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Modelling Nematode Infections in Sheep and Parasite Control Strategies

Yan Christian Stephen Mountfort Laurenson

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Declaration of originality

I hereby declare that the research described in this thesis and the thesis itself was composed and originated entirely by myself, except where otherwise stated.

Yan Christian Stephen Mountfort Laurenson

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List of Publications

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Conferences:

Laurenson Y.C.S.M., Kyriazakis, I., and Bishop, S.C. 2012. To graze together or to graze apart: the epidemiological consequences of host resistance to *Teladorsagia circumcincta* in lambs. *Proceedings of the British Society of Animal Science and the Association of Veterinary Teaching and Research Work*. Nottingham, UK. ID#166

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Laurenson Y.C.S.M., Bishop, S.C., and Kyriazakis, I. 2011. The epidemiological consequences of resistance to *Teladorsagia circumcincta* in grazing sheep as assessed using a mathematical model. *Proceedings of the 23rd International Conference of the World Assocaiation for the Advancement of Veterinary Parasitology*. Buenos Aires, Argentina. p16

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Abstract

Gastrointestinal parasitism in grazing lambs adversely affects animal performance and welfare, causing significant production losses for the sheep industry. Control of gastrointestinal parasitism using chemotherapeutic treatment is under threat due to the emergence of anthelmintic resistance, thus stimulating research into alternative control strategies. Whilst investigating control strategies experimentally can be costly and time consuming, using a mathematical modelling approach can reduce such constraints. A previously developed model which describes the impact of host nutrition, genotype and gastrointestinal parasitism in a growing lamb, provided an appropriate starting point to explore control strategies and their impact on host-parasite interactions.

Two contrasting mechanisms have previously been proposed to account for the occurrence of anorexia during parasitism. These were reductions in either intrinsic growth rate or relative food intake. Thus, the existing individual lamb model was modified to evaluate these mechanisms by exploring the relationship between anorexia and food composition (**Chapter 2**). For foods that did not constrain food intake, published data was found to be consistent with the predictions that arose from anorexia being modelled as a reduction in relative food intake.

Reported genetic parameter estimates for resistance and performance traits appear to vary under differing production environments. In order to explore the impact of epidemiological effects and anthelmintic input on genetic parameter estimates the model was extended to simulate a population of lambs in a grazing scenario (**Chapter 3**). Whilst estimates of heritabilities and genetic correlations for drenched lambs remained constant, for lambs given no anthelmintic treatment, the heritability of empty body weight (EBW) reduced and the genetic correlation between faecal egg count (FEC) and EBW became increasingly negative with increasing exposure to infective larvae. Thus differences in anthelmintic input and pasture larval contamination (PC) may provide plausible causes for the variation in

genetic parameter estimates previously reported.

To investigate the interactions between host resistance and epidemiology (**Chapter 4**) a population of 10,000 lambs were simulated and FEC predictions used to assign the 1,000 lambs with the highest and lowest predicted FEC to 'susceptible' (S) and 'resistant' (R) groups, respectively. R and S groups were then simulated to graze separate pastures over 3 grazing seasons. The average FEC and PC predictions of these groups diverged during the first 2 grazing seasons and stabilised during the third, such that the difference in FEC predictions between R and S groups were double those predicted when grazed with the population. This was found to be consistent with experimental data. Further, anthelmintic treatment and grazing strategies were predicted to have no impact on the EBW of resistant lambs, suggesting that control strategies should be targeted towards susceptible animals.

Targeted selective anthelmintic treatment (TST) has been proposed to reduce risks of anthelmintic resistance with minimal impacts on performance. To describe the short- and long-term impacts of TST and drenching frequency on sheep production and the emergence of anthelmintic resistance, the model was extended to include a description of anthelmintic resistance genotypes within the nematode population (**Chapter 5**). Reducing the proportion of treated animals was predicted to increase the duration of anthelmintic efficacy, whilst reducing the drenching frequency increased the long-term benefits of anthelmintic on sheep production. Various determinant criteria for use in TST regimes were compared (**Chapter 5**) including performance traits such as live weight and growth rate, and parasitological traits such as FEC. Using FEC as the TST criterion was predicted to allow the greatest reduction in the number of anthelmintic treatments administered whilst maintaining the highest average EBW, whilst live weight and growth rate were predicted to give little to no improvement in comparison to selecting animals at random for TST. Using estimated breeding values (EBVs) for FEC as the determinant criterion for TST regimes was compared to using measured FEC (**Chapter 6**). The EBV for true FEC across the entire growth period, akin to perfect genomic selection, was predicted to be a better criterion than measured time-specific FEC (including a sampling error) for a TST regime. EBVs calculated using measured

time-specific FEC showed little benefit compared to measured FEC.

The information gained from these simulation studies increases our understanding of control strategies and their impact on host-parasite interactions under various scenarios that may not have been possible using experimental methods. It is important to remember that the aim of alternative or complimentary control strategies is to maintain the sustainability of sheep production systems, and as such the production gain of any control strategy needs to be weighed against the financial, labour and time costs involved in implementation.

Chapter One

General Introduction

1.1 Overview

Gastrointestinal parasitism is one of the most pervasive challenges to the health and welfare of ruminants, and causes significant production losses for the sheep industry (Larsen *et al.*, 1995). The economic cost of gastrointestinal parasitism of grazing sheep in the UK has previously been estimated at about £84 million per year (Nieuwhof and Bishop, 2005). Effective control of nematode infections currently relies heavily on the use of anthelmintic drugs (Barger, 1997; Sargison, 2012). However, the extensive use of anthelmintics (Hein and Harrison, 2005), often in combination with poor management practices (Wolstenholme *et al.*, 2004; Leathwick *et al.*, 2009; Waghorn *et al.*, 2009), has led to the emergence of anthelmintic resistance in nematode populations. Consequently, anthelmintic resistance is now a major problem in small ruminant nematodes in several parts of the world (Jackson and Coop, 2000; Bartley *et al.*, 2003; Kaplan, 2004; Wolstenholme *et al.*, 2004), with resistance being recorded throughout Europe (Papadopoulos *et al.*, 2012). Thus, reduced efficacy due to the evolution of drug resistance threatens the sustainability of livestock systems (Waller, 2006a; Besier, 2007; Papadopoulos, 2008), and has stimulated the search for alternative control strategies.

1.2 The impact of gastrointestinal parasitism on sheep

Whilst high levels of gastrointestinal parasitism may result in clinical symptoms and death of the host (Reid *et al.*, 1970; Horak, 1971; Saad *et al.*, 1984; Gulland, 1992), infection more commonly leads to sub-clinical disease causing significant production losses for the sheep industry in the UK (Nieuwhof and Bishop, 2005). Within the UK, the most numerous sheep breed is the Scottish Blackface (National Sheep

Association, 1998), and the most abundant parasite is the nematode *Teladorsagia circumcincta* (Stear *et al.*, 2007). *T. circumcincta* infection in sheep has previously been shown to depress food intake (Coop *et al.*, 1982; Greer *et al.*, 2008) and food digestibility (Coop and Holmes, 1996), impair the efficiency of nutrient utilisation (Coop *et al.*, 1985), cause protein loss via damage to the epithelium of the gastrointestinal tract (Yakoob *et al.*, 1983; Holmes, 1987; Houdijk *et al.*, 2001), and reduce growth rate in lambs (Thompson and Callinan, 1981; Coop *et al.*, 1982; Coop *et al.*, 1985). Further, these impacts have previously been associated with the severity of *T. circumcincta* infection, with increasing parasitic burdens leading to greater reductions in food intake and growth rate (Coop *et al.*, 1982; 1985).

The reduction in food intake, also known as parasite-induced anorexia, may present up to a 20% reduction in the food intake of parasitised animals, compared with their non-infected counterparts (Coop *et al.*, 1982; Sandberg *et al.*, 2006; Greer *et al.*, 2008), and accounts for much of the observed reduction in weight gain (Kyriazakis *et al.*, 1998). This phenomenon appears to be paradoxical, as animals reduce food intake during a period of protein deficiency (Kyriazakis *et al.*, 1998). However, parasite-induced anorexia may have a functional purpose in reducing further infection (Kyriazakis *et al.*, 1998) and minimising food intake to facilitate the repair of the gastrointestinal tract (Stear *et al.*, 2003).

Infection with *T. circumcincta* results in considerable damage to the abomasal mucosa (McKellar, 1993), causing breaches in the epithelium, an increase in mucosal pH and mucus production (Playford, 1995), and protein loss via plasma leakage (Yakoob *et al.*, 1983; Houdijk *et al.*, 2001). Thus, damage to the mucosal architecture may decrease food digestibility (Coop and Holmes, 1996). Further, the combination of protein loss, reduced food digestibility and the investment of protein in the repair of gastrointestinal damage and immune responses may be considered to lead to the reductions in efficiency of food utilisation (protein, fat and mineral deposition) and growth rate (Coop *et al.*, 1982; 1985).

1.3 Host immune response to gastrointestinal parasitism

Persistent ingestion of *T. circumcincta* larvae presents nematode antigens to the host eliciting a T helper 2 cell (Th2) immune response. Th2 lymphocytes secrete cytokines (IL-4, IL-5, IL-9, IL-10 and IL-13) which moderate eosinophilia (Korenaga and Tada, 1994; Miller, 1996), mucosal mastocytosis (Miller, 1996; Stear *et al.*, 1995) and immunoglobulin responses (Huntley *et al.*, 1998; Smith *et al.*, 1983). This immune response has previously been shown to reduce the establishment of infective larvae, increase nematode mortality, retard the growth of adult nematodes and reduce fecundity (Smith *et al.*, 1985; Miller, 1996; Stear *et al.*, 1999; Stear *et al.*, 2004). Thus, sheep mount a specific immunological response in order to expel nematodes and reduce the impacts of parasitism described above. However, the level of immune response to *T. circumcincta* infection has previously been shown to vary across host age, with greater immune responses being observed in older sheep (Smith *et al.*, 1985). This suggests that resistance to nematodes is an acquired immune function, where sheep are born naïve and immunity develops with increasing exposure to nematodes (Stear *et al.*, 1999). Further, between-animal variation in the rate of immune acquisition and hence observed resistance has previously been reported in growing lambs (Bishop *et al.*, 1996).

