorexia in periparturient ewes is related to the developing immune responses which eventually lead to the restoration of immunity.

5.5.3. Effects of protein supplementation on food intake and performance

The extent of PPRI in ewes is sensitive to dietary protein intake (Donaldson et al., 1998; Houdijk et al., 2000). For that reason we also investigated how protein supplementation affected the degree of anorexia in periparturient ewes. G ewes were heavier and lambs nursed by G ewes had significantly higher body weight and weight gain than lambs nursed by B ewes. These differences imply that G ewes had a greater MP demand for maintenance, late pregnancy and lactation than B ewes (Robinson et al., 1969; AFRC, 1993), supporting the rationale for feeding a higher protein supplement to G ewes on the HP treatment. However, in order to be able to measure anorexia as a result of infection, all ewes were fed *ad libitum*. G ewes on the HP treatments did indeed consume more basal diet than B ewes and all HP groups achieved an MP intake that was more than adequate in relation to their requirements, as intended.

Unexpectedly, LP ewes of both breeds consumed similar amounts of basal diet, which resulted in G ewes consuming a lower proportion of their MP requirements than B ewes during late pregnancy and during lactation (see Table 5.2). Therefore, over the entire periparturient period, MP supply was more limiting in G ewes than in B ewes. Since the AFRC (1993) system does not take into account effects of urea recycling on MP scarcity, it seems likely that on the LP treatment MP supply was underestimated (Van Soest, 1988). Performance of B ewes and their lambs was similar in supplemented and unsupplemented treatments, suggesting that the MP limitation must have been small. In contrast, unsupplemented G ewes lost more weight (Figure 5.3) and their lambs gained less weight than their counterparts in the supplemented treatments. These observations agree with the MP supply data that the MP limitation was much more severe in G than in B ewes

Protein supplementation had no significant effect on the degree of anorexia in either breed because the reduction in ADFI was similar in the HP and LP treatments. This finding does, therefore, not support the hypothesis that protein supplementation can lower the extent

of anorexia in a manner similar to its effect on PPRI. In the only other study that investigated effects of food quality on anorexia, the degree of anorexia following trypanosome infection was also similar in goats that received either a high or a low protein food (Van Dam et al. 1998). We conclude that protein supplementation affected PPRI as measured by FEC but not anorexia and that the latter two variables are, therefore, not strongly related.

The significant interactions between breed and protein supplementation on ewe BW and whole litter BWG provide indirect evidence of differences between breeds in the extent to which their protein demands were met. However, these results may not have been the exclusive effect of differences in protein scarcity because they could well have been affected by the differences in total ADFI between G ewes on the HP and LP treatments. The differences between breeds in protein scarcity as a result of the LP treatment were, to some extent, also reflected in the lower muscle depth in G ewes, but not in B ewes, when compared to their HP counterparts, since it has been shown that MP undernutrition reduces the weights of a range of proteinaceous body components (Sykes et al., 1972; Houdijk et al., 2001a). In addition, excess MP can improve abomasal integrity restoration which in turn leads to lower plasma pepsinogen concentrations in infected ewes (Houdijk et al., 2003). Accordingly, the absence of a significant effect of protein supplementation on pepsinogen concentrations in B ewes, in contrast to G ewes, could also be a reflection of the differences in protein scarcity between the two breeds.

5.5.4. Effects of infection on PLC in relation to back-fat reserves

An important role in the relationship between nutritional status and immune function has been recently ascribed to leptin (Lord et al, 1998). Changes in PLC in response to inflammation have been suggested to be important for the animals' ability to cope with, and survive, infections (La Cava and Matarese, 2004). Therefore, we tested the hypothesis that nematode infection of periparturient ewes will result in increased PLC.

Data obtained in a recent study (Valderrabano et al., 2006) suggested that differences in the immune response appeared to be associated with differences in serum leptin levels in periparturient ewes infected with *H. contortus*. Although this study was the first to address the possible implication of leptin during the periparturient immune response in ewes, the results were difficult to interpret because of the absence of any non-infected control ewes in that experiment. The experimental design of our study allowed a direct comparison of PLC between infected and non-infected ewes during the periparturient period. Since a positive relationship between adiposity and PLC exists in ruminants (Chilliard et al., 1999; Delavaud et al., 2002) the statistical model for PLC analysis included body fat depth as covariable to take this into account. Our results showed that PLC was affected significantly by time. These results are in accordance with the finding that maternal PLC in ewes declines from mid-pregnancy to early lactation where it reaches a nadir and increases gradually thereafter (Ehrhardt et al., 2001). Our results also show that PLC was positively correlated with back-fat depth, which is consistent with previous observations. Infected ewes tended to have lower back-fat depth and significantly lower BCS. However, when differences in back-fat reserves were accounted for, infected ewes did not differ in their PLC from non-infected ewes, which suggests that PLC are unlikely to be increased as a result of infection in periparturient ewes. In addition, despite protein supplementation that resulted in increased albumin and lower pepsinogen levels and reduced the extent of PPRI in periparturient ewes, PLC was not significantly affected by the level of protein supplementation. This further suggests that PLC are unlikely to be involved in MP partitioning towards the physiological functions of the host. Reduced appetite in ruminants has previously been ascribed primarily to physical limitations of the gastrointestinal tract, but metabolic signals may play an equally important role (Ingvartsen and Andersen, 2000; Tolkamp et al., 2006). Although a role for leptin for the anorexia of infection has been proposed in other models of disease (Grunfeld et al., 1996; Barbier et al., 1998), our results suggest that leptin is unlikely to contribute to the reduction in appetite in infected

periparturient ewes because PLC did not differ between infected and control ewes and PLC was not strongly associated with RADFI.

5.5.5. Conclusions

This study showed that *T. circumcincta* infection resulted in anorexia in periparturient ewes of each of two breeds differing in production potential, and that the anorexia can occur before any increase in FEC is observed. The differential breed responses to nematode infection were not associated with breed differences in anorexia. The results add to the growing body of evidence that where breakdown of immunity to *T. circumcincta* infection occurs under conditions of protein scarcity, the supplementation with protein can lower the extent of the breakdown. However, the hypothesis that dietary protein supplementation can reduce the magnitude of anorexia in periparturient ewes had to be rejected. The results were also not consistent with the hypothesis that nematode infection of periparturient ewes would result in increased plasma leptin concentrations and it is unlikely that leptin is involved in the occurrence of anorexia of nematode infected periparturient ewes.

CHAPTER SIX

General discussion

6.1. Introduction

This thesis describes studies on the interactions between the effects of host genotype, immune status and protein supplementation on the parasite-induced anorexia of sheep. In the first place, the hypothesis tested was that the degree of anorexia following a primary infection will be greater in a genotype that has been selected more intensively for growth than a genotype that has been selected less intensively. This hypothesis was tested also during secondary infections (re-infections) in lambs and ewes. Re-infection of lambs started 2, 4 or 8 weeks after finishing the primary infection. In ewes the effect of re-infection and its interaction with the genotype on anorexia was tested at the time of periparturient relaxations of immunity (PPRI). In addition to the interaction between effects of genotype (i.e. intensively selected for growth or not) and immune status (i.e. primary or secondary infection), the effect of protein supplementation on the parasite induced anorexia in sheep, was also investigated.

The thesis also examined the role of leptin in the parasite-induced anorexia of sheep. In view of the scant evidence as to whether leptin is involved in the regulation of immune responses in parasitised sheep, the first aim of the study was to investigate whether nematode infection results in increased plasma leptin concentration, as it has been shown for murine models (Barbier et al., 1998). It was hypothesized that plasma leptin concentration is increased as a result of infection with nematodes, when variation in of food intake or body fat reserves was accounted for. The hypothesis was tested during the acquisition phase of the immune response and during the expression of immunity in lambs (Chapters 3 and 4), but also during the occurrence of PPRI in ewes (Chapter 5).

The principal issues raised by the experimental studies, which warrant further discussion in this Chapter are:

- The effects of breed on immune response to infection and on the parasite-induced anorexia
- Effects of dietary protein supplementation on parasite-induced anorexia
- The role of leptin in the immune response and anorexia of parasitised sheep.

Subsequently, practical implications of these findings and suggestions for further research are proposed.

6.2. The effects of breed on immune response to infection and on the parasite-induced anorexia

In order to study the effect of breed and its interaction with protein supplementation and immune status on the parasite-induced anorexia in sheep, the design of each experiment involved the use of two breeds of sheep that differed in their production potential. The breeds selected for experimentation were Suffolk × Greyface (S) or Greyface cross (G) and Scottish Blackface (B). Lambs of the S or B breed are known to vary in their intrinsic capacity for growth and mature size in a common environment (Emmans and Friggens, 1995; Lewis et al., 2004). Also, as discussed in Chapter 5, G and B ewes differed in their production potential and mature BW as it is evidenced by the differences in their BW, milk production, and differences in litter BW at birth. In addition, lambs nursed by G ewes were growing faster than the lambs nursed by the B ewes, at least within the high protein treatment. Overall the results from the three experimental Chapters are in support of the previous suggestion that these breeds differ in their production potential (Emmans and Friggens, 1995; Lewis et al., 2004), and therefore, the selection of these breeds for experimentation was sufficiently robust to enable sound investigation of the hypotheses developed.

The experiments of the present thesis showed that animals of the B breed were more resistant to infection than that of the S or G breed, as judged by the FEC. The significant breed difference in resistance was observed during the acquisition of immunity, following a primary nematode infection, but not during the expression of immunity following a secondary nematode infection (see Chapters 3 and 4), in lambs. In addition, the breed differences in IgA response to nematode infection was more pronounced during the acquisition of immunity (primary infection experiment I, Chapter 4) rather than during the

expression of immunity (secondary infection experiment III, Chapter 4). However, the breed differences in the acquisition phase of the immune response diminish, when breed comparison in resistance was made within older lambs (see Chapter 4). As shown by the data of the non-infected lambs, growth rates of the young lambs (i.e. during the primary infections) were significantly higher from that of the older lambs (i.e. during the secondary infections), and this effect was more pronounced in the lambs of the S breed (see Chapters 3 and 4). This finding suggests that as the growth rate of the lambs is reduced, their ability to deal with the incoming larvae is increased. These results suggest that the undesirable effects that selection for enhanced productivity can have on immune response are associated with the acquisition of immunity in growing lambs, rather than with the expression of immunity. However, breed differences in the expression of immunity can be observed, in circumstances when animals have to face, at the same time, additional physiological functions such as pregnancy and lactation (Chapter 5). This was evident from the fact that G ewes showed a greater degree of PPRI than B ewes, following nematode infection. Therefore, overall the results of the three experimental chapters provide support of the view that the function of growth, pregnancy and lactation are prioritised over that of immune response, as proposed from the nutrient partitioning framework of Coop and Kyriazakis (1999), and as reviewed recently by Golditz (2008).