Whilst immune responses may present as a means of controlling the nematode burden of a host thereby reducing the impacts of parasitism, components of the activation of the immune response (e.g. cytokines) may also be the cause of the reduction in food intake observed during gastrointestinal parasitism (Langhans, 2000; Plata-Salaman, 2001). This causal hypothesis is supported by experimental observations (Greer *et al.*, 2008).

1.4 Alternative control strategies

Various strategies have previously been proposed as an alternative to using anthelmintics for the control of gastrointestinal parasites (Waller, 2003; Sayers and Sweeney, 2005). Such control measures include nutrient supplementation (Coop and Kyriazakis, 2001; Houdijk *et al.*, 2005), selective breeding for host resistance

(Bishop and Stear, 1997; Stear *et al.*, 2001), grazing management (Githigia *et al.*, 2001; Niven *et al.*, 2002) and targeted selective treatment (Kenyon *et al.*, 2009). The purpose of these strategies is to reduce the reliance on anthelmintic drugs by minimising the number of treatments required for effective control of parasitism (Larsen *et al.*, 2006), which should therefore also reduce the rate of development for resistance to anthelmintics (Prichard *et al.*, 1980).

1.4.1 Nutritional control

Protein supplementation has previously been suggested as a means of maintaining production and enhancing the immune response (Kyriazakis *et al.*, 1996a; Houdijk *et al.*, 2005; Greer *et al.*, 2009). Supplying supplementary protein during a period of protein deficiency may reduce the deleterious impacts of parasitism (MacRae, 1993) and provide the host with sufficient protein to allow a maximal rate of acquisition of immunity (Coop and Kyriazakis, 1999). Greater control of parasitism by the host, combined with reduced impacts on host performance, thereby reduce the need for anthelmintic treatment. Further, the provision of food which supplies sufficient nutrients to fulfil the host requirements for growth and immune response is a control strategy that can be used in combination with other control methods without negating any further benefits that may be derived from additional control strategies.

1.4.2 Selective breeding for host resistance

Breeding for host resistance to nematodes has become an increasingly attractive alternative control strategy (Woolaston and Baker, 1996; Bishop *et al.*, 2011), supported by evidence of heritable variation for faecal egg count (FEC (Bishop and Morris, 2007)) and results from selection in practice (Kemper *et al.*, 2010). Improvements in the overall flock immunity to gastrointestinal parasitism may be expected to reduce the need for anthelmintic treatment due to the greater host control of parasitic burdens. However, commercial sheep breeding programmes are likely to also include production trait goals within their selection objectives. Hence, in order to design appropriate breeding strategies, genetic parameter estimates are required for host resistance and production traits including the heritability of individual traits

and the genetic correlation between traits. Whilst the reported heritabilities for FEC and body weight (BW) are relatively consistent (Safari and Fogarty, 2003; Safari *et al.*, 2005; Bishop and Morris, 2007), estimates of genetic correlations between these traits are variable, ranging from -0.8 (Bishop *et al.*, 1996) to +0.4 (McEwan *et al.*, 1992; 1995). Variation observed in such correlations may be due to interactions between host resistance genotype, the intensity of infection and anthelmintic drenching practices (Coop and Kyriazakis, 1999). According to a previous study (Doeschl-Wilson *et al.*, 2008), variation in observed correlations could also be due to interaction between host genotypes and nutrition.

1.4.3 Grazing management

Reductions in FEC derived from breeding for host resistance may have a beneficial impact on the larval contamination of pasture and hence host exposure to infective larvae. Thus, the combination of selection for resistance and other control measures, such as grazing management (Githigia *et al.*, 2001; Niven *et al.*, 2002), may provide further complementary benefits and lead to reduced anthelmintic use (Coop and Kyriazakis, 2001). However, predicting the benefits of selection for host resistance in grazing ruminants can be difficult due to complex interactions between parasite epidemiology and host resistance to nematode infections (Bishop and Stear, 1997). Therefore, a greater understanding of these interactions is required before implementing grazing management strategies aimed at controlling nematode populations by exploiting the benefits of selective breeding.

1.4.4 Targeted selective treatment

Maintaining a proportion of the nematode population *in refugia* (unexposed to anthelmintic) preserves susceptible parasite genotypes, thus slowing the development of anthelmintic resistance (Michel, 1985; van Wyk, 2001). Targeted selective treatment (TST) provides a control strategy capable of increasing the nematode population *in refugia* by selective treatment of only those animals that will benefit most from treatment whilst leaving the rest of the flock untreated (van Wyk *et al.*, 2006; Kenyon *et al.*, 2009), thus exploiting between-animal variation in immune

responses. Hence, the implementation of a TST strategy requires determinant criteria for the identification of animals susceptible to parasitism. Such determinant traits include FEC as an indicator of host resistance (Cringoli *et al.*, 2009; Gallidis *et al.*, 2009), and performance traits such as live weight (Leathwick *et al.*, 2006a; b) and weight gain (Waghorn *et al.*, 2008; Stafford *et al.*, 2009; Gaba *et al.*, 2010) as indicators of the ability of the host to cope with the deleterious impacts of parasitism (resilience). However, no comparison of these determinant criteria has currently been made to ascertain which trait allows for the greatest reduction in the number of anthelmintic treatments administered whilst maintaining effective parasite control.

Previously, field studies used to investigate TST regimes have focussed on the short-term impact upon flock performance (Kenyon and Jackson, 2012), whilst epidemiology based simulation studies have been used to provide insights into the long-term impact of drenching frequency on the emergence of anthelmintic resistance (Barnes *et al.*, 1995; Leathwick *et al.*, 1995). However, the differing focus of such studies makes it difficult to compare the impacts of such control strategies on flock performance and the emergence of anthelmintic resistance.

1.5 Identification of resistant and resilient animals

Both selective breeding programs and TST regimes require the ability to identify resistant and resilient animals (Bisset and Morris, 1996; Stear *et al.*, 2001). Traditionally this has been achieved by using different phenotypic traits (Hunt *et al.*, 2008) as described above. Such traits require frequent measurements that may be costly and labour intensive and which may be prone to sampling errors and environmental variation. However, it is now possible to obtain an estimated breeding value (EBV) of individual animals based upon FEC data (Morris *et al.*, 1998; Bisset *et al.*, 2001; Woolaston and Windon, 2001). Further, with DNA tests now available for supposed resistance to parasites (http://www.pfizeranimalgenetics.co.uk/default.aspx) and promising advances in quantitative trait loci mapping and whole genome selection (Hunt *et al.*, 2008), in the near future it may be possible to identify animals for selective breeding programs and TST regimes using a genomic approach.

1.6 Thesis aims

Experimental investigations assessing the impact of alternative control strategies, either separately or in combination, can be costly and time consuming. Further, monitoring complex systems involving numerous interactions between the host and parasite populations, environment, grazing management and parasite control strategies may be technically difficult (Barnes *et al.*, 1995). However, using a mathematical modelling approach may provide a way to explore such interactions whilst considerably reducing the financial and diuternal costs associated with experimental investigation. Thus, a previously developed model (Vagenas *et al.*, 2007a) that describes the impact of host nutrition, genotype and gastrointestinal parasitism in a growing lamb, provided an appropriate starting point to explore control strategies and their impact on host-parasite interactions.

The overall aim of this thesis was to use a mathematical modelling approach to investigate the relationship between host-parasite interactions and parasite control strategies, identify potential causes of variation in the reported results from experimental studies, and provide a description of the potential impact of control strategies on epidemiological, parasitological and performance traits for a population of grazing lambs. The specific objectives of the thesis were:

1. To evaluate the model of Vagenas *et al.* (2007a) to determine its appropriateness for use in the exploration of host parasite interactions and parasite control strategies (the subsequent objectives). In addition to updating the model and refining its parameterisation, a specific focus was directed towards the differing hypotheses proposed to explain the occurrence of parasite-induced anorexia as described above, and the impact of the nutritional composition of food on parasitological and performance traits (**Chapter 2**).

2. Variation in reported genetic correlations between FEC and BW may be due to interactions between host resistance genotype, the intensity of infection and anthelmintic drenching practices (Coop and Kyriazakis, 1999).

Consequently, the mathematical model was used to investigate the impact of the level of exposure to *T. circumcincta* larvae and anthelmintic treatment on genetic parameter estimates for performance and resistance traits in sheep (**Chapter 3**).

3. Predicting the benefits of selection for host resistance in grazing ruminants can be difficult due to complex interactions between parasite epidemiology and host resistance to nematode infections (Bishop and Stear, 1997). Further, selection for resistance may be combined with grazing management to provide further complementary benefits and lead to reduced anthelmintic use (Coop and Kyriazakis, 2001). Thus, the mathematical model was used to quantify the interaction between host resistance genotype, control interventions and parasite epidemiology (**Chapter 4**).

4. Targeted selective treatment regimes require determinant criteria for the identification of animals susceptible to parasitism. Numerous determinant criteria have previously been proposed as indicators of both host resistance and resilience (Kenyon and Jackson, 2012); however, no comparison of the implications of using these determinant criteria have currently been carried out. Further, experimental studies investigating TST approaches have focussed on the short-term impacts upon flock performance, whilst simulation studies have focussed on the long-term impacts on the emergence of anthelmintic resistance. Hence, the mathematical model was used to make a comparison of traits previously proposed for use as determinant criteria in TST regimes and investigate the short- and long-term impacts of TST regimes and drenching frequency on both sheep performance and the emergence of anthelmintic resistance (**Chapter 5**).

5. Phenotypic traits previously proposed for use as determinant criteria within TST strategies are prone to environmental variation and sampling errors. Therefore, estimated breeding values for individual animals based upon FEC data (Woolaston and Windon, 2001), such as may be used in a selective breeding program, along with genetic markers for host resistance (Kemper *et al.*, 2011) may provide a better means of identifying susceptible animals for treatment. Thus the mathematical model was used to assess the potential

future use of EBVs for FEC and genomic selection to identify susceptible animals for treatment in a TST regime (**Chapter 6**).