To date, there was no information to link host genotype to the features of anorexia during exposure to nematodes in sheep. This was despite the fact that breeds vary markedly in their resistance to infection (Gray 1997) and that selection for enhanced productivity can have adverse effects on resistance to gastrointestinal infection (Woolaston and Baker, 1996; Bisset et al, 2001). As evidenced by several animal models, there is a link between anorexia and the stimulation of the (acquired) immune response of the host by pathogens (Plata-Salaman, 1996; Hart, 1998; Langhans 2000). In sheep, this suggestion is supported by Greer et al., (2005) who found that immuno-suppression during the phase of acquisition of

immunity, prevented immunologically naïve lambs challenged with gastrointestinal nematodes to develop any anorexia. It is possible, therefore, that differences in the ability of sheep to cope with nematodes also lead to differences in the rate of development, extent and duration of anorexia (Sandberg et al., 2006). The duration of anorexia may reflect the time taken by the immune system to begin controlling and subsequently eliminating the pathogens and thus it is seen as a reflection of the rate of acquisition of acquired immunity (Kyriazakis and Houdijk, 2006). By definition, a resistant genotype may be able to acquire immunity towards the pathogen at a faster rate than a susceptible genotype (Woolaston and Baker, 1996; Baker, 1998; Coop and Sykes, 2002). This in turn, may result in an earlier occurrence of anorexia but of shorter duration in a resistant genotype, whereas in a susceptible genotype, anorexia may occur slightly later but will be of longer duration (Sandberg et al 2006).

In growing lambs, the hypothesis that the degree of anorexia following a primary infection will differ between breeds that differ in their production potential is supported by the results of experiment I (Chapter 3). As discussed in Chapter 3, the absence of anorexia in B lambs could be related to the fact that the lambs were not entirely parasite naïve prior to infection. Because B lambs are more resistant to infection (as shown by the results of the present thesis), it is possible that these lambs acquired immunity faster due to their preliminary exposure to infective larvae, and thus the primary trickle infection did not result in anorexia. In support of this suggestion are the results of the subsequent experiment (experiment I, Chapter 4). In this experiment, all lambs were entirely parasite naïve prior to the start of the infection and anorexia observed in both breeds, although the breed difference in anorexia was not significant. The absence of a significant breed effect on anorexia could be related to the apparently small reduction in food intake (approximately 6%) that the primary infection resulted in both breeds, in this experiment (see table 4.1). There is no clear explanation for this result, but it seems unlikely to be related to the protein content of the food (see discussion of Chapter 4 and part 6.3 of this Chapter). It is also not likely to be

related to the infectivity of the administrated larvae, as the same strain of L3 was used in all experiments of the present thesis.

In experiment II (Chapter 4), primary infection did not result in a statistically significant reduction in intake in the in the 6-month old lambs. The ADFI data, however, could suggest a tendency ($P = 0.08$) for the infected S lambs to have reduced intake (see Figure 4.5a). The fact that this was not statistically significant is possibly related to the smaller number of lambs per treatment and to the shorter duration of this experiment compared to the experiment I of both 3 and 4 Chapters. Therefore, based on the results of these experiments the hypothesis tested cannot be rejected. However, further investigation into breed effects on anorexia is required, as the current work is the first to study the effect of genotype on the parasite-induced anorexia of sheep.

In the present thesis it was also tested whether infection of previously infected sheep results in renewed anorexia. The expectation was that the degree of the renewed anorexia would increase in accordance to the length of the parasite free period, as acquired immunity diminishes with time, in the absence of nematode challenge (Dineen and Wagland, 1966; Jackson et al 2004). Following re-infection animals would have to re-acquire immunity, which in turn is expected to result in anorexia, since anorexia is associated with the acquisition phase of the immune response (Greer et al, 2005). In Chapter 3, re-exposure to infection after 2 weeks of the end of the primary tended to affect ADFI of the S breed (Figure 3.3a). However, in this experiment the initiation of the secondary infection was relatively close to the end of the primary infection. Therefore, the tendency of the re-infected S lambs to eat less relative to B lambs, could be viewed either as a renewed anorexia or as a failure of these lambs to recover their ADFI following de-worming. The later view is rather likely since ADFI in the non-re-infected S lambs increased, while ADFI of re-infected lambs did not decrease, but, remained constant (Figure 3.3.a). The effect of re-infection of lambs in

the two breeds was examined also in a subsequent experiment (Chapter 4) in which, lambs were re-infected after 4 or 8 weeks of a parasite free period. The results showed that anorexia did not occur in either of these treatments in either breed. Kimambo et al (1998) have also found that anorexia did not occur in re-infected sheep after a prolonged (6 months) parasite-free period. Therefore, the suggestion that anorexia may occur in re-infected lambs is not supported by the results of the present thesis. As discussed in Chapter 4, it is possible that a pro-inflammatory type reaction evolves more quickly to a specific type immune reaction in older and/or previously infected lambs, thus the extent of anorexia following (re)infection is less pronounced or even absent.

The present thesis revealed evidence that re-infection does result in anorexia in previously infected ewes, during the periparturient period. The results showed that the degree of anorexia in periparturient ewes was not associated with the degree of the breakdown of immunity as judged by FEC, and was not associated with the breed differences in the extent of PPRI (Chapter 5). The effect of infection on anorexia in periparturient ewes has not been studied extensively, and therefore information about the mechanisms responsible for the anorexia of periparturient ewes was not available. The fact that anorexia does not occur in previously infected non -pregnant -lactating ewes (Kimambo et al 1998; Greer et al 2005), but it does occur in periparturient ewes, raises the question whether the loss of the acquired immunity is responsible for the occurrence of anorexia *per se*. Studies with immuno-suppressed sheep can be helpful in the attempt to answer this question. For example, immuno-suppression of non -pregnant -lactating ewes can be viewed as an equivalent to the loss of acquired immunity in periparturient ewes. The failure of immuno-suppressed ewes to express immunity to nematode infection resulted in an increase in FEC, but not in anorexia (Greer et al 2005) and therefore, it can be suggested that the occurrence of anorexia in periparturient ewes cannot be related to their inability to express their acquired immunity. The data of the present thesis suggest that anorexia can occur in periparturient

ewes before any increase in FEC was observed. In addition, given the significant breed differences in the extent of PPRI, it can be argued that the magnitude but also the duration of anorexia in periparturient ewes are not strongly associated with the extent of PPRI that the periparturient ewes display, as judged by the rise in FEC. Because, the occurrence of anorexia in sheep is associated with the acquisition phase of the immune response (Greer et al, 2005) and ewes restore their immunity following the PPRI, it is possible that the occurrence of anorexia in periparturient ewes is related to the developing immune responses that alleviate the extent of PPRI and which eventually lead to the restoration of immunity.

The findings of the present thesis show that anorexia develops during the acquisition phase of the immune response in previously naïve lambs and during the developing immune responses in periparturient ewes. Expression of immunity was not associated with anorexia in re-infected lambs and, as mentioned previously, anorexia does not occur during the expression of immunity in non -pregnant -lactating ewes (Kimambo et al 1998; Greer et al 2005). Although it may be apparent from this series of experiments that the developing immune response is involved in the reduction of appetite, this does not explain why animals have evolved a mechanism which reduces nutrient intake at a time when demands for energy and protein for the development of immune function and repair of damaged tissue are increased. In many animal models, the occurrence of anorexia following infection has been suggested to serve a function, rather than being simply a by-product of the immune response to infection (Murray et al 1979; Symons, 1985; Hart, 1998; Kyriazakis et al. 1998). Kyriazakis et al (1998), in a comprehensive review have developed several functional hypotheses to account for the reduced feed intake in parasitized animals. According to this review, the most plausible hypothesis is that i) food intake decreases for the purpose of promoting an effective immune response in the host or ii) anorexia allows the host to become more selective in its diet, and thus select foods that either minimize the risk of infection or are high in antiparasitic compounds. The role of anorexia in early immune response to

infection is supported by the experimental evidence which shows that force feeding of infected mice to a "normal" energy intake (i.e. that of their uninfected controls) led to an increased mortality, and shortened survival time, compared with infected mice that were allowed to become anorexic (Murray et al 1979). This outcome could well be seen as the host's impaired ability to mount an effective immune response (Kyriazakis et al 1998). Because in the present experiments anorexia occurred when animals had to develop, rather than to express immune response, it suggests that anorexia during nematode infection has an "immunopromotionary" role. On the other hand, if the role of anorexia was to help animals to become more selective in their diet, it would be expected on different diets, animals will show different degrees of anorexia. In case of the dietary protein content, the results of the present thesis show that this is not likely to be the case. The effect of protein supplementation on anorexia are summarised in the next section.

6.3. Effects of protein supplementation on parasite-induced anorexia

Several studies with ewes (Donaldson et al., 1998, Houdijk et al., 2000, 2001a,b), goats (Chartier et al., 2000) and more recently with rats (Houdijk et al., 2005a) have shown that dietary metabolisable protein (MP) scarcity exaggerates the extent of PPRI while an increased supply of, or a decreased demand for, MP can lower the extent of PPRI. Thus the present thesis investigated whether protein supplementation can alleviate also the degree of anorexia in periparturient ewes, in a similar way to its effects on PPRI. In agreement with previous studies (Donaldson et al., 1998, Houdijk et al., 2000, 2001b), the results of the present thesis showed that protein supplementation limited the extent of PPRI in ewes, as judged by FEC results. The results showed that infection imposed a significant reduction in ADFI in both breeds, but this reduction was not affected by protein supplementation as shown by the non significant interaction between infection, protein supplementation and time (Chapter 5). Similar was the effect of infection when data were analyzed on a RADFI basis (ADFI data scaled relative to ewe BW; RADFI, $g \cdot kg^{-1} \cdot d^{-1}$), which accounted for differences in mature size between breeds and treatments. The data suggest that protein supplementation can alleviate the extent of PPRI but not the degree of anorexia in periparturient ewes.