The general discussion (**Chapter 7**) combines the findings of the simulation studies carried out here with the available literature on the impact of control strategies on host performance, parasite epidemiology and the emergence of anthelmintic resistance, in order to clarify our understanding of such interactions and identify further gaps in our knowledge. Further, it considers the potential of combining the various options available for parasite control into an integrated approach with the aim of reducing the need for anthelmintic treatment and thereby delaying the development of anthelmintic resistance and ensuring the long-term sustainability of livestock systems.

Chapter Two

Exploration of the mechanisms that underlie parasite-induced anorexia in sheep

2.1 Introduction

Gastrointestinal parasitism is one of the most pervasive challenges to the health and welfare of mammalian hosts. One of the main consequences of gastrointestinal parasitism is the occurrence of a reduction in voluntary food intake, henceforth called anorexia, which under sub-clinical infections accounts for up to a 20% reduction in the food intake of parasitised animals, compared with their non-infected counterparts (Coop *et al.*, 1982; Sandberg *et al.*, 2006; Greer *et al.*, 2009). Clinical helminth infections, however, may lead to a complete cessation of eating (Reid *et al.*, 1970; Horak, 1971; Saad *et al.*, 1984). Given the fact that gastrointestinal parasitism imposes nutritional penalties on the host (Sykes and Kyriazakis, 2007), anorexia and its subsequent consequences are the major contributors to the impacts of parasitism on host performance and overall fitness. The effects on host performance can be large; for example, the economic cost of gastrointestinal parasitism of grazing sheep in the UK was estimated at about £84 million per year (Nieuwhof and Bishop, 2005). In this chapter we deal with the issue of anorexia during gastrointestinal parasitism in sheep because of its apparent economic significance and the consequent attention the phenomenon has received in these hosts. We are of the view, however, that the principles we develop will be relevant to other parasite-mammalian host systems.

There are both functional and causal hypotheses to account for the occurrence of anorexia during parasitism in most mammalian hosts, including sheep. For example, Kyriazakis *et al.* (1998) have suggested that anorexia develops in order to allow hosts to cope with the exposure to pathogens. Langhans (2000) and Plata-Salaman (2001), on the other hand, have suggested that anorexia is a consequence of

the activation of the immune response by anorexinogenic cytokines, which are produced in response to infection. However, neither of these two groups of hypotheses is able to adequately account for or predict the extent of anorexia, nor do they provide a framework for predicting consequences on the food intake and performance of parasitised animals. Thus, this phenomenon continues to be paradoxical (Kyriazakis *et al.*, 1998).

There are at least two ways to account for the reduction in the food intake of growing animals during exposure to environmental and other stressors. Wellock *et al*. (2003) have suggested that the reduction in food intake is a consequence of a reduction in the intrinsic growth rate of the animals; the implication of this is that the reduction in food intake may be modelled as a consequence of a reduction in growth (mechanism 1). This mechanism was implemented by Vagenas *et al.* (2007a; b) when modelling host-parasite interactions in growing lambs, and implies that the animals' intrinsic growth capability changes, for example, in a manner akin to the gene expression alterations seen with foetal programming (Burdge *et al.*, 2007). Sandberg *et al.* (2006), on the other hand, have suggested that anorexia could be modelled as a direct reduction in the relative food intake of the parasitised animals (mechanism 2), which may be due to inappetence caused by immune response components such as cytokines. These authors have used this approach to model the time trend of food intake during the course of infection, mainly in pigs. A third approach has been proposed by Black *et al.* (1999) in which the above two mechanisms were used in equal proportion to account for the reduction in food intake. This approach is not considered any further in this chapter, due to it simply being a composite of the other two proposed mechanisms. To date, no attempt has been made to compare and consider the consequences of the above mechanisms on the extent of anorexia and its consequences on the performance of parasitised animals.

Further gaps exist in our understanding of the relationship between food composition and anorexia. For example, currently there is little known on whether and how food composition affects the characteristics of anorexia, such as its extent,

duration and impact on animal performance. A recent paper by Kyriazakis (2010) extensively reviewed the literature on this issue and concluded that there was a significant lack of experimental evidence that would allow us to reach unequivocal conclusions about this relationship, and use it to the advantage of parasitised hosts. One issue that was specifically identified by the review was the lack of evidence on the nature of parasite-induced anorexia on low-quality foods, irrespective of whether such foods were low in nitrogen or energy.

The aim of this chapter was to investigate, *in silico*, the consequences of the above two mechanisms on the food intake of parasitised sheep. Parasitism was through infection with *Teladorsagia circumcincta*, which is the most prevalent parasite of sheep in temperate climates (Stear *et al.*, 2007). We used the model of Vagenas *et al.* (2007a; b) as our starting point, as it is capable of accounting for the interaction between nutrition and gastrointestinal parasitism for growing and immunologically naïve sheep. Several modifications were made to the model, which are described below, to alter both the cause and the mechanism of impact of anorexia. To model the consequences of *T. circumcinta* infection, anorexia was parameterised as a function of immune responses, rather than the worm mass parameterisation used by Vagenas *et al.* (2007a). Further, the model was altered to allow for the investigation of the two proposed mechanisms by which anorexia leads to reduced food intake. The outcomes of this exercise were compared with existing data on the food intake of parasitised sheep in order to provide insights into the nature of anorexia during gastrointestinal parasitism in sheep and its likely impacts on host performance. In addition, we investigated the relationship between anorexia and food composition. Our hypothesis was that we would observe interactions between food composition and the mechanism of anorexia, in terms of observed anorexia, total food intake and level of parasitism. The outcomes of this exercise were expected to have heuristic value, giving insight into the design of future experiments that wish to address this issue.

2.2 Materials and Methods

2.2.1 Host-parasite interaction model

A previously developed model (Vagenas *et al.*, 2007a) that describes the impact of host nutrition, genotype and gastrointestinal parasitism in a growing lamb was modified and used to explore two mechanisms to account for parasite-induced anorexia. A schematic diagram describing the structure of the model is provided in Figure 2.1. A description of each component of the model is given below. Equations and relationships previously published are given in the corresponding sections of Appendix 1 (section 2.6), whereas modifications to the model remain in the main body of the text. First, the model for daily growth of an unchallenged animal is described for both non-limiting and nutritionally limiting conditions; the model is then extended to accommodate parasitic challenges, host immunity and host-parasite interactions, and to predict the growth of the lamb and its parasitic burden over time.
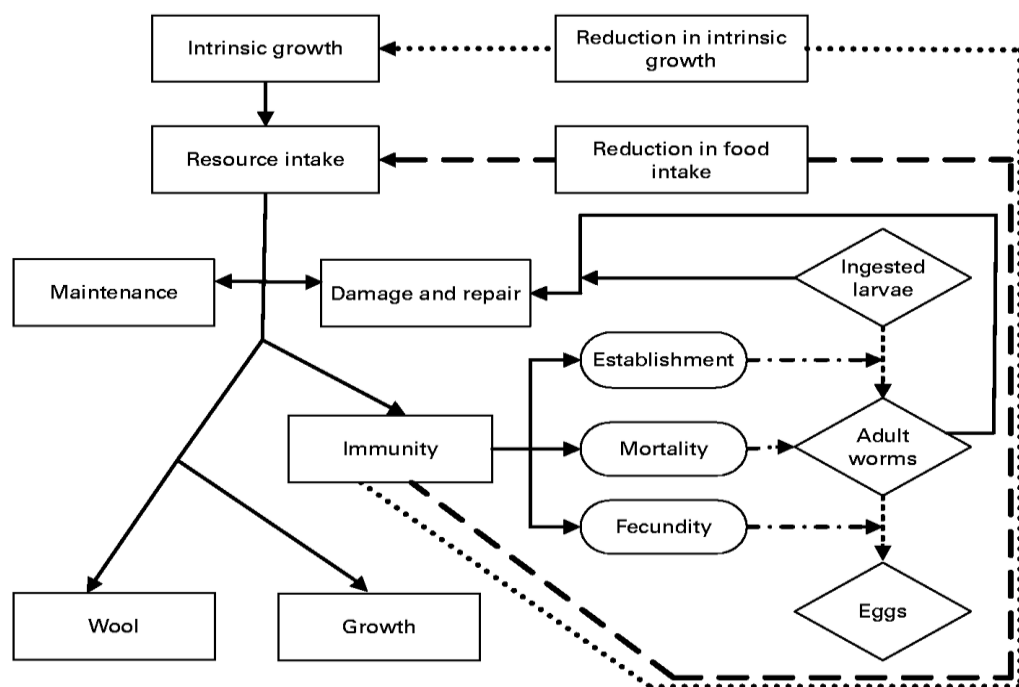
Figure 2.1. Schematic description of the host-parasite interactions and parasite-induced anorexia mechanisms (mechanism 1: intrinsic growth reduction mechanism (········); mechanism 2: food intake reduction mechanism (– – –)) in sheep infected with gastrointestinal nematodes. Rectangular boxes indicate the flow of food resources, rounded boxes indicate host-parasite interactions and diamond boxes indicate key quantifiable parasite lifecycle stages.

2.2.2 The parasite-free animal

2.2.2.1 Intrinsic growth model

The growing lamb is described by the initial fleece-free empty body weight (body weight minus gut fill and wool) and the expected protein and lipid body content at maturity (P_m and L_m, respectively). The intrinsic growth rate of the lamb (B) is given as equation 1 in Appendix 1 (section 2.6.1).

The fleece-free empty body is considered to be the sum of the body protein, ash, water and lipid, and it is assumed that the lamb aims to achieve its expected intrinsic growth for these components. The desired maximum daily protein growth (ΔPG_{max}; kg/day), desired daily lipid growth (ΔL_{des}; kg/day), daily accretion of ash (ΔAsh; kg/day), and daily accretion of water ($\Delta Water$; kg/day) are given as equations 2, 3, 4 and 5, respectively, in Appendix 1 (section 2.6.1).

The live weight of the lamb is given by the fleece-free empty body weight plus wool and gut fill. The expected maximum daily wool growth ($\Delta PWool_{max}$; kg/day) and gut fill (GF; kg) are given as equations 6 and 7 in Appendix 1 (section 2.6.1).

2.2.2.2 Resource requirements and food Intake

The protein and energy requirements to fulfil the expected growth rates are subsequently estimated. Only the protein and energy requirements have been considered (Wellock *et al.*, 2004), as all other nutrient requirements are assumed to be satisfied by the diet. The daily protein requirements for maintenance, growth and wool are estimated by equations 8, 9 and 10 in Appendix 1 (section 2.6.2). Energy requirements are estimated assuming that the deposition of wool protein has the same energy requirement per unit mass as body protein (AFRC, 1993). The daily energy requirements for maintenance, growth and wool are estimated by equations 11, 12 and 13 in Appendix 1 (section 2.6.2). Total protein requirements (PR) and total energy requirements (ER) are simply the sum of the individual requirements for maintenance, growth and wool.

It is assumed that the lamb will attempt to ingest sufficient nutrients to meet its expected requirements for growth. The desired food intake is therefore the food intake necessary to meet the expected requirements. Desired food intake for meeting, separately, the energy (FI_E) and protein (FI_P) requirements of the lamb are estimated by equations 14 and 15 in Appendix 1 (section 2.6.2). The desired food intake of the animal is calculated as the higher of FI_E and FI_P. The energy requirements of the lamb were expressed in terms of effective energy (*EE*; MJ/kg) in accordance with Emmans (1994). These *EE* requirements were linked to the metabolisable energy (*ME*; MJ/kg) yielded by a feed using equation 16 in Appendix 1 (section 2.6.2).

2.2.2.3 Constrained resources

Under many circumstances resources may be constrained or insufficient to meet requirements. The procedure described above results in a food intake that increases as the quality (protein and energy content) of the feed decreases. However, it has been observed that the rate of increase in daily food intake declines as feed quality declines, and daily food intake may decrease for feeds with a low energy content (Kyriazakis and Emmans, 1995) due to an assumed maximum capacity for bulk. To represent this, a quantity called constrained food intake (*CFI*) is defined by equation 17 in Appendix 1 (section 2.6.3). This relationship implies that the capacity of the animal for daily indigestible organic matter (*CAP*; kg) and the energy content of the food jointly determine the constrained food intake. *CAP* in young lambs has been found to increase linearly, equal to proportionally 0.0223 of the current body weight up to 0.51 of the mature body weight, and to remain constant thereafter (Lewis *et al.*, 2004). Thus, *CAP* is given by equation 18 in Appendix 1 (section 2.6.3).

Actual food intake is then the lower of desired food intake and *CFI*. Efficiency of digestion, accounting for level of feeding (*LF*), rumen outflow rate and current state of the lamb, and hence metabolisable protein (MP) available to the animal, were calculated using the equations described by the Agricultural and Food Research Council (1993).

2.2.2.4 Allocation of nutrients

Ingested protein and energy are allocated to various bodily functions. The maintenance needs of the lamb are assumed to be satisfied first and remaining nutrients are allocated to production (body protein, body lipid and wool growth). The energy remaining after allocation to maintenance and production is subsequently stored as additional lipid. The daily lipid deposited ($\Delta Lipid$) is given by equation 19 in Appendix 1 (section 2.6.4). If $\Delta Lipid$ is negative, then lipid will be catabolised to satisfy the animal's energetic needs for other functions as given by equation 20 in Appendix 1 (section 2.6.4).

If the lamb has a MP intake that is below its maintenance requirements, it is assumed to use its body reserves to cover its maintenance functions. If this protein inadequacy is prolonged the lamb will catabolise body protein, eventually leading to death. The quantity of protein that the animal can mobilize from its body, i.e. labile protein (P_{Labile}) (Houdijk *et al.*, 2001; Sykes, 2000), is defined by equation 21 in Appendix 1 (section 2.6.4).

The baseline body lipid level (L_{base}), i.e. the minimum body lipid content essential for animal survival, is estimated as a proportion of its body protein content (P) as given by equation 22 in Appendix 1 (section 2.6.4). If the energy intake of the lamb is not sufficient to meet this baseline body lipid level, then energy allocated towards protein growth is retracted and reallocated to lipid accretion. This scenario, which was absent from the previous model (Vagenas *et al.*, 2007a), is modelled by first calculating the required protein reduction (PR_{Red}) that would be sufficient to fulfil the L_{base} energy requirement. This is estimated as:

$$PR_{Red} = \frac{\left(\left(\frac{bl}{bp}\right)\cdot\left(\left(L_{base}\cdot P\right)-L\right)\right)}{\left(\left(L_{base}\cdot\left(\frac{bl}{bp}\right)\right)+1\right)} \ (kg)$$

where P = current body protein (kg), L = current body lipid (kg), bl = energetic cost per kg of lipid deposition (56 MJ/kg) (Emmans, 1994), and bp = energetic cost per kg of protein deposition (50 MJ/kg) (Emmans, 1994).

The energy lipid shortfall (E_{LS}) is therefore calculated as:

$$E_{LS} = PR_{Red} \cdot bp \text{ (MJ)}$$

Subsequently, daily protein (ΔP) and daily lipid (ΔL) deposited are given as:

$$\Delta P = \Delta PG - PR_{Red} \text{ (kg)}$$

$$\Delta L = \Delta Lipid + \left(\frac{E_{LS}}{bl} \right) \text{ (kg)}$$

where ΔPG = protein growth (kg).

2.2.3 The parasitised animal

2.2.3.1 Protein loss

Parasitism leads to protein loss in animals through damage to the gastrointestinal tract by ingested larvae and adult worms that develop from such larvae. Ingested larvae have a cost to the host manifested by protein loss, for example, tissue loss or plasma loss (Houdijk *et al.*, 2001). The potential protein loss (PLI_{Pot}) due to larval intake (LI) when there is no immune response is given by equation 23 in Appendix 1 (section 2.6.5).

The animal is able to reduce the damage caused by LI through its immune response. Thus in the presence of an immune response the protein loss due to LI (PLI) is assumed to decrease. Actual protein loss due to LI is therefore given by equation 24 in Appendix 1 (section 2.6.5).

A proportion of the ingested larvae (LI) will establish in the host gastrointestinal tract and develop to adult worms (see below). The adult worms will also cause protein loss to the host, for example, via damaged tissue or reduced absorption. However, the total number of adult worms present in the gastrointestinal tract (worm burden; WB) does not provide a complete description of the parasitic burden of the lamb (Bishop and Stear, 1997), because this does not take into account

the mass of the adult worm burden or the effects of population density. To fully account for this, it is necessary to account for the total mass of the worm burden. Worm length has been shown to be strongly positively correlated with the fecundity (*F*) of the worm (number of eggs produced) (Stear and Bishop, 1999). Further, it is assumed that worm length is closely related to, and hence may be used as a proxy for, worm mass. Therefore, the worm mass (*WM*) of a population of worms can be approximated as:

$$WM \approx F \cdot WB$$

To take into account the density-dependence effects upon the worm population, in which individual worm size and fecundity decrease with increasing worm burden, fecundity was scaled as an inverse function of worm burden, assuming a mean worm burden of 2,500. Thus, fecundity was scaled as given by equation 26 in Appendix 1, and worm mass given by equation 27 in Appendix 1 (section 2.6.5).

The protein loss caused by worm mass (*PWM*) is given by equation 28 in Appendix 1 (section 2.6.5). Total protein loss (*PLoss*) due to parasitism is therefore estimated as the sum of protein loss due to *LI* (*PLI*) and protein loss caused by worm mass (*PWM*).

2.2.3.2 Immune response

The lamb is assumed to invest in the immune response in order to reduce the impact of parasitism. Lambs are initially naïve to parasites and they develop immunity as a function of their exposure to infective larvae. The immune response is represented by the host controlled traits of nematode establishment (*ε*), fecundity (*F*) and mortality (*μ*). The functions used to describe these three immune response traits, modified from those described by Louie *et al.* (2005) and Vagenas *et al.* (2007a), are given by the following sigmoidal relationships:

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$$\varepsilon = \left(\frac{\varepsilon_{max} \cdot K_\varepsilon{}^3}{K_\varepsilon{}^3 + \left(\sum_t LI^* \right)^3} \right) + \varepsilon_{min} \text{ (proportion of larvae establishing/day)}$$

$$\mu = \left(\frac{\mu_{max} \cdot \left(\sum_t LI^* \right)^3}{mi^3 + \left(\sum_t LI^* \right)^3} \right) + \mu_{min} \text{ (proportion of adult worms/day)}$$

$$F = \left(\frac{F_{max} \cdot fi^3}{fi^3 + \left(\sum_t LI^* \right)^3} \right) + F_{min} \text{ (eggs/worm/day)}$$

where ε_{max}, μ_{max} and F_{max} = maximum establishment (0.7 (Jackson *et al.*, 2004)), mortality (0.11 (Jackson *et al.*, 2004)) and fecundity(20 (Bishop and Stear, 1997)) rates, respectively, ε_{min}, μ_{min} and F_{min} = minimum establishment (0.06 (Jackson *et al.*, 2004)), mortality (0.01 (Kao *et al.*, 2000)) and fecundity (5 (Vagenas *et al.*, 2007a; b)) rates, respectively, $\sum_t LI^*$ = scaled cumulative *LI* (see below), and K_ε, *mi* and *fi* = rate constants for establishment (200,000), mortality (450,000), and fecundity (230,000), respectively.

Scaled cumulative *LI* ($\sum_t LI^*$) is given as:

$$\sum_t LI^* = \sum_{t-1} LI^* + \left(\left(LI_{max} \cdot \frac{LI}{LI + cli} \right) \cdot \left(\frac{PAC_{Imm}}{(PRQ_{Imm})_{Tot}} \right) \right)$$

where *cli* = constant of relationship between $\sum_t LI^*$ and $\sum_{t-1} LI^*$ (2000 (Vagenas *et al.*, 2007a)).

The immune response is assumed to be predominantly driven by protein; as such, the protein requirements for immunity are calculated separately for *LI* and *WM*

and are given as equations 29 and 30 in Appendix 1 (section 2.6.6). Thus, the total protein required for immunity ((PRQ_{Imm})$_{Tot}$) is given by the sum of requirements for *LI* and *WM*.