Protein supplementation does not seem to have a significant effect on the degree of anorexia in parasitised lambs either. That protein supplementation can affect the degree of anorexia was proposed in the study of Bown et al (1991), who found that abomasal infusion of casein decreased the degree of anorexia in lambs. Subsequent studies however, (Kyriazakis et al., 1994, 1996b) did not provide support for this suggestion. van Dam et al, (1998), also found that the degree of anorexia following trypanosome infection was similar in goats that received either a high or a low protein food. In the present thesis, the effect of

protein supplementation on the degree of anorexia in lambs was not investigated. However, as discussed in Chapter 4, it is unlikely that the dietary protein content of the food could have contributed in the differences in the degree of anorexia, as shown by the results of the primary infected S lambs in Chapter 3 and 4.

There is evidence from studies in pigs that protein supplementation can affect the characteristics of anorexia in a different manner. The data from the experiments of Williams *et al.* (1997) suggested that the degree of anorexia diminishes, as the protein content of the food (lysine) is decreased; food intake between the control and "immune stimulated" pigs was identical at the lowest level of lysine. The results of the present thesis showed that ewes in the LP treatment, although received well below their requirements in MP (Table 5.2) did show anorexia. This was in magnitude, similar to that observed in the ewes of the HP treatment, which over covered the MP requirements of the ewes. It seems therefore that there are contradictory findings on the effect of protein supplementation on anorexia following infection in reproductive animals. The fact that protein supplementation does not seem to affect the degree of anorexia in periparturient ewes or lambs, at least as evidenced by the available studies so far, could suggest that protein supplementation does not have a direct effect on the mechanism responsible for the occurrence of anorexia in sheep. However, the effects of protein supplementation on parasite-induced anorexia merit further investigation especially in the view of the little information available. In an attempt to understand better the mechanisms involved in the parasite-induced anorexia, the present thesis put forward novel hypotheses that tested the role of leptin in the immune responses and anorexia during the gastrointestinal infection of sheep. These findings are discussed in the next section.

6.4. The role of leptin in anorexia and immune responses of sheep infected with gastrointestinal nematodes

The adipocyte hormone leptin is involved in the regulation of immune responses and its plasma concentrations increase acutely following infection in many disease models. An increase in plasma leptin concentration could itself be responsible for the anorexia of parasitised sheep, or could act indirectly, through the stimulation of excretion of pro-inflammatory cytokines that are known to act in the hypothalamus and promote anorexia (Spurlock, 1997; Plata-Salaman, 1998, 2004; Ingvartsen and Andersen, 2000). Evidence for an involvement of leptin in the nematode infected animal is, however, scant. Thus, the major challenge of the current thesis was to investigate, in the first place, whether nematode infection results in increased plasma leptin concentration in sheep. The hypothesis tested was that plasma leptin concentrations are increased as a result of the immune response to nematode infection in sheep, when accounted for the level of food intake or body fat reserves. This hypothesis was based mainly on the existing evidence that studies in rodents and humans have revealed, but also on the current -admittedly very limited- evidence on the role of leptin in immune response in farm animals.

In general, the results of the present thesis show that plasma leptin concentrations do not increase acutely in lambs following a primary or a secondary infection with *T. circumcincta*, neither in ewes during the occurrence of PPRI. These findings are in contrast to the acute increase in PLC that has been observed in earlier studies during nematode infection (Mercer et al., 2000; Roberts et al., 1999), acute intestinal inflammation (Barbier et al., 1998), and other disease models (Faggioni et al., 1997; Grunfeld et al., 1996; Sarraf et al., 1997) in rodents. The failure to detect an acute increase in plasma leptin concentration in response to nematode infection could be due to species differences as the majority of

evidence for such an effect for leptin comes from studies in rodents and humans (Daniel et al 2003; Kulcsar et al, 2004). It is also possible that this result is due to the nature of infection; however, this suggestion is less likely in view of the evidence in other models of disease than parasitism, in ruminants. Daniel et al (2003) report that peripheral administration of lipopolysaccharide (LPS) or inflammatory cytokines such as TNF or IL-1 did not alter circulating concentrations of leptin in ewes, in contrast with studies in mice (Finck et al., 1998) and rats (Roelfsema et al., 2001). Acute endotoxemia also failed to result in an increased PLC in sheep (Soliman et al., 2001) and cows (Soliman et al., 2002) and similar was the effect of prion disease on PLC on ewes (Viguié et al 2004). With regards to the effect of nematode infection on PLC in sheep, Valderrabano, et al, (2006) recently suggested that leptin may be involved in the protective immune reactivity in periparturient ewes. However, these findings are not supported by those from Fox et al (2006) who found that a single dose of *T. circumcincta* did not result in significant changes in PLC, in Suffolk–Romney cross lambs. It should be noted here that both of these studies did not involve control (non-infected) lambs to allow for a direct comparison in PLC between infected and non-infected sheep and, therefore, the results are difficult to interpret. Recently, Liu et al (2007) reported that infection with a combination of *T. circumcincta* and *T. colubriformis* did not result in an increase in PLC in restrictedly fed Merino lambs. In the experiments of the present thesis, infection with *T. circumcincta* did not result in acute increase in PLC in ad libitum fed lambs or ewes. This was shown in all experiments by a direct comparison in PLC between infected and non-infected sheep. In accordance with other observations, gastrointestinal nematode infection does, therefore, not seem to result in an acute increase of PLC in *ad libitum* fed sheep.

However, the failure to observe an acute increase in PLC following infection, does not necessarily imply that PLC are unaffected by infection, as its regulation can be influenced simultaneously by other mechanisms (Kulcsar et al, 2004; Batra and Siegmund,

2007). For example, Barb et al (2001) suggested that leptin regulation in pigs during endotoxin challenge is under the control of two opposing mechanisms: one stimulatory (i.e. by inflammatory mediators) and one inhibitory (i.e. due to changes in energy metabolism). It could be well argued therefore, that this might be also the case during infections in sheep (Kulcsar et al. 2005). For many animal species, including sheep, it is well recognized that PLC are reduced with starvation (Blache et. al., 2000; Delavaud et al, 2000; Adam et al, 2002; Altmann et al, 2005). Because parasitised sheep are expected to show anorexia, this decrease in intake could have itself an effect on PLC. Changes in body fatness as a result of infection could also affect PLC in sheep. Therefore, in order to prove a critical role for leptin in parasitised sheep, experiments that account for these opposing mechanisms are required. The design of the experiments of the present thesis allowed comparisons in PLC between infected and non-infected sheep with similar food intake and/or body fatness, as measured by condition score or back-fat depth. The results of Chapter 3, showed that PLC were affected by infection with *T. circumcincta* when the statistical model accounted for differences in the RADFI of infected and non-infected lambs. This effect was significant during both the primary and secondary infection. These results suggest that leptin may be involved in the immune response to nematode infection in lambs, even when nematode infection does not provoke an acute increase in PLC.

The results of the subsequent experiments (Chapter 4), however, did not show a similar effect of infection in PLC and do not provide strong support of the hypothesis tested. The discrepancy in the findings of Chapter 3 and 4 is difficult to explain. Because the same strain and dose of T. circumcincta larvae was used in all experiments, it is unlikely that the severity of infection have contributed to this outcome. This suggestion is supported also by the similar FEC results in these experiments. It is proposed, however, that this outcome could be related to the different approaches adopted in the two Chapters, in order to account for the effect of food intake level on PLC. This explanation relies on the fact that RADFI of

the pair-fed lambs in Chapter 4 was not significantly reduced in order to affect PLC and thus when RADFI data were used as a co-variable did not provide significant explanation in PLC variation. In contrast, the RADFI of the restrictedly fed treatments in Chapter 3 was significantly reduced and PLC were highly positive correlated with RADFI (Tables 3.2 and 3.3). The differences in the severity of food intake reduction in the non-infected lambs between the two experiments may have resulted in the inconsistency of the effect of infection on PLC.

Similar to previous observations, the experiment with the ewes (Chapter 5) suggests that PLC are not acutely affected by nematode infection during the periparturient period. Valderrabano et al., (2006) recently suggested that differences in the immune response appeared to be associated with differences in serum leptin levels in periparturient ewes infected with *H. contortus*. However, such an effect was not observed in the present study. This was shown by the finding that observed PLC did not differed between infected and non infected ewes in either breeds or treatments. In addition, the effect of infection on PLC remained non significant when the statistical model accounted for the variation in back-fat depth across breeds and treatments. The results show that during the periparturient period, PLC are unlikely to be affected by nematode infection.

The results of the present thesis are consistent with the observation that maternal PLC decline progressively through late pregnancy and remain at low levels during lactation (Ingvartsen and Boisclait 2001; Ehrhardt et al, 2001). The decrease in leptin production during late-pregnancy/early-lactation could result from negative energy balance and lower body condition score (BCS) of ruminants during that period (see review from Chilliard et al., 2005). Low leptin levels during this period could serve to increase metabolic efficiency and energy conservation (Chilliard et al., 2000) that favour nutrient partitioning to the mammary gland for increased milk production (Vernon et al, 2002). In accordance, eliminating the

energetic costs of lactation by preventing milk delivery induced dramatic increases in plasma leptin and insulin levels and also increased adiposity (Woodside et al, 2000). Therefore, results of the present work that nematode infection failed to increase PLC during the periparturient ewes could reflect the view that the function of pregnancy and lactation is prioritised over that of the immune response, as suggested in the nutrient partitioning framework of Coop and Kyriazakis (1999).

Several factors have been proposed as the specific cause of reduction in food intake during pathogen challenges (Plata-Salaman, 1996; Johnson, 1998; Langhans, 2000). These factors are strongly related with the developing immune responses and are members of the cytokine family (Weingarden, 1995; Spurlock, 1997; Plata-Salaman, 1998; 2004; Ingvartsen and Andersen, 2000; Langhans, 2000). Leptin and its receptor belong to the cytokine family. In several disease models there was an undoubted positive correlation between the elevated circulating leptin levels and reduced intake (Barbier, et al, 1998; Grunfeld et al, 1996; Sarraf et al, 1997; Mercer et al 1999; Niswender et al 2001). Thus a role for leptin in the anorexia of infection and inflammation was proposed (Grunfeld et al 1996; Barbier et al., 1998; Plata-Salaman, 1998). The results of the present thesis show that infection did not result in an acute increase in PLC during primary and secondary infections in lambs and ewes. In Chapter 3, although infected lambs had significantly higher PLC than non-infected lambs with similar RADFI in both breeds, anorexia was observed in lambs of the S breed only. In the subsequent experiments with lambs (Chapter 4) the association between RADFI and changes in PLC were not clear. In periparturient ewes, despite the significant reduction in food intake, PLC did not differ between infected and control ewes and PLC was not strongly associated with RADFI. Overall, these results show that leptin alone is unlikely to be responsible for the anorexia that is observed in parasitized sheep. Since recent evidence suggests that cytokines are involved in the regulation of immune responses in sheep

(Pernthaner et al 2005, 2006). It is therefore possible that cytokines are part of the mechanism responsible for the occurrence of anorexia in parasitised sheep.

6.5. Conclusions - Future directions

The major consequence of gastrointestinal infections in ruminants is the reduction of the voluntary food intake, which in turn results in impaired animal productivity and economic losses in ruminant production systems. Some of the principal approaches in the control of parasitism are based upon the exploitation of the genetic resistance of livestock, and improved nutrition (e.g. protein supplementation) of the host. The present thesis presents a series of experiments that studied the interaction between the effects of host genotype, immune status and protein supplementation on the parasite-induced anorexia of sheep. In relation to the mechanism(s) involved in the occurrence of anorexia, the study put forward a novel hypothesis and tested whether leptin concentrations in plasma are increased as a result of infection and whether this increase is associated with the reduced food intake.

This work provided novel evidence that anorexia does occur in ewes during the PPRI and that the degree of anorexia they display is not influenced by dietary protein supplementation. The results show that the effect of protein supplementation on the degree of anorexia is not influenced by the host genotype. The results further suggest that protein supplementation is unlikely to act directly on the mechanism responsible for the occurrence of anorexia in periparturient ewes. Whether the degree of anorexia is affected by food characteristics other than dietary protein, merits further investigation.

The present study is the first to investigate whether breeds of different production potential differ in the degree of anorexia, following nematode infection. The results show that there were phenotypic differences in resistance to infection between those breeds, with high producing genotypes showing less resistance. The breed differences in the phenotypic resistance to infection were not associated with breed differences in the degree of anorexia in

periparturient ewes and in lambs during secondary infections. Overall, these results suggest that the degree of anorexia is unlikely to be a correlated trait to the phenotypic resistance that animals show through their ability to limit FEC. However, more attention to the genotype effects on anorexia is required because the data from the lamb experiment (Chapter 3) show that breed differences in the degree of anorexia can exist during the acquisition phase of the immune response.

The experiments presented in the present thesis have provided novel evidence that leptin is unlikely to be itself responsible for the occurrence of anorexia in parasitised sheep. The data show that nematode infection (primary or secondary) does not result in an acute increase in plasma leptin concentrations in sheep, in contrast to findings in other disease models in murine studies. The novelty of the experimental design allowed for comparisons in plasma leptin concentrations between infected and non-infected sheep with similar food intakes. These data showed that leptin is probably involved in the regulation of immune responses, as the effect of infection on plasma leptin concentrations was significant, when differences in intake were accounted for. However, the suggestion that leptin is regulated by two opposing mechanisms is open to future investigation. Further elucidation of the mechanism as well as the functional significance of anorexia in sheep in response to nematode infection would assist us in devising control strategies that could either overcome or minimise its impact.

Bibliography

Bibliography

Abbott EM & Holmes PH (1983) The Influence of Dietary-Protein on the Resistance of Sheep to Haemonchus-Contortus. *Parasitology* **87**, R16-R16.

Abbott EM, Parkins JJ & Holmes PH (1985) Influence of Dietary-Protein on Parasite Establishment and Pathogenesis in Finn Dorset and Scottish Blackface Lambs Given a Single Moderate Infection of Haemonchus-Contortus. *Research in Veterinary Science* **38**, 6-13.

Abbott EM, Parkins JJ & Holmes PH (1988) Influence of Dietary-Protein on the Patho-Physiology of Haemonchosis in Lambs Given Continuous Infections. *Research in Veterinary Science* **45**, 41-49.

AFRC (1993) *Energy and Protein Requirements of Ruminants.* An Advisory Manual Prepared by the AFRC Technical Committee on Responses to Nutrients. Wallingford, UK: CAB International.

Ahima RS & Flier JS (2000) Adipose tissue as an endocrine organ. *Trends in Endocrinology and Metabolism* **11**, 327-332.

Altaif KI & Dargie JD (1978) Genetic Resistance to Helminths - Influence of Breed and Hemoglobin Type on Response of Sheep to Primary Infections with Haemonchus-Contortus. Parasitology 77, 161-175.

Anderson N, Hansky J, & Titchen DA (1981) Effects of *Ostertagia circumcincta* infections on plasma gastrin in sheep. *Parasitology*, **82**, 401-410.

Amarante AF, Bricarello PA, Rocha RA & Gennari SM (2004) Resistance of Santa Ines, Suffolk and Ile de France sheep to naturally acquired gastrointestinal nematode infections. *Veterinary Parasitology* **120**, 91-106.

Amarante AFT, Barbosa MA, Deoliveira M & Siqueira ER (1992) Changes in Fecal Egg Counts of Gastrointestinal Nematodes by Ewes of 4 Breeds in Different Reproductive Status. *Pesquisa Agropecuaria Brasileira* **27**, 47-51.

Baker RL (1998) A review of genetic resistance to gastrointestinal nematode parasites in sheep and goats in the tropics and evidence for resistance in some sheep and goat breeds in sub-humid coastal Kenya. *Animal Genetics Resources Information Bulletin* **24**, 13-30.

Balic A, Bowles VM, Liu YS & Meeusen ENT (2003) Local immune responses in sensitized sheep following challenge infection with Teladorsagia circumcincta. *Parasite Immunology* **25**, 375-381.

Balic A, Bowles VM & Meeusen ENT (2000) The immunobiology of gastrointestinal nematode infections in ruminants. *Advances in Parasitology, Vol 45* **45**, 181-241.

Barb CR, Hausman GJ & Houseknecht KL (2001) Biology of leptin in the pig. *Domestic Animal Endocrinology* **21**, 297-317.

Barb CR, Yan X, Azain MJ, Kraeling RR, Rampacek GB & Ramsay TG (1998) Recombinant Porcine Leptin Reduces Feed Intake and Stimulates Growth Hormone Secretion in Swine. *Domestic Animal Endocrinology* **15**, 77-86.

Barbier M, Cherbut C, Aube AC, Blottiere HM & Galmiche JP (1998) Elevated plasma leptin concentrations in early stages of experimental intestinal inflammation in rats. *Gut* **43**, 783-790.

Barger IA (1982) Helminth parasites and animal production. In: Biology and Control of Endoparasites (Edited by Symons L.E.A., Donald A.D. and Dineen J.K.). pp. 133-155 Academic Press, Sydney.

Barger IA (1988) Resistance of Young Lambs to Haemonchus-Contortus Infection, and Its Loss Following Anthelmintic Treatment. *International Journal for Parasitology* **18**, 1107-1109.

Barger IA (1993) Influence of Sex and Reproductive Status on Susceptibility of Ruminants to Nematode Parasitism. *International Journal for Parasitology* **23**, 463-469.

Barnes EH & Dobson RJ (1993) Persistence of Acquired-Immunity to Trichostrongylus-Colubriformis in Sheep after Termination of Infection. *International Journal for Parasitology* **23**, 1019-1026.

Barrachina MD, Martinez V, Wei JY & Tache Y (1997) Leptin acts in the brain to reduce food intake and gastric emptying in male Long-Evans rats. *Gastroenterology* **112**, A861-A861.

Bellmann O, Wegner J, Teuscher F, Schneider F & Ender K (2004) Muscle characteristics and corresponding hormone concentrations in different types of cattle. *Livestock Production Science* **85**, 45-57.

Bishop SC & Stear MJ (1997) Modelling responses to selection for resistance to gastro-intestinal parasites in sheep. *Animal Science* **64**, 469-478.

Bishop SC & Stear MJ (1999) Genetic and epidemiological relationships between productivity and disease resistance: gastro-intestinal parasite infection in growing lambs. *Animal Science* **69**, 515-524.

Bisset SA, Morris CA, McEwan JC & Vlassoff A (2001) Breeding sheep in New Zealand that are less reliant on anthelmintics to maintain health and productivity. *New Zealand Veterinary Journal* **49**, 236-246.

Blache D, Tellam RL, Chagas LM, Blackberry MA, Vercoe PE & Martin GB (2000) Level of nutrition affects leptin concentrations in plasma and cerebrospinal fluid in sheep. *Journal of Endocrinology* **165**, 625-637.

Blaxter KL (1989) *Energy metabolism in animals and man.* Cambridge, UK: Cambridge University Press.

Bocquier F, Bonnet M, Faulconnier Y, Guerre-Millo M, Martin P & Chilliard Y (1998) Effects of photoperiod and feeding level on perirenal adipose tissue metabolic activity and leptin synthesis in the ovariectomized ewe. *Reproduction Nutrition Development* **38**, 489-498.

Bown, M.D., Poppi, D.P., & Sykes, A.R. (1989). The effects of a concurrent infection of *Trichostrongylus colubriformis* and *Ostertagia circumcincta* on calcium, phosphorus and magnesium transactions along the digestive tract of lambs. *Journal of Comparative Pathology*, 101, 11-20.

Bown MD, Poppi DP & Sykes AR (1991) The Effect of Post-Ruminal Infusion of Protein or Energy on the Pathophysiology of Trichostrongylus-Colubriformis Infection and Body-Composition in Lambs. *Australian Journal of Agricultural Research* **42**, 253-267.

Bueno L, Dakkak A & Fioramonti J (1982) Gastroduodenal Motor and Transit Disturbances Associated with Haemonchus-Contortus Infection in Sheep. *Parasitology* **84**, 367-374.

Campfield LA, Smith FJ, Guisez Y, Devos R & Burn P (1995) Recombinant Mouse Ob Protein - Evidence for a Peripheral Signal Linking Adiposity and Central Neural Networks. *Science* **269**, 546-549.

Chandra RK (1993) Nutrition and the Immune-System. *Proceedings of the Nutrition Society* **52**, 77-84.

Chartier C, Etter E, Hoste H, Pors I, Mallereau MP, Broqua C, Mallet S, Koch C & Masse A (2000) Effects of the initial level of milk production and of the dietary protein intake on the course of natural nematode infection in dairy goats. *Veterinary Parasitology* **92**, 1-13.

Chilliard Y, Bocquier F, Delavaud C, Faulconnier Y, Bonnet M, Guerre-Millo M, Martin P & Ferlay A (1999) Leptin in ruminants. Nutritional and physiological factors of variation. *Productions Animales* **12**, 225-237.

Chilliard Y, Bocquier F & Doreau M (1998) Digestive and metabolic adaptations of ruminants to undernutrition, and consequences on reproduction. *Reproduction Nutrition Development* **38**, 131-152.