2.2.3.3. Effect of parasitism on protein partitioning

As in the case of no parasitic challenge, it is assumed that the maintenance needs of the lamb will be satisfied first. If the available protein is less than the requirements for maintenance then the lamb must catabolise protein. In this case, no protein is allocated to immunity or production. Otherwise, nutrients remaining after allocation to maintenance are allocated to immunity and production (body and wool growth) in proportion to their requirements. Protein allocated to production (PAC_{Growth}) and protein allocated to immunity (PAC_{Imm}) are given by equations 31 and 32 in Appendix 1 (section 2.6.7). This approach is different from the traditional view of considering the requirements for the immune response as part of the maintenance requirements (Coop and Kyriazakis, 1999). Metabolised protein allocated to immunity is assumed to be used with an efficiency of 0.59 (AFRC, 1993); thus the quantity of protein associated with the immune function per day is given by equation 33 in Appendix 1 (section 2.6.7).

Due to protein being allocated to immunity there will be a reduction in protein loss due to parasitism. Protein loss due to worm mass is then re-estimated after the reduction in fecundity and recalculating worm mass, and the protein loss spared added back to the available protein (P_{Avail}). Subsequently the final protein allocated to production (PAC_{Prod}^{F}) is estimated as:

$$PAC_{Prod}^{F} = P_{Avail} - \left(PAC_{Imm} + PLoss \right) \text{ (kg/day)}$$

2.2.3.4 Effect of parasitism on food intake

In the model of Vagenas *et al.* (2007a), anorexia was assumed to be a function of adult worm burden; however, this mechanism leads to anorexia commencing too late (i.e. after 21 days post-infection) for *T. circumcincta*; Coop *et al.* (1982) and Greer *et al.* (2008) reported that anorexia became apparent 7 to 10 days after initial challenge with *T. circumcincta*. Because immune response components such as cytokines cause inappetance, we modelled anorexia as a direct function of the rate of acquisition of immunity as this formulation captures the time-dependent dynamics of *T. circumcincta*–induced anorexia. Anorexia was then applied to either the intrinsic growth rate or actual food intake, as described below, through a reduction parameter (*RED*). *RED* is calculated as a direct function of the rates (i.e. 1st derivatives) of immune response acquisition as:

$$RED = R_c \cdot \left(\frac{d\varepsilon}{dx} + \frac{d\mu}{dx} + \frac{dF}{dx} \right)$$

where R_c = constant linking the reduction to the immune response (2.5), ε = establishment, F = fecundity, μ = mortality, and x = scaled cumulative larval intake.

During the course of an infection *RED* will start at zero, rise to a maximum and then decline towards zero as immunity is fully acquired.

Anorexia was then implemented through the two mechanisms as follows.

Mechanism 1: The reduction was applied to the intrinsic growth rate; thus the reduction of food intake may be modelled as a consequence of a reduction in growth. In order to represent the reduction in intrinsic growth the reduction calculated above is implemented as (Vagenas *et al.*, 2007a):

$$B_{New} = B \cdot RED$$

where B_{New} = new rate of tissue mass retention.

Mechanism 2: The reduction equation was applied directly to the food intake (*FI*) of the lamb to obtain:

$$FI_{New} = FI \cdot RED$$

FI$_{New}$ therefore gives the food intake of the lamb as a consequence of parasite-induced anorexia.

2.2.4 Experimental design

The model was used to explore the consequences of the two different mechanisms for parasite-induced anorexia. For both mechanisms, the model was used to investigate the effect of nutrition and varying levels of challenge with *T. circumcincta* on the performance of a lamb growing from 2 to 6 months of age. This time period was chosen to represent the period in which the lambs are growing at their maximum rate whilst not being fully immune, and thus the period in which parasitism can be expected to have its greatest impact upon weight gain. The model predicts events for time increments of one day and it was updated on a daily basis, with predictions from the previous day being used as the starting point for the current day.

The lambs were simulated to have an initial live weight of around 20kg corresponding to an initial empty body weight of 12.73kg and an initial body protein weight of 2.03kg. The genotype traits of the lamb were a protein weight at maturity (*P$_m$*) of 9.525kg, a lipid weight at maturity (*L$_m$*) of 40.11kg and a growth rate parameter (*B*) of 0.0125. These parameter values were chosen to give growth characteristics similar to those of Scottish Blackface lambs, a common British breed.

For both mechanisms of parasite-induced anorexia, lambs were given a trickle challenge infection of *T. circumcincta* L$_3$ larvae of either control, 1,000 or 5,000 L$_3$ per day, from day one. Numerous levels of larval challenge were initially investigated; the 5,000 L$_3$ per day challenge is reported here as it corresponds to the high level of sub-clinical *T. circumcincta* infections investigated by Coop *et*

al.(1985), which lead to parasite-induced anorexia and reduced growth rate. The 1,000 L_3 per day challenge was chosen to represent a lower challenge level as used by Valderrábano *et al.* (2002) and Coop *et al.* (1982), and for comparison with the previously published model of Vagenas *et al.* (2007a; b). This level of infection also has consequences on the food intake predicted by the two different mechanisms and therefore has a heuristic value.

This scenario was simulated for each of seven different grass qualities (Table 2.1, Figure 2.2).

Table 2.1. Composition of the foods used in the experiments

LF	Description	Feed						
		1	**2**	**3**	**4**	**5**	**6**	**7**
	CP (g/kg DM)	90	115	140	140	140	165	190
	ME (MJ/kg DM)	7.50	10.0	8.75	10.0	11.25	10.0	12.5
	FME (MJ/kg	6.45	8.95	7.70	8.95	10.2	8.95	11.45
6	RP (g/kg DM)	31.2	56	67.1	72.1	80.4	88.2	100.7
	UP (g/kg DM)	56.1	52.1	65.0	59.3	50.3	67.9	84.7
	DUP (g/kg DM)	46.6	42.7	52.2	47.9	40.1	55.8	70.1
	MP (g/kg DM)	66.4	78.4	95.0	93.8	91.4	112	134.3
	MP:ME	8.85	7.84	10.86	9.38	8.12	11.2	10.74

LF, level of feeding as multiples of energy requirements for maintenance; CP, crude protein; ME, metabolisable energy; FME, fermentable metabolisable energy; RP, rumen degradable protein; UP, undegradable protein; DUP, digestible undegradable protein; MP, metabolisable protein; DM, dry matter.
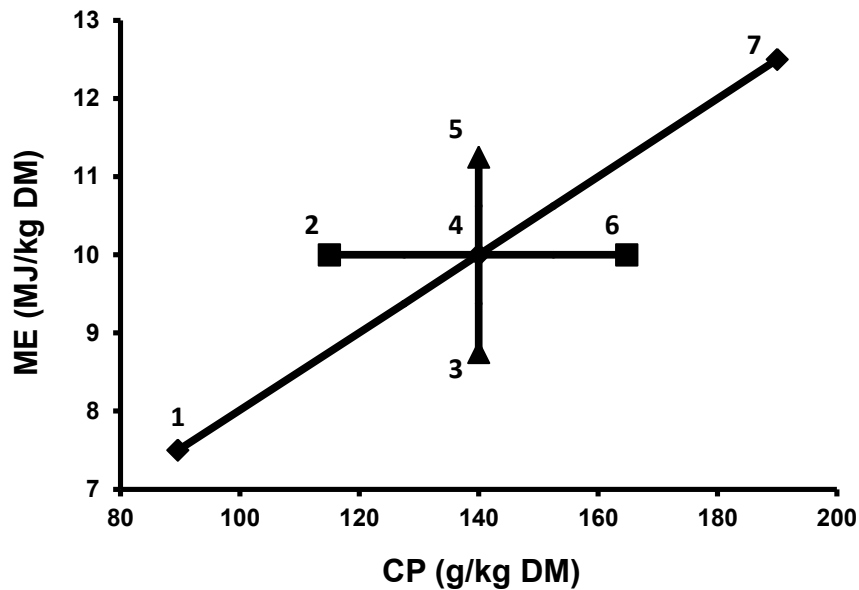
Figure 2.2. Nutritional space occupied by the foods used in the simulated experiments. Foods 2, 4 and 6 differed in their crude protein (CP) contents, Foods 3, 4 and 5 differed in their metabolisable energy (ME) contents and Foods 1, 4 and 7 differed in both their CP and ME contents. DM, dry matter.

Foods 1, 4 and 7 were taken from appendix 1 of the AFRC manual (1993) as being representative of poor-, medium- and good-quality grasses; they were equally spaced in terms of energy and nitrogen contents. Foods 1 and 7 have been previously used in the same model (Vagenas *et al.*, 2007a; b; Doeschl-Wilson *et al.*, 2008) to investigate the impact of food composition on the extent of parasitism in growing lambs. Four additional 'grasses' (foods 2, 3, 5 and 6) were also used for the purposes of the simulations; their composition was considered appropriate and within realistic bounds to investigate the impact of food energy or protein content alone on anorexia during parasitism. Foods 2 and 6 were isoenergetic in terms of ME to grass 4 (10 MJ ME/kg DM), but contained nitrogen levels which were placed between foods 1, 4 and 7, whereas foods 3 and 5 were isonitrogenous in terms of crude protein (CP) to grass 4 (140 g CP/kg DM), but contained ME levels that were placed between the same foods. The composition of foods 2, 3, 5 and 7 was also estimated in accordance with AFRC recommendations (1993). All foods were assumed to have the same ash content (70 g/kg DM) and the same fat content (30 g/kg DM (Noci *et al.*, 2005)). For

the calculation of the MP content of the foods, the assumptions made regarding the impact of the feeding level in relation to maintenance were adjusted as the animals grew, but the yields of protein given in Table 2.1 are for a level of feeding of six times maintenance.

The outputs from the model are presented for food intake (kg/day) and daily worm egg count (eggs/day). The food intake was reported in order to present the differences that occur for the two mechanisms of parasite-induced anorexia. The live weight predictions are not presented here, as they are a direct consequence of the food intake of the lamb. The daily worm egg count, which is the total number of eggs produced per day, was chosen to present the parasitological outcomes of our predictions. This was preferred over faecal egg count (FEC), which is the number of eggs per g of faeces, in order to overcome the dilution effect of the quantity of faeces produced upon the parasitological predictions.