Chilliard Y, Bonnet M, Delavaud C, Faulconnier Y, Leroux C, Djiane J & Bocquier F (2001) Leptin in ruminants. Gene expression in adipose tissue and mammary gland, and regulation of plasma concentration. *Domestic Animal Endocrinology* **21**, 271-295.

Chilliard Y, Delavaud C & Bonnet M (2005) Leptin expression in ruminants: Nutritional and physiological regulations in relation with energy metabolism. *Domestic Animal Endocrinology* **29**, 3-22.

Christie M & Jackson F (1982) Specific Identification of Strongyle Eggs in Small Samples of Sheep Feces. *Research in Veterinary Science* **32**, 113-117.

Claerebout E & Vercruysse J (2000) The immune response and the evaluation of acquired immunity against gastrointestinal nematodes in cattle: a review. *Parasitology* **120**, S25-S42.

Colditz IG (2002). Effects of the immune system on metabolism: implications for production and disease resistance in livestock. *Livestock Production Science*, **75**, 257-268.

Colditz IG (2008) Six costs of immunity to gastrointestinal nematode infections. Parasite Immunology 30, 63-70.

Considine RV & Caro JF (1996) Leptin in humans: Current progress and future directions. *Clinical Chemistry* **42**, 843-844.

Coop RL & Holmes PH (1996) Nutrition and parasite interaction. *International Journal for Parasitology* **26**, 951-962.

Coop RL, Huntley JF & Smith WD (1995) Effect of Dietary-Protein Supplementation on the Development of Immunity to Ostertagia-Circumcincta in Growing Lambs. *Research in Veterinary Science* **59**, 24-29.

Coop RL & Kyriazakis I (1999) Nutrition-parasite interaction. *Veterinary Parasitology* **84**, 187-204.

Coop RL & Sykes AR (2002) Interactions between gastrointestinal parasites and nutrients. *In* :M. Freer and H. Dove (Eds), *Sheep Nutrition* (pp. 313-331). Wallingford, U.K.: CAB international.

Coop RL, Sykes AR & Angus KW (1977) Effect of a Daily Intake of Ostertagia-Circumcincta Larvae on Body-Weight, Food-Intake and Concentration of Serum Constituents in Sheep. *Research in Veterinary Science* **23**, 76-83.

Coop RL, Sykes AR & Angus KW (1982) The Effect of 3 Levels of Intake of Ostertagia-Circumcincta Larvae on Growth-Rate, Food-Intake and Body-Composition of Growing Lambs. *Journal of Agricultural Science* **98**, 247-255.

Courtney CH, Parker CF, Mcclure KE & Herd RP (1984) A Comparison of the Periparturient Rise in Fecal Egg Counts of Exotic and Domestic Ewes. *International Journal for Parasitology* **14**, 377-381.

Daniel JA, Whitlock BK, Baker JA, Steele B, Morrison CD, Keisler DH & Sartin JL (2002) Effect of body fat mass and nutritional status on 24-hour leptin profiles in ewes. *Journal of Animal Science* **80**, 1083-1089.

Delavaud C, Bocquier F, Chilliard Y, Keisler DH, Gertler A & Kann G (2000) Plasma leptin determination in ruminants: effect of nutritional status and body fatness on plasma leptin concentration assessed by a specific RIA in sheep. *Journal of Endocrinology* **165**, 519-526.

Delavaud C, Ferlay A, Faulconnier Y, Bocquier F, Kann G & Chilliard Y (2002) Plasma leptin concentration in adult cattle: Effects of breed, adiposity, feeding level, and meal intake. *Journal of Animal Science* **80**, 1317-1328.

Dineen JK & Wagland BM (1966) Dynamics of Host-Parasite Relationship .4. Response of Sheep to Graded and to Repeated Infection with Haemonchus Contortus. *Parasitology* **56**, 639-

Dobson RJ, Barnes EH & Windon RG (1992) Population-Dynamics of Trichostrongylus-Colubriformis and Ostertagia-Circumcincta in Single and Concurrent Infections. *International Journal for Parasitology* **22**, 997-1004.

Donald AD, Morley FHW, Waller PJ, Axelsen A, Dobson RJ & Donnelly JR (1982) Effects of Reproduction, Genotype and Anthelmintic Treatment of Ewes on Ostertagia Spp Populations. *International Journal for Parasitology* **12**, 403-411.

Donaldson J, van Houtert MFJ & Sykes AR (1998) The effect of nutrition on the periparturient parasite status of mature ewes. *Animal Science* **67**, 523-533.

Donaldson J, van Houtert MFJ & Sykes AR (2001) The effect of dietary fish-meal supplementation on parasite burdens of periparturient sheep. *Animal Science* **72**, 149-158.

Douch PGC, Green RS, Morris CA & Hickey SM (1995) Genetic-Factors Affecting Antibody-Responses to 4 Species of Nematode Parasite in Romney Ewe Lambs. *International Journal for Parasitology* **25**, 823-828.

Douch PGC, Morum PE & Rabel B (1996) Secretion of anti-parasite substances and leukotrienes from ovine gastrointestinal tissues and isolated mucosal mast cells. *International Journal for Parasitology* **26**, 205-211.

Dynes RA, Poppi DP, Barrell GK & Sykes AR (1998) Elevation of feed intake in parasite-infected lambs by central administration of a cholecystokinin receptor antagonist. *British Journal of Nutrition* **79**, 47-54.

Encyclopedia of Parasitology (2007) Mehlhorn Heinz (Ed.), Third edition, Volume 2. Springer, New York, USA

Ehrhardt RA, Greenwood PL, Bell AW & Boisclair YR (2003) Plasma leptin is regulated predominantly by nutrition in preruminant lambs. *Journal of Nutrition* **133**, 4196-4201.

Ehrhardt RA, Slepetis RM, Bell AW & Boisclair YR (2001) Maternal leptin is elevated during pregnancy in sheep. *Domestic Animal Endocrinology* **21**, 85-96.

Ehrhardt RA, Slepetis RM, Siegal-Willott J, Van Amburgh ME, Bell AW & Boisclair YR (2000) Development of a specific radioimmunoassay to measure physiological changes of circulating leptin in cattle and sheep. *Journal of Endocrinology* **166**, 519-528.

Emmans GC & Friggens NC (1995) The effects of liveweight, and of the quality of the feed given previously, on the ad libitum intakes of a poor quality hay by sheep of 5 breeds of different mature liveweights. *Annales De Zootechnie* **44**, 269.

Faggioni R, Fantuzzi G, Fuller J, Dinarello CA, Feingold KR & Grunfeld C (1998) IL-1 beta mediates leptin induction during inflammation. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology* **43**, R204-R208.

Faggioni R, Feingold KR & Grunfeld C (2001) Leptin regulation of the immune response and the immunodeficiency of malnutrition. *Faseb Journal* **15**, 2565-2571.

Faggioni R, Fuller J, Moser A, Feingold KR & Grunfeld C (1997) LPS-induced anorexia in leptin-deficient (ob/ob) and leptin receptor-deficient (db/db) mice. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology* **42**, R181-R186.

Fantuzzi G (2005) Adipose tissue, adipokines, and inflammation. *Journal of Allergy and Clinical Immunology* **115**, 911-919.

Fantuzzi G & Faggioni R (2000) Leptin in the regulation of immunity, inflammation, and hematopoiesis. *Journal of Leukocyte Biology* **68**, 437-446.

Flier JS (1998) What's in a name? In search of leptin's physiologic role. *Journal of Clinical Endocrinology and Metabolism* **83**, 1407-1413.

Fox MT (1997) Pathophysiology of infection with gastrointestinal nematodes in domestic ruminants: recent developments. *Veterinary Parasitology* **72**, 285-297.

Fox MT, Gerrelli D, Pitt SR, Jacobs DE, Gill M & Gale DL (1989) Ostertagia-Ostertagi Infection in the Calf - Effects of a Trickle Challenge on Appetite, Digestibility, Rate of Passage of Digesta and Liveweight Gain. *Research in Veterinary Science* **47**, 294-298.

Fox MT, Pitt SR, Gerrelli D & Jacobs DE (1988) Effect of Diet on Gastrin-Response to Ostertagia-Ostertagi Infection in the Calf. *International Journal for Parasitology* **18**, 547-548.

Fox MT, Reynolds GW, Scott I, Simcock DC & Simpson HV (2006) Vagal and splanchnic afferent nerves are not essential for anorexia associated with abomasal parasitism in sheep. *Veterinary Parasitology* **135**, 287-295.

Friedman JM & Halaas JL (1998) Leptin and the regulation of body weight in mammals. *Nature* **395**, 763-770.

Gasbarre LC, Leighton EA & Sonstegard T (2001) Role of the bovine immune system and genome in resistance to gastrointestinal nematodes. *Veterinary Parasitology* **98**, 51-64.

Glasbey CA, Abdalla I & Simm G (1996) Towards automatic interpretation of sheep ultrasound scans. *Animal Science* **62**, 309-315.

Good B, Hanrahan JP, Crowley BA & Mulcahy G (2006) Texel sheep are more resistant to natural nematode challenge than Suffolk sheep based on faecal egg count and nematode burden. *Veterinary Parasitology* **136**, 317-327.

Gray GD, Barger IA, Le Jambre LF & Douch PGC (1992) Parasitological and immunological responses of genetically resistant Merino sheep on pastures contaminated with parasitic nematodes. *International Journal for Parasitology* **22**: 411-425.

Greer AW, Stankiewicz M, Jay NP, McAnulty RW & Sykes AR (2005) The effect of concurrent corticosteroid induced immuno-suppression and infection with the intestinal parasite Trichostrongylus colubriformis on food intake and utilization in both immunologically naive and competent sheep. Animal Science 80, 89-99.

Greer AW (2008) Trade-offs and benefits: implications of promoting a strong immunity to gastrointestinal parasites in sheep. *Parasite Immunology* **30**, 123-132.

Grunfeld C, Zhao C, Fuller J, Pollock A, Moser A, Friedman J & Feingold KR (1996) Endotoxin and cytokines induce expression of leptin, the ob gene product, in

hamsters - A role for leptin in the anorexia of infection. *Journal of Clinical Investigation* **97**, 2152-2157.

Harrison GBL, Pulford HD, Gatehouse TK, Shaw RJ, Pfeffer A & Shoemaker CB (1999) Studies on the role of mucus and mucosal hypersensitivity reactions during rejection of Trichostrongylus colubriformis from the intestine of immune sheep using an experimental challenge model. *International Journal for Parasitology* **29**, 459-468.

Harrison GBL, Pulford HD, Hein WR, Barber TK, Shaw RJ, Mcneill M, Wakefield SJ & Shoemaker CB (2003) Immune rejection of Trichostrongylus colubriformis in sheep; a possible role for intestinal mucus antibody against an L3-specific surface antigen. *Parasite Immunology* **25**, 45-53.

Hart BL (1988) Biological Basis of the Behavior of Sick Animals. *Neuroscience and Biobehavioral Reviews* **12**, 123-137.

Henry BA, Goding JW, Alexander WS, Tilbrook AJ, Canny BJ, Dunshea F, Rao A, Mansell A & Clarke IJ (1999) Central Administration of Leptin to Ovariectomized Ewes Inhibits Food Intake without Affecting the Secretion of Hormones from the Pituitary Gland: Evidence for a Dissociation of Effects on Appetite and Neuroendocrine Function. *Endocrinology* **140**, 1175-1182.

Henry BA, Goding JW, Tilbrook AJ, Dunshea FR, Blache D & Clarke IJ (2004) Leptin-mediated effects of undernutrition or fasting on luteinizing hormone and growth hormone secretion in ovariectomized ewes depend on the duration of metabolic perturbation. *Journal of Neuroendocrinology* **16**, 244-255.

Henry BA, Goding JW, Tilbrook AJ, Dunshea FR & Clarke IJ (2001) Intracerebroventricular infusion of leptin elevates the secretion of luteinising hormone without affecting food intake in long-term food-restricted sheep, but increases growth hormone irrespective of bodyweight. *Journal of Endocrinology* **168**, 67-77.

Higashiyama Y, Abe H, Hayashi M & Hodate K (2003) The comparison of plasma level and mRNA expression of leptin from Japanese Black steers and Holstein steers. *Livestock Production Science* **81**, 247-255.

Holmes PH, (1985) Pathogenesis of Trychostrongylosis. *Veterinary Parasitology* **18** (2): 89-101.

Houdijk JG, Jackson F, Coop RL & Kyriazakis I (2006) Rapid improvement of immunity to Teladorsagia circumcincta is achieved through a reduction in the demand for protein in lactating ewes. *International Journal for Parasitology* **36**, 219-227.

Houdijk JG, Kyriazakis I, Jackson F, Huntley JF & Coop RL (2000) Can an increased intake of metabolizable protein affect the periparturient relaxation in immunity against Teladorsagia circumcincta in sheep? *Veterinary Parasitology* **91**, 43-62.

Houdijk JGM, Jessop NS, Knox DP & Kyriazakis I (2003) Breakdown of immunity to Nippostrongylus brasiliensis in lactating rats. *British Journal of Nutrition* **90**, 809-814.

Houdijk JGM, Jessop NS, Knox DP & Kyriazakis I (2005a) Nutritional control of gastointestinal parasitism in lactating rats. *Proceedings- British Society of Animal Science*, 11.

Houdijk JGM, Jessop NS, Knox DP & Kyriazakis I (2005b) Secondary infection of Nippostrongylus brasiliensis in lactating rats is sensitive to dietary protein content. *British Journal of Nutrition* **93**, 493-499.

Houdijk JGM, Kyriazakis I, Coop RL & Jackson F (2001a) The expression of immunity to Teladorsagia circumcincta in ewes and its relationship to protein nutrition depend on body protein reserves. *Parasitology* **122**, 661-672.

Houdijk JGM, Kyriazakis I, Jackson F & Coop RL (2001b) The relationship between protein nutrition, reproductive effort and breakdown in immunity to Teladorsagia circumcincta in periparturient ewes. *Animal Science* **72**, 595-606.

Houseknecht KL, Baile CA, Matteri RL & Spurlock ME (1998) The biology of leptin: A review. *Journal of Animal Science* **76**, 1405-1420.

Huntley JF, Newlands GFJ, Jackson F & Miller HRP (1992) The Influence of Challenge Dose, Duration of Immunity, or Steroid Treatment on Mucosal Mast-Cells and on the Distribution of Sheep Mast-Cell Proteinase in Haemonchus-Infected Sheep. *Parasite Immunology* **14**, 429-440.

Huntley JF, Schallig HDFH, Kooyman FNJ, Mackellar A, Millership J & Smith WD (1998) IgE responses in the serum and gastric lymph of sheep infected with Teladorsagia circumcincta. *Parasite Immunology* **20**, 163-168.

Ingvartsen KL & Andersen JB (2000) Integration of metabolism and intake regulation: A review focusing on periparturient animals. *Journal of Dairy Science* **83**, 1573-1597.

Ingvartsen KL & Boisclair YR (2001) Leptin and the regulation of food intake, energy homeostasis and immunity with special focus on periparturient ruminants. *Domestic Animal Endocrinology* **21**, 215-250.

Jackson F, Greer AW, Huntley J, McAnulty RW, Bartley DJ, Stanley A, Stenhouse L, Stankiewicz M & Sykes AR (2004) Studies using Teladorsagia circumcincta in an in vitro direct challenge method using abomasal tissue explants. *Veterinary Parasitology* **124**, 73-89.

Janeway CA (2001) How the immune system protects the host from infection. *Microbes and Infection* **3**, 1167-1171.

Jankovic D, Liu ZG & Gause WC (2001) Th1-and Th2-cell commitment during infectious disease: asymmetry in divergent pathways. *Trends in Immunology* **22**, 450-457.

Jeffcoate IA, Wedrychowicz H, Fishwick G, Dunlop EM, Duncan JL & Holmes PH (1992) Pathophysiology of the Periparturient Egg Rise in Sheep - a Possible Role for Iga. *Research in Veterinary Science* **53**, 212-218.

Jenkins NL, Turner JL, Dritz SS, Durham SK & Minton JE (2004) Changes in circulating insulin-like growth factor-I, insulin-like growth factor binding proteins, and leptin in weaned pigs infected with Salmonella enterica serovar Typhimurium. *Domestic Animal Endocrinology* **26**, 49-60.

Johnson LN, Kotz S & Balakrishnan N (1998) *Continuous Univariate Distributions*, Second ed. Boston: PWS-KENT Publishing.

Kadokawa H, Briegel JR, Blackberry MA, Blache D, Martin GB & Adams NR (2003) Relationships between plasma concentrations of leptin and other metabolic hormones in GH-transgenic sheep infused with glucose. *Domestic Animal Endocrinology* **24**, 219-229.

Kahn LP, Knox MR, Gray GD, Lea JM & Walkden-Brown SW (2003) Enhancing immunity to nematode parasites in single-bearing Merino ewes through nutrition and genetic selection. *Veterinary Parasitology* **112**, 211-225.

Kahn LP, Kyriazakis I, Jackson F & Coop RL (2000) Temporal effects of protein nutrition on the growth and immunity of lambs infected with Trichostrongylus colubriformis. *International Journal for Parasitology* **30**, 193-205.

Kaibara A, Moshyedi A, Auffenberg T, Abouhamze A, Copeland EM, Kalra S & Moldawer LL (1998) Leptin produces anorexia and weight loss without inducing an acute phase response or protein wasting. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology* **43**, R1518-R1525.

Kambara T, Mcfarlane RG, Abell TJ, Mcanulty RW & Sykes AR (1993) The Effect of Age and Dietary-Protein on Immunity and Resistance in Lambs Vaccinated with Trichostrongylus-Colubriformis. *International Journal for Parasitology* **23**, 471-476.

Keith KA, Duncan MC, Murray M, Bairden K & Tait A (1990) Stage-Specific Cuticular Proteins of Ostertagia-Circumcincta and Ostertagia-Ostertagi. *International Journal for Parasitology* **20**, 1037-1045.

Kershaw EE & Flier JS (2004) Adipose tissue as an endocrine organ. *Journal of Clinical Endocrinology and Metabolism* **89**, 2548-2556.

Kimambo AE, Macrae JC, Walker A, Watt CF & Coop RL (1988) Effect of Prolonged Subclinical Infection with Trichostrongylus-Colubriformis on the Performance and Nitrogen-Metabolism of Growing Lambs. *Veterinary Parasitology* **28**, 191-203.

Kulcsar M, Janosi S, Lehtolainen T, Katai L, Delavaud D, Balogh O, Chilliard Y, Pyorala S, Rudas P & Huszenicza G (2005) Feeding-unrelated factors influencing the plasma leptin level in ruminants. *Domestic Animal Endocrinology* **29**, 214-226.

Kyriazakis I, Anderson DH, Coop RL & Jackson F (1996a) The pathophysiology and development of immunity during long-term subclinical infection with Trichostrongylus colubriformis of sheep receiving different nutritional treatments. *Veterinary Parasitology* **65**, 41-54.

Kyriazakis I, Anderson DH, Oldham JD, Coop RL & Jackson F (1996b) Long-term subclinical infection with Trichostrongylus colubriformis: effects on food intake, diet selection and performance of growing lambs. *Veterinary Parasitology* **61**, 297-314.

Kyriazakis I & Houdijk J (2006) Immunonutrition: Nutritional control of parasites. *Small Ruminant Research* **62**, 79-82.

Kyriazakis I, Oldham JD, Coop RL & Jackson F (1994) The effect of subclinical intestinal nematode infection on the diet selection of growing sheep. *British Journal of Nutrition* **72**, 665.

Kyriazakis I, Tolkamp BJ & Hutchings MR (1998) Towards a functional explanation for the occurrence of anorexia during parasitic infections. *Animal Behaviour* **56**, 265-274.

La Cava A & Matarese G (2004) The weight of leptin in immunity. *Nature Reviews Immunology* **4**, 371-380.

Lewis RM, Macfarlane JM, Simm G & Emmans GC (2004) Effects of food quality on growth and carcass composition in lambs of two breeds and their cross. *Animal Science* **78**, 355-368.

Leyva V, Henderson AE & Sykes AR (1982) Effect of Daily Infection with Ostertagia-Circumcincta Larvae on Food-Intake, Milk-Production and Wool Growth in Sheep. *Journal of Agricultural Science* **99**, 249-259.

Liu SM, Adams NR, Briegel JB, Smith TL & Martin GB (2007) Effect of genetic resistance to gastrointestinal nematodes on plasma concentrations of insulin-like growth factor-1 and leptin in Merino sheep. *Australian Journal of Experimental Agriculture* **47**, 905-911.

Lord GM, Matarese G, Howard LK, Baker RJ, Bloom SR & Lechler RI (1998) Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. *Nature* **394**, 897-901.