2.2.5 Model validation

The model was parameterised using the results of Coop *et al.* (1982). However, to ensure that the values predicted by the model were representative of values reported in experiments other than Coop *et al.* (1982), a search was carried out for published comparable experiments. Experiments investigating the impacts of *T. circumcincta* infection on growing lambs were checked against selected criteria. These criteria were: sufficient information on food composition, *ad libitum* feeding and the use of non-parasitised control animals. Two experiments met these criteria and contained sufficient information to enable simulations to be carried out for comparison; these were experiments described by Greer *et al.* (2008) and Valderrábano *et al.* (2002).

Greer *et al.* (2008) infected immunologically naïve Coopworth ewe lambs with either control or 4,000 *T. circumcincta* L_3 per day for 9 weeks, and offered them *ad libitum* access to food containing 10.5MJ ME/kg DM and 146g CP/kg DM. Coopworth lambs were assumed to be similar in terms of their growth characteristics to the sheep used for our simulations.

Valderrábano *et al.* (2002) infected immunologically naïve Rasa Aragonesa female lambs with either 0 or 1,000 *T. circumcincta* L_3 per day for 6 weeks, and offered *ad libitum* access to food containing 13.25MJ ME/kg DM and 175.5g CP/kg DM. Genetic descriptions of Rasa Aragonesa lambs in the terms required by our model do not appear to exist in the literature. For this reason the model was calibrated for the growth rates and food intake of the non-infected sheep; thus the performance and food intake of the infected sheep was a model prediction.

2.3 Results

2.3.1 Validation

The model predictions for anorexia and FEC were close to those reported by Greer *et al.* (2008); however, impacts of parasitism on growth rate were under-predicted. The model predicted a reduction in the food intake of infected lambs of 0.16 from 15 to 28 days post-infection in comparison with uninfected lambs; this is similar to the reported 0.17 reduction in the intake of the infected lambs over the same time period. Infected lambs were predicted to have a 26% slower growth rate than uninfected lambs until day 38 post-infection, after which growth rates became similar. Infected lambs were predicted to remain proportionately 0.09 lighter than control lambs at 63 days post-infection. The reported growth rate for infected sheep was 43% lower than that of uninfected sheep up to day 35 of infection and infected lambs were proportionately 0.11 lighter at 63 days post-infection. FEC was predicted to peak 30 days post-infection, and the observed FEC peak occurred 28 days post-infection.

Simulations reproducing the experiment of Valderrábano *et al.* (2002) predicted an average food intake of 1,060g/day for uninfected and 999g/day for infected sheep, showing a 6% reduction in the average food intake of infected lambs in comparison with their uninfected counterparts over the experimental period. These compare favourably with the reported intakes of 1,070 and 960 g/day, respectively, although the observed reduction in the average food intake of infected lambs was slightly higher (10%). Some of this difference could be due to the assumptions made to convert the reported CP content of the feed to MP.

2.3.2 Food intake

The daily food intakes for uninfected lambs are shown in Figure 2.3 for foods differing in both energy and protein content (a), for the three isoenergetic foods (b) and for the three isonitrogenous foods (c). Figure 2.4 shows the food intake for lambs challenged with control, 1,000 or 5,000 larvae per day offered access to either Food 4 for mechanism 1 (Figure 2.4(a)) and for mechanism 2 (Figure 2.4(b)) or food 1 for mechanism 1 (Figure 2.4(c)) and for mechanism 2 (Figure 2.4(d)). Average food intake predictions for uninfected lambs, and the relative food intake of lambs challenged with 1,000 or 5,000 larvae per day (given as a proportion of uninfected lambs) for both anorexia mechanisms and for all foods are summarised in Table 2.2. The maximum extent of anorexia (largest reduction predicted in comparison with uninfected lambs), including day at which the maximum extent was observed are given in Table 2.3., and the duration of anorexia is summarised in Table 2.4. Duration was defined as the number of days during which the proportional reduction in food intake was greater than 0.05.
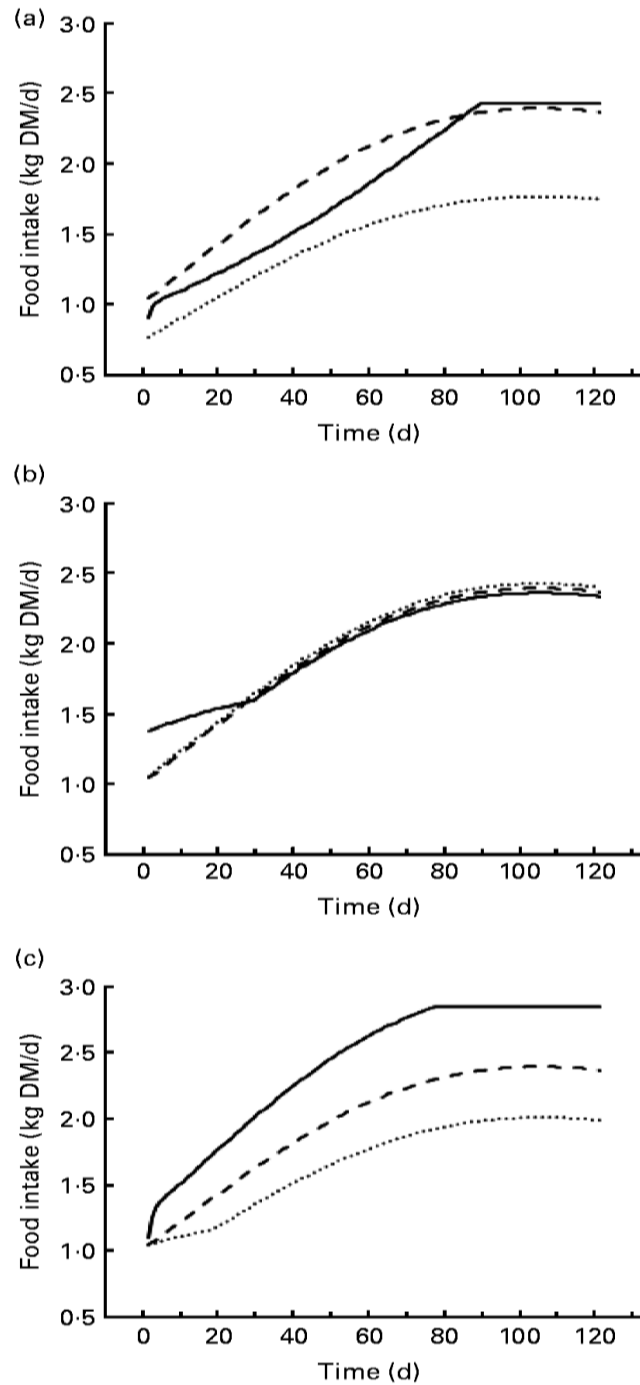
Figure 2.3. Food intake predictions for uninfected lambs given *ad libitum* access to foods of different crude protein (CP) and metabolisable energy (ME) content (for details of foods, see Table 2.1). (a) Foods of different CP and ME contents (——— , food 1; – – – , food 4; ········, food 7). (b) Foods of different CP content but the same ME content (10MJ/kg DM) (——— , food 2; – – – , food 4; ········ , food 6). (c) Foods of different ME content but the same CP content (140g/kg DM) (——— , food 3; – – – , food 4; ········ , food 5).
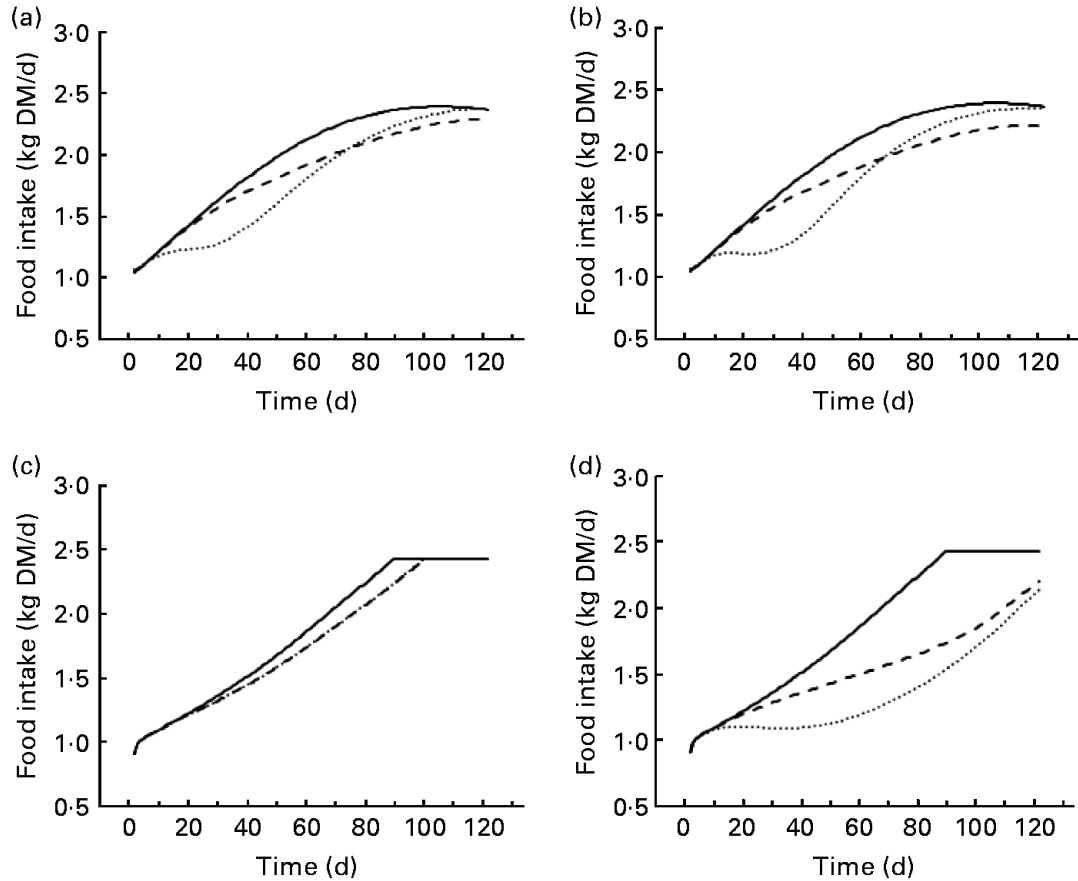
Figure 2.4. (a) Food intake predictions for mechanism 1 (reduction in the intrinsic capacity for growth) for lambs given access to food 4 (crude protein = 140g/kg DM; metabolisable energy = 10 MJ/kg DM), whilst exposed to control (——), 1,000 (– – –) or 5,000 (·······) *Teladorsagia circumcincta* L₃ per day. (b) Food intake predictions for mechanism 2 (direct reduction in food intake) for lambs given access to food 4. (c) Food intake predictions for mechanism 1 for lambs given access to food 1 (crude protein = 90g/kg DM; metabolisable energy = 7.5 MJ/kg DM). (d) Food intake predictions for mechanism 2 for lambs given access to food 1. DM, dry matter.