Macrae JC, Walker A, Brown D & Lobley GE (1993) Accretion of Total Protein and Individual Amino-Acids by Organs and Tissues of Growing Lambs and the Ability of Nitrogen-Balance Techniques to Quantitate Protein Retention. *Animal Production* **57**, 237-245.

Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S, Kern PA & Friedman JM (1995) Leptin Levels in Human and Rodent - Measurement of Plasma Leptin and Ob Rna in Obese and Weight-Reduced Subjects. *Nature Medicine* **1**, 1155-1161.

Maizels RM & Yazdanbakhsh M (2003) Immune regulation by helminth parasites: Cellular and molecular mechanisms. *Nature Reviews Immunology* **3**, 733-744.

Maratos-Flier E (2008) The long reach of leptin. *Nature Medicine* **14**, 604-606.

Marie M, Findlay PA, Thomas L & Adam CL (2001) Daily patterns of plasma leptin in sheep: effects of photoperiod and food intake. *Journal of Endocrinology* **170**, 277-286.

Matarese G, La Cava A, Sanna V, Lord GM, Lechler RI, Fontana S & Zappacosta S (2002) Balancing susceptibility to infection and autoimmunity: a role for leptin? *Trends in Immunology* **23**, 182-187.

Matarese G, Moschos S & Mantzoros CS (2005) Leptin in immunology. *Journal of Immunology* **174**, 3137-3142.

McAnulty RW, Familton AS, Sedcole RD & Sykes AR (2001) Changes in the resistance of the ewe to infection with Teladorsagia circumcincta during late pregnancy and lactation. *Animal Science* **72**, 159-168.

McEwan JC, Bisset SA, Morris CA (1997) The selection of sheep for natural resistance to internal parasites. In: Barrell GK (ed). *Sustainable Control of Internal Parasites in Ruminants - an Animal Industries Workshop.* pp 161–82. Lincoln University, Lincoln.

McEwan JC, Mason P, Baker RL & Clarke JN (1992) Effect of selection for production traits on internal parasite resistance in sheep. *Proceedings- New Zealand Society of Animal Production* **52**, 53.

Meeusen ENT (1999) Immunology of helminth infections, with special reference to immunopathology. *Veterinary Parasitology* **84**, 259-273.

Mercer JG, Mitchell PI, Moar KM, Bissett A, Geissler S, Bruce K & Chappell LH (2000) Anorexia in rats infected with the nematode, Nippostrongylus brasiliensis: experimental manipulations. *Parasitology* **120**, 641-648.

Miller JE, Bahirathan M, Lemarie SL, Hembry FG, Kearney MT & Barras SR (1998) Epidemiology of gastrointestinal nematode parasitism in Suffolk and Gulf Coast Native sheep with special emphasis on relative susceptibility to Haemonchus contortus infection. *Veterinary Parasitology* **74**, 55-74.

Minson J (1990) *Forage in Ruminant Nutrition.* San Diego, CA: Academic Press.

Montague CT, Farooqi IS, Whitehead JP, Soos MA, Rau H, Wareham NJ, Sewter CP, Digby JE, Mohammed SN, Hurst JA, Cheetham CH, Earley AR, Barnett AH, Prins JB & ORahilly S (1997) Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature* **387**, 903-908.

Morris CA, Bisset SA, Vlassoff A, Baker RL, Watson TG, Leathwick DM & Wheeler M (1997) Correlated responses in fleece weight to selection for divergence in faecal nematode egg count in New Zealand Romneys and Perendales. *Proceedings of the New Zealand Society of Animal Production*, **57**, 26-28.

Morris CA, Clarke JN, Watson TG, Wrigglesworth AL & Dobbie JL (1996) Faecal egg count and food intake comparisons of Romney singletrait selection and control lines. *New Zealand Journal of Agricultural Research*, **39**, 371-378.

Morris CA, Vlassoff A, Bisset SA, Baker RL, Watson TG, West CJ & Wheeler M (2000) Continued selection of Romney sheep for resistance or susceptibility to nematode infection: estimates of direct and correlated responses. *Animal Science*, **70**, 17-27.

Morrison CD, Daniel JA, Holmberg BJ, Djiane J, Raver N, Gertler A & Keisler DH (2001) Central infusion of leptin into well-fed and undernourished ewe lambs: effects on feed intake and serum concentrations of growth hormone and luteinizing hormone. *Journal of Endocrinology* **168**, 317-324.

Moshyedi AK, Josephs MD, Abdalla EK, Mackay SLD, Edwards CK, Copeland EM & Moldawer LL (1998) Increased leptin expression in mice with bacterial peritonitis is partially regulated by tumor necrosis factor alpha. *Infection and Immunity* **66**, 1800-1802.

Murray MJ & Murray AB (1979) Anorexia of infection as a mechanism of host defense. *American Journal of Clinical Nutrition*, **32**, 593–596.

Niswender KD, Morton GJ, Stearns WH, Rhodes CJ, Myers MG & Schwartz MW (2001) Intracellular signalling - Key enzyme in leptin-induced anorexia. *Nature* **413**, 794-795.

Paynter DI (1992) Pepsinogen Activity; Determination in Serum and Plasma. Australian Standard Diagnostic tehniques. Standing Committee of Agriculture. Australia: CSIRO.

Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T & Collins F (1995) Effects of the Obese Gene-Product on Body-Weight Regulation in Ob/Ob Mice. *Science* **269**, 540-543.

Pernthaner A, Cole SA, Morrison L, Green R, Shaw RJ & Hein WR (2006) Cytokine and antibody subclass responses in the intestinal lymph of sheep during repeated experimental infections with the nematode parasite Trichostrongylus colubriformis. *Veterinary Immunology and Immunopathology* **114**, 135-148.

Pernthaner A, Cole SA, Morrison L & Hein WR (2005) Increased expression of interleukin-5 (IL-5), IL-13, and tumor necrosis factor alpha genes in intestinal lymph cells of sheep selected for enhanced resistance to nematodes during infection with Trichostrongylus colubriformis. *Infection and Immunity* **73**, 2175-2183.

Plata-Salaman CR (1998) Cytokines and anorexia: A brief overview. *Seminars in Oncology* **25**, 64-72.

Plata-Salaman CR (2004) Leptin and anorexia in renal insufficiency. *Nephron Clinical Practice* **97**, 73-75.

Poppi DP, MacRae JC, Brewer A, & Coop R.L (1986) Nitrogen transactions in the digestive tract of lambs exposed to the intestinal parasite *Trichostrongylus colubriformis. British Journal of Nutrition*, **55**, 593-602.

Rauw WM, Kanis E, Noordhuizen-Stassen EN & Grommers FJ (1998) Undesirable side effects of selection for high production efficiency in farm animals: a review. *Livestock Production Science* **56**, 15-33.

Ren MQ, Wegner J, Bellmann O, Brockmann GA, Schneider F, Teuscher F & Ender K (2002) Comparing mRNA levels of genes encoding leptin, leptin receptor, and lipoprotein lipase between dairy and beef cattle. *Domestic Animal Endocrinology* **23**, 371-381.

Roberts HC, Hardie LJ, Chappell LH & Mercer JG (1999) Parasite-induced anorexia: leptin, insulin and corticosterone responses to infection with the nematode, Nippostrongylus brasiliensis. *Parasitology* **118**, 117-123.

Robinson JJ, Foster WH & Forbes TJ (1969) Estimation of Milk Yield of a Ewe from Body Weight Data on Suckling Lamb. *Journal of Agricultural Science* **72**, 103-&.

Russel AJF, Doney JM & Gunn RG (1969) Subjective Assessment of Body Fat in Live Sheep. *Journal of Agricultural Science* **72**, 451-

Sandberg FB, Emmans GC & Kyriazakis I (2006) A model for predicting feed intake of growing animals during exposure to pathogens. *Journal of Animal Science* **84**, 1552-1566.

Sarraf P, Frederich RC, Turner EM, Ma G, Jaskowiak NT, Rivet DJ, Flier JS, Lowell BB, Fraker DL & Alexander HR (1997) Multiple cytokines and acute inflammation raise mouse leptin levels: potential role in inflammatory anorexia. *Journal of Experimental Medicine* **185**, 171-175.

Seaton DS, Jackson F, Smith WD & Angus KW (1989) Development of Immunity to Incoming Radiolabeled Larvae in Lambs Continuously Infected with Ostertagia-Circumcincta. *Research in Veterinary Science* **46**, 241-246.

Simcock DC, Joblin KN, Scott I, Burgess DM, Rogers CW, Pomroy WE & Simpson HV (1999) Hypergastrinaemia, abomasal bacterial population densities and pH in sheep infected with Ostertagia circumcincta. *International Journal for Parasitology* **29**, 1053-1063.

Smith RM, Hocking Edwards JE, Dynes RA., (1996) Protected protein reduces the faecal egg count of weaners exposed to gastrointestinal parasites. *Proceedings of the Australian Society of Animal Production*, **21**, 386

Smith WD, Jackson F, Jackson E & Williams J (1985) Age Immunity to Ostertagia-Circumcincta - Comparison of the Local Immune-Responses of 4-1/2-Month-Old and 10-Month-Old Lambs. *Journal of Comparative Pathology* **95**, 235-245.

Smith WD, Jackson F, Jackson E, & Williams J (1983) Local immunity and *Ostertagia circumcincta*: Changes in the lymph node of immune sheep after a challenge infection. *Journal of Comparative Pathology*, **93**, 479-488.

Smith WD, Jackson F, Graham R., Jackson E, & Williams, J. (1987). Mucosal IgA production and lymph cell traffic following prolonged low level infections of *Ostertagia circumcincta*. *Research in Veterinary Science*, **43**, 320-326.

Soliman M, Abdelhady S, Fattouh I, Ishioka K, Kitamura H, Kimura K & Saito M (2001) No alteration in serum leptin levels during acute endotoxemia in sheep (NOTE). *Journal of Veterinary Medical Science* **63**, 1143-1145.

Soliman M, Ishioka K, Kimura K, Kushibiki S & Saito M (2002) Plasma leptin responses to lipopolysaccharide and tumor necrosis factor alpha in cows. *Japanese Journal of Veterinary Research* **50**, 107-114.

Spurlock ME (1997) Regulation of metabolism and growth during immune challenge: An overview of cytokine function. *Journal of Animal Science* **75**, 1773-1783.

Stankiewicz M, Pernthaner A, Cabaj W, Jonas WE, Douch PGC, Bisset SA, Rabel B, Pfeffer A & Green RS (1995) Immunization of Sheep against Parasitic Nematodes Leads to Elevated Levels of Globule Leukocytes in the Small-Intestine Lumen. *International Journal for Parasitology* **25**, 389-394.