Table 2.2. Average food intake (kg) predictions for uninfected (control) lambs, and the relative food intake predictions for mechanisms 1 and 2 for lambs given access to foods of different crude protein and metabolisable energy content and exposed to 1,000 or 5,000 *Teladorsagia circumcincta* L_3 per day over 121 days.

Food	Average food intake of controls (kg DM)	Relative food intake † (proportion of control)			
		Mechanism 1 ‡		Mechanism 2 §	
		1,000 L_3	5,000 L_3	1,000 L_3	5,000 L_3
1	1.849	0.96	0.96	0.83	0.73
2	1.999	0.94	0.90	0.92	0.88
3	2.419	0.95	0.91	0.92	0.87
4	1.982	0.94	0.90	0.92	0.89
5	1.670	0.93	0.89	0.91	0.87
6	2.010	0.94	0.90	0.92	0.90
7	1.462	0.94	0.90	0.92	0.90

* For details of foods, see Table 2.1.

† Total food intake given as a proportion of that of uninfected lambs.

‡ Reduction in the intrinsic capacity for growth.

§ Direct reduction in food intake.

Table 2.3. Maximum extent of anorexia predictions for mechanisms 1 and 2 for lambs given access to foods of different crude protein and metabolisable energy content and exposed to 1,000 or 5,000 *Teladorsagia circumcincta* L_3 per day over 121 days.

Food*	Mechanism 1 †				Mechanism 2 ‡			
	1,000 L_3		5,000 L_3		1,000 L_3		5,000 L_3	
	LRP	Day of max	LRP	Day of max	LRP	Day of max	LRP	Day of max
1	0.08	89	0.08	89	0.28	89	0.38	76
2	0.10	66	0.23	35	0.12	66	0.26	41
3	0.10	66	0.23	35	0.11	68	0.36	33
4	0.10	67	0.23	35	0.11	68	0.27	36
5	0.11	65	0.24	35	0.13	64	0.27	43
6	0.10	66	0.23	35	0.11	68	0.25	33
7	0.10	66	0.23	35	0.11	68	0.26	33

LRP, largest reduction predicted

* For details of foods, see Table 2.1.

† Reduction in the intrinsic capacity for growth.

‡ Direct reduction in food intake

Table 2.4. Duration of anorexia predictions (number of days during which the reduction in food intake was greater than 0.05) for mechanisms 1 and 2 for lambs given access to foods of different crude protein and metabolisable energy content and exposed to 1,000 or 5,000 *Teladorsagia circumcincta* L_3 per day over 121 days.

Food*	Mechanism 1 †		Mechanism 2 ‡	
	1,000 L_3	**5,000 L_3**	**1,000 L_3**	**5,000 L_3**
1	48	49	94+	116+
2	75	79	91+	84
3	57	73	89+	77
4	75	80	89+	78
5	82	82	95+	87
6	75	80	89+	74
7	75	80	89+	76

* For details of foods, see Table 2.1.

† Reduction in the intrinsic capacity for growth.

‡ Direct reduction in food intake

+ Anorexia still present at day 121.

Across all foods investigated, with the exception of the food low in both protein and energy (food 1), food intake for infected lambs presented similar patterns for both mechanisms of anorexia, differing only in the predicted duration and extent of anorexia; the extent of anorexia being greater for larger levels of challenge. In general, mechanism 2 had a greater maximum extent of anorexia and lower relative food intake than mechanism 1 for both levels of challenge and for all foods. For mechanism 1 the duration of anorexia remained similar for both levels of infection, whilst for mechanism 2 for all foods the duration of anorexia was longer for lambs challenged with 1,000 larvae per day than for lambs challenged with 5,000 larvae per day.

Comparisons between foods for both mechanisms are explored in more detail below.

2.3.2.1 Effect of nitrogen content on food intake (foods 2, 4 and 6)

The effect of nitrogen content on food intake of uninfected lambs was small (Figure 2.3(b)), with the exception of the food intake of the lambs on the lowest nitrogen food during the early stages of growth (food 2). The model predicted that lambs offered access to food 2 would compensate for the food nitrogen content by increasing their food intake for the first 28 days of the simulated experiment, when this food was first limiting in MP.

For infected lambs, nitrogen content of the food had different impacts on relative food intake, and the maximum extent and duration of anorexia for the two mechanisms. Little effect of the nitrogen content of the food was seen on relative food intake, and the maximum extent and duration of anorexia for mechanism 1. However, for mechanism 2 the nitrogen content of the food affected the duration of anorexia for lambs challenged with 5,000 larvae per day. The duration of anorexia decreased from 84 to 74 days as food CP content increased from 115 to 165g/kg DM, although the maximum extent of anorexia was not affected significantly by food nitrogen content, remaining at about 0.26 for all three foods. For lambs challenged with 5,000 larvae per day, the relative food intake was predicted to increase from 0.88 to 0.9 as food CP content increased from 115 to 165g/kg DM. However, similar relationships between anorexia traits and nitrogen content of the food were not observed in lambs challenged with 1,000 larvae per day, with anorexia still being present at the end of the simulation.

2.3.2.2 Effect of energy content on food intake (foods 3, 4 and 5)

The effect of energy content on food intake of uninfected lambs was large (Figure 2.3(c)). This was due to the model predicting that lambs would compensate for a reduction in food energy content by increasing food intake.

For mechanism 1, the energy content of the food had little impact on the maximum extent of anorexia for both levels of parasitic challenge (Table 2.3); however, differences were predicted in the duration of anorexia for both levels of

challenge (Table 2.4). For lambs challenged with 1,000 larvae per day the duration of anorexia was predicted to increase from 57 to 82 days as food ME content increased from 8.75 to 11.25MJ/kg DM, whilst for lambs challenged with 5,000 larvae per day the equivalent predicted increase in the duration of anorexia was from 73 to 82 days. Likewise, for lambs challenged with 1,000 larvae per day the relative food intake was predicted to decrease from 0.95 to 0.93 as food ME content increased from 8.75 to 11.25MJ/kg DM. For lambs challenged with 5,000 larvae per day the equivalent predicted decrease in relative food intake was from 0.91 to 0.89.

For mechanism 2, energy content of the feed affected the maximum extent and duration of anorexia for lambs challenged with 5,000 larvae per day. The maximum extent of anorexia was predicted to be the same for foods 4 and 5 (0.27), whilst for food 3 the maximum extent of anorexia increased to 0.36. The duration of anorexia was similar (77 to 78 days) for foods 3 and 4, whilst for food 5 the duration of anorexia increased to 87 days. The relative food intakes of foods 3 and 5 were the same (0.87), whilst for food 4 (10MJ/kg DM) the relative food intake was predicted to be 0.89. Similar relationships were not observed in lambs challenged with 1,000 larvae per day, as anorexia was still present at the end of the simulation.

2.3.2.3 Effect of varying both energy and protein content on food intake (foods 1, 4 and 7)

For uninfected lambs, food intakes when offered foods 4 and 7 reflected the relative energy densities of the diets; however, bulk constraints due to the maximum capacity of the gastrointestinal tract were observed for food 1 (Figure 2.3(a)).

For mechanism 1, there was little difference between the food intake characteristics of lambs offered food 4 or 7 when challenged with 1,000 larvae per day, or between these foods when challenged with 5,000 larvae per day. However, predicted food intake patterns differed substantially in the case of lambs offered food 1. Both larval challenge levels were predicted to result in the same maximum extent (0.08), relative food intake (0.96) and duration of anorexia (48 to 49 days). For food 1, the severity and extent of anorexia were less than those seen for the other foods.

For mechanism 2, lambs challenged with 5,000 larvae per day and offered either food 4 or 7 had similar food intake and anorexia characteristics, as did lambs challenged with 1,000 larvae per day. However, the food intake predictions for lambs offered food 1 differed substantially, as shown in Figure 2.4(d). Lambs challenged with 5,000 larvae per day and offered food 1 were predicted to have a maximum extent of 0.38, whilst lambs challenged with 1,000 larvae per day were predicted to have a maximum extent of anorexia of 0.28. For both levels of challenge, the duration of anorexia was longer for food 1 than for all other foods, such that anorexia was still present at the end of the simulated time period. Lambs challenged with 5,000 larvae per day and offered food 1 had a relative food intake of 0.73, whilst lambs challenged with 1,000 larvae per day were predicted to have a relative food intake of 0.83.