Stear MJ, Bishop SC, Duncan JL, Mckellar QA & Murray M (1995) The Repeatability of Fecal Egg Counts, Peripheral Eosinophil Counts, and Plasma Pepsinogen Concentrations during Deliberate Infections with Ostertagia-Circumcincta. *International Journal for Parasitology* **25**, 375-380.

Stear MJ, Strain S & Bishop SC (1999) Mechanisms underlying resistance to nematode infection. *International Journal for Parasitology* **29**, 51-56.

Steel JW, Jones WO & Symons LEA (1982) Effects of a Concurrent Infection of Trichostrongylus-Colubriformis on the Productivity and Physiological and Metabolic Responses of Lambs Infected with Ostertagia-Circumcincta. *Australian Journal of Agricultural Research* **33**, 131-140.

Steel JW, Symons LEA & Jones WO (1980) Effects of Level of Larval Intake on the Productivity and Physiological and Metabolic Responses of Lambs Infected with Trichostrongylus-Colubriformis. *Australian Journal of Agricultural Research* **31**, 821-838.

Strain SAJ & Stear MJ (2001) The influence of protein supplementation on the immune response to Haemonchus contortus. *Parasite Immunology* **23**, 527-531.

Sykes AR (1991) Nutrition and parasites. *Recent Advances in Herbivore Nutrition*, **29**, 283-290.

Sykes AR (1982) Nutritional and Physiological Aspects of Helminthiasis in Sheep. In *Biology and Control of Endoparasites: McMaster Laboratory 50th Anniversary Symposium in Parasitology* [LEA Symons, AD Donald and JK Dineen, editors]. New York: Academic Press Inc.

Sykes, A.R., & Coop, R.L. (1976). Intake and utilisation of food by growing lambs with parasitic damage to the small intestine caused by daily dosing with *Trichostrongylus colubriformis* larvae. *Journal of Agricultural Science, Cambridge,* 86, 507-515.

Sykes AR & Coop RL (1977) Intake and Utilization of Food by Growing Sheep with Abomasal Damage Caused by Daily Dosing with Ostertagia-Circumcincta Larvae. *Journal of Agricultural Science* **88**, 671-677.

Sykes AR, & Coop RL (2001) Interaction between nutrition and gastrointestinal parasitism in sheep. *New Zealand Veterinary Journal*, **49**, 222-226.

Sykes AR & Field AC (1972) Effects of Dietary Deficiencies of Energy, Protein and Calcium on Pregnant Ewe .1. Body Composition and Mineral Content of Ewes. *Journal of Agricultural Science* **78**, 109-117.

Sykes AR, Xie HL, Stankiewicz M, Huntley JF, Mackellar A, Sedcole JR, McAnulty RW & Green R (2007) The effect of vaccinating infection during pregnancy and dietary protein supply on the peri-parturient immune response of sheep to infection with Teladorsagia circumcincta and Trichostrongylus colubriformis larvae. *Animal* **1**, 249-260.

Symons LEA, (1985) Anorexia; occurrence, pathophysiology, and possible causes in parasitic infection. *Advances in Parasitology.* **24**, 103–133.

Symons LEA, Steel JW & Jones WO (1981) Effects of Level of Larval Intake on the Productivity and Physiological and Metabolic Responses of Lambs Infected with Ostertagia-Circumcincta. *Australian Journal of Agricultural Research* **32**, 139-148.

Tang-Christensen M, Havel PJ, Jacobs RR, Larsen PJ & Cameron J (1999) Central administration of leptin inhibits food intake and activates the sympathetic nervous system in rhesus macaques. *Journal of Clinical Endocrinology and Metabolism* **84**, 711-717.

Thatcher EF, Gershwin LJ & Baker NF (1989) Levels of Serum Ige in Response to Gastrointestinal Nematodes in Cattle. *Veterinary Parasitology* **32**, 153-161.

Thomas L, Wallace JM, Aitken RP, Mercer JG, Trayhurn P & Hoggard N (2001) Circulating leptin during ovine pregnancy in relation to maternal nutrition, body composition and pregnancy outcome. *Journal of Endocrinology* **169**, 465-476.

Thomas RJ & Ali DA (1983) The Effect of Haemonchus-Contortus Infection on the Pregnant and Lactating Ewe. *International Journal for Parasitology* **13**, 393-398.

Tokuda T, Delavaud C & Chilliard Y (2003) Plasma leptin concentration in pre- and post-weaning lambs. *Animal Science* **76**, 221-227.

Tolkamp BJ, Emmans GC & Kyriazakis I (2006) Body fatness affects feed intake of sheep at a given body weight. *Journal of Animal Science* **84**, 1778-1789.

Tolkamp BJ, Yearsley JM, Gordon IJ, Illius AW, Speakman JR & Kyriazakis I (2007) Predicting the effects of body fatness on food intake and performance of sheep. *British Journal of Nutrition* **97**, 1206-1215.

Urquhart GM, Armour J, Duncan JL, Dunn AM & Jennings FW (1996) Veterinary Parasitology (2nd ed.), Blackwell Science Ltd., Oxford, England.

Valderrabano J, Gomez-Rincon C & Uriarte J (2006) Effect of nutritional status and fat reserves on the periparturient immune response to Haemonchus contortus infection in sheep. *Veterinary Parasitology* **141**, 122-131.

Valderrabano J & Uriarte J (2003) Effect of nutrition in early pregnancy on the periparturient relaxation of immunity to gastro-intestinal parasitism in prolific ewes. *Animal Science* **76**, 481-489.

Van Dam JTP, vanderHeide D, vanderHel W, vandenIngh TSGAM, Verstegen MWA, Wensing T & Zwart D (1996) The effect of Trypanosoma vivax infection on energy and nitrogen metabolism and serum metabolites and hormones in west African dwarf goats on different food intake levels. *Animal Science* **63**, 111-121.

Van Houtert MFJ & Sykes AR (1996) Implications of nutrition for the ability of ruminants to withstand gastrointestinal nematode infections. *International Journal for Parasitology* **26**, 1151-1168.

Van Houtert MFJ, Barger IA, Steel JW, Windon RG & Emery DL (1995) Effects of Dietary-Protein Intake on Responses of Young Sheep to Infection with Trichostrongylus-Colubriformis. *Veterinary Parasitology* **56**, 163-180.

Van Soest PJ (1988) *Nutritional Ecology of the Ruminant:* Ruminant Metabolism, Nutritional Strategies, the Cellulolytic Fermentation and the Chemistry of Forages and Plant Fibres. Oregon, USA: O & B Books.

Wakelin D, (1996) "Immunity to parasites: How parasitic infections are controlled". Cambridge University Press, Cambridge.

Wallace DS, Bairden K, Duncan JL, Fishwick G, Gill M, Holmes PH, Mckellar QA, Murray M, Parkins JJ & Stear MJ (1995) Influence of Supplementation with Dietary Soybean-Meal on Resistance to Hemonchosis in Hampshire down Lambs. *Research in Veterinary Science* **58**, 232-237.

Watson, T.G., Hosking, B.C., Hurford, A.P., & Mather, B.C. (1992). Developments in breeding Perendale sheep for resistance or susceptibility to internal nematode parasites. *Proceedings of the New Zealand Society of Animal Production*, 52, 61-64.

Williamson JF, Blair HT, Garrick DJ, Pomroy WE, Dough PGC, Green RS & Simpson HV (1995) Parasitism and Production in Fleece-Weight-Selected and Control Sheep. *New Zealand Journal of Agricultural Research* **38**, 381-387.

Wilson WD, & Field AC (1983) Absorption and secretion of calcium and phosphorus in the alimentary tract of lambs infected with daily doses of *Trichostrongylus colubriformis* or *Ostertagia circumcincta* larvae. *Journal of Comparative Pathology*, **93**, 61-71.

Wing E & Kelley V (1982) Loss of Bacterial-Resistance in Mrl Autoimmune Mice - Protection by Prostaglandin-E. *Federation Proceedings* **41**, 938-938.

Woolaston RR & Baker RL (1996) Prospects of breeding small ruminants for resistance to internal parasites. *International Journal for Parasitology* **26**, 845-855.

Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. 1994 Positional cloning of the mouse ob gene and its human homologue. Nature. **372**:425– 432.

List of publications

Refereed publications

Zaralis, K., Tolkamp, B.J., Houdijk, J.G.M., Wylie, A.R.G., Kyriazakis, I. (2008) Changes in food intake and circulating leptin due to gastrointestinal parasitism in lambs of two breeds. *Journal of Animal Science*, **86**: 1891-1903

Zaralis, K., Tolkamp, B.J., Houdijk, J.G.M., Wylie, A.R.G., Kyriazakis, I. (2008) Consequences of protein supplementation for anorexia, expression of immunity and plasma leptin concentrations in parasitized ewes of two breeds. *British Journal of Nutrition*, doi:10.1017/S000711450802401X **(in press)**

Zaralis, K., Tolkamp, B. J., Houdijk, J. G. M. and Kyriazakis, I. (2007). Protein supplementation consequences on anorexia and expression of immunity of parasitized ewes of two breeds. In: *12th Seminar on Sheep and Goat Nutrition: Nutritional and foraging ecology of sheep and goats*. Thessaloniki, Greece **(in press)**

Zaralis, K., Tolkamp, B.J., Houdijk, J.G.M., Wylie, A.R.G., Kyriazakis, I. (2007). Effects of nematode infection on anorexia and leptin levels in lambs of two breeds. In: *Journal of Animal and Feed Sciences, 16, Suppl. 2, 405 – 410. (Proceedings of the VII International Symposium of the Nutrition of Herbivores, Beijing, China)*

Conference papers

Zaralis, K., Tolkamp, B.J., Houdijk, J.G.M., Wylie, A.R.G., Kyriazakis, I. (2006). Effects of parasitic infection on anorexia and leptin levels in lambs of two different breeds. Proceedings- Nutrition Society of London 65, 73A.

Zaralis, K., Tolkamp, B.J., Wylie, A.R.G., Houdijk, J.G.M., Kyriazakis, I. (2007). Effects of secondary nematode infection on anorexia and leptin levels in growing lambs of two different breeds. Proceedings- British Society of Animal Science, 091.

Zaralis, K., Tolkamp, Houdijk, J.G.M., Kyriazakis, I. (2007). Effects of protein supplementation on anorexia and expression of immunity in parasitised periparturient ewes of two breeds. Proceedings- British Society of Animal Science, 094.