2.3.2.4 Effect of differing levels of parasitic challenge on anorexia

The maximum extent of anorexia for lambs given access to food 4 for increasing levels of parasite challenge is given in Figure 2.5, with predictions given for both mechanisms. The maximum extent of anorexia showed a non-linear increase with increasing challenge level, being on average 17% greater for mechanism 2 than mechanism 1.
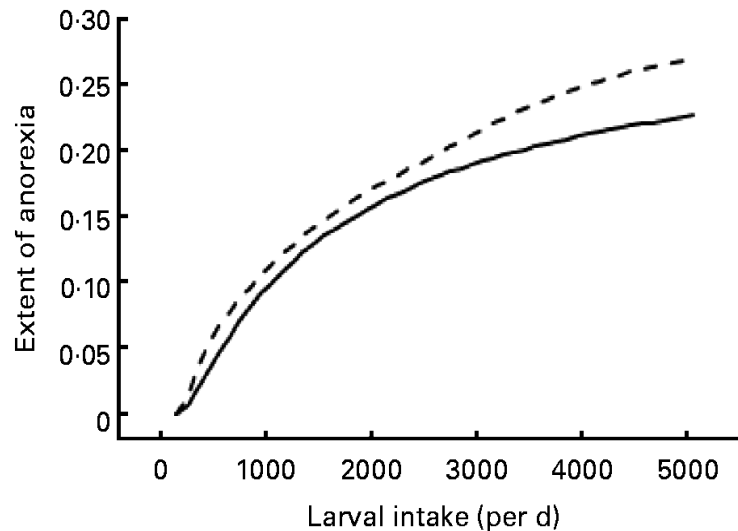
Figure 2.5. Maximum extent of anorexia (i.e. largest reduction predicted in comparison to uninfected lambs) predictions for mechanism 1 (reduction in the intrinsic capacity for growth; ——) and mechanism 2 (direct reduction on food intake; − − −) for lambs given access to food 4 (crude protein = 140g/kg DM, metabolisable energy = 10MJ/kg DM), whilst exposed to increasing levels of larval challenge. Data presented is for increments of 100 larvae per day.

2.3.3 Daily egg counts

The predicted daily egg counts for infected lambs for food 4 (mechanism 2) are provided in Figure 2.6, as an example of the profile for all foods. Whilst total egg count was always higher for the higher challenge level, differences between foods were often small. The maximum daily egg count predicted (including day of occurrence) for mechanisms 1 and 2 for lambs offered access to all foods, whilst challenged with either 1,000 or 5,000 larvae per day, are summarised in Table 2.5.
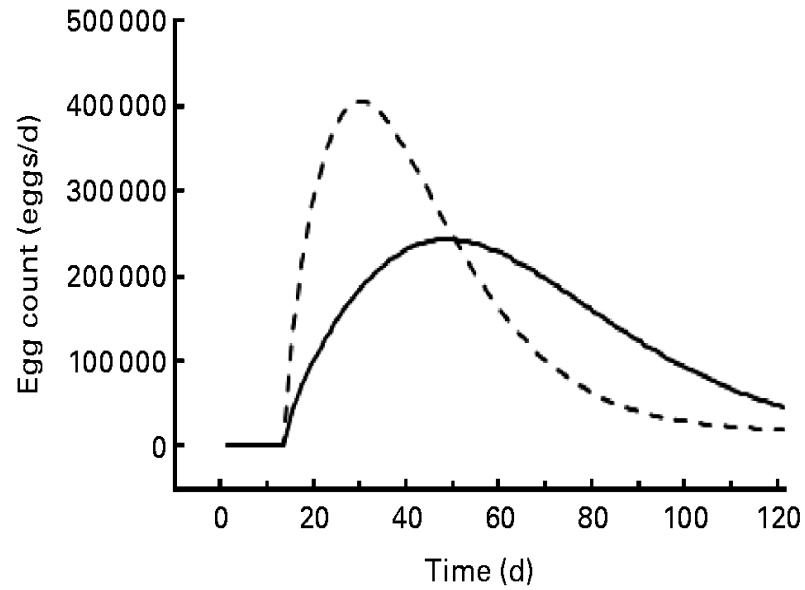
Figure 2.6. Daily egg count (eggs/day) prediction for lambs given access to food 4 (crude protein = 140g/kg DM, metabolisable energy = 10MJ/kg DM) for mechanism 2 (direct reduction in food intake), whilst exposed to either 1,000 (——) or 5,000 (– – –) *Teladorsagia circumcincta* L_3 per day.

Table 2.5. Maximum daily egg count (10^{-3}eggs/day) predictions for mechanisms 1 and 2 for lambs given access to foods of different crude protein and metabolisable energy content and exposed to 1,000 or 5,000 *Teladorsagia circumcincta* L_3 per day.

| Food* | Mechanism 1 † | | | | Mechanism 2 ‡ | | | |
| | 1000 L_3 | | 5000 L_3 | | 1000 L_3 | | 5000 L_3 | |
	Egg count	Day of max	Egg count	Day of max	Egg count	Day of max	Egg count	Day of max
1	292	55	534	33	305	59	624	39
2	243	48	399	29	243	48	423	31
3	243	48	399	29	243	48	399	29
4	243	48	400	29	243	48	405	30
5	246	49	411	30	247	49	458	32
6	243	48	399	29	243	48	399	29
7	243	48	399	29	243	48	399	29

* For details of foods, see Table 2.1.

† Reduction in the intrinsic capacity for growth.

‡ Direct reduction in food intake.

2.3.3.1 Effect of nitrogen content on daily egg count (foods 2, 4 and 6)

Results from mechanism 1 suggested that the protein content of the food would have no impact upon the maximum daily egg count predicted for either level of larval challenge. Results from mechanism 2 similarly predicted no impact on maximum daily egg count for lambs given a challenge of 1,000 larvae per day. However, for the lambs challenged with 5,000 larvae per day, the protein content of the food had a small impact upon the predicted maximum daily egg count, with a 1.5% increase for food 4 and a 6% increase for food 2 in comparison with food 6.

2.3.3.2 Effect of energy content on daily egg count (foods 3, 4 and 5)

For lambs challenged with 1,000 larvae per day, essentially no differences were predicted in maximum daily egg count for foods 3, 4 and 5, for both mechanisms. For lambs challenged with 5,000 larvae per day, mechanism 1 resulted in a 3% increase in predicted maximum egg counts for food 5 in comparison with foods 3 and 4. Mechanism 2 led to a 1.5% increase for food 4 and a 15% increase for food 5 in comparison with the maximum daily egg count predicted for food 3.

2.3.3.3 Effect of varying both energy and protein content on daily egg count (foods 1, 4 and 7)

For mechanism 1, no differences in maximum daily egg count were predicted for foods 4 and 7. However, for food 1, daily egg counts were predicted to be 20% and 34% greater for lambs challenged with 1,000 and 5,000 larvae per day, respectively. For mechanism 2, lambs challenged with 1,000 larvae per day were predicted to have no differences in maximum daily egg count for foods 4 and 7 and a 26% increase for food 1 in comparison with the other foods. Lambs challenged with 5,000 larvae per day were predicted to have a 1.5% increase for food 4 and a 56% increase for food 1 in comparison with the maximum daily egg count predicted for food 7.

2.4 Discussion

The aim of this chapter was to investigate the consequences of two proposed mechanisms for parasite-induced anorexia on the food intake of parasitised sheep, and to explore the relationship between anorexia and food composition. In addition to exploring these results we will also compare our predictions to appropriate, published experimental data. The comparisons are qualitative as there are no experiments in the literature that have investigated the effect of food composition on the food intake of sheep infected with *T. circumcincta*; however, they do enable us to draw conclusions about the nature of anorexia in parasitised sheep. We conclude by proposing experiments that need to be performed in order to gain further understanding of the nature of parasite-induced anorexia and its relationship to feed composition.

2.4.1 Accounting for the predictions made by each mechanism

2.4.1.1 Mechanism 1

Anorexia was observed on all foods with the exception of food 1. This was due to the energy content of the food being low, so that although lambs attempted to eat sufficient quantities of the food to meet energy requirements, they were constrained in doing so by their maximum gastrointestinal tract capacity. Whilst the desired food intake for growth was reduced due to parasitism, the maximum gastrointestinal tract capacity caused a constraint greater than this and consequently anorexia was not observed. However, a 4% reduction in total food intake (Table 2.2) was still predicted for food 1. This was due to the lamb being unable to compensate for nutrient loss due to parasitism because of the maximum gastrointestinal tract capacity. Therefore the implication of mechanism 1 is that the food intake of the animals will be dictated by the first operating constraint, which in this case was gut fill (Kyriazakis, 2003).

For lambs challenged with 5,000 larvae per day the maximum extent of anorexia was about 0.23 in all remaining foods, in comparison with uninfected

control lambs, and for lambs challenged with 1,000 larvae per day the maximum extent of anorexia was about 0.10. The marginally increased maximum reduction in food intake and duration of anorexia predicted for food 5 is discussed below. The duration of anorexia for both levels of larval challenge was unaffected by the nitrogen content of the food; however, in both cases the duration of anorexia increased with the increasing energy content of the food.

The maximum daily egg count for infected lambs on all foods was the same except for foods 1 and 5. For lambs offered food 1, the increases predicted for both challenge levels arise from the maximum gastrointestinal tract capacity. Due to this, the food intake of the lambs did not meet the intake required for growth rate and the acquisition of immunity. Therefore a reduction in the rate of acquisition of immunity allowed more worms to establish, survive and produce eggs in comparison to foods 2, 3, 4, 6 and 7; subsequently the daily egg count increased. Intake of food 5 was not constrained by the maximum gastrointestinal tract capacity; nevertheless increases of 1% and 3% in the maximum daily egg count were predicted for challenge levels of 1,000 and 5,000 larvae per day, respectively, in comparison with foods 2, 3, 4, 6 and 7. For each of these latter diets, if a lamb eats to meet its desired energy intake then it receives an excess of protein. Hence, with the reduction in the intrinsic growth rate with mechanism 1, the immune function will receive sufficient protein to achieve the optimal rate of acquisition even when anorexia is present. However, if the ME content of the food is high and the MP:ME ratio is low, as seen for food 5, a marginal deficiency in protein intake occurs in our model when anorexia is present. As a consequence, with this diet the model predicted a reduction in both growth rate and the acquisition rate of immunity (hence an increased duration of anorexia), leading to an increase in parasite burden.

2.4.1.2 Mechanism 2

Anorexia was observed for all foods at both challenge levels. For lambs given a challenge of 1,000 larvae per day no differences were predicted in the maximum extent of anorexia for foods 3, 4, 6 and 7. The maximum extent of anorexia was

largest for food 1 due to the added impact of the gut capacity, whilst foods 2 and 5 were predicted to have a small increase in comparison to foods 3, 4, 6 and 7. Similar differences were predicted for the duration of anorexia; however, further conclusions could not be drawn for this level of challenge, as anorexia was not complete by the end of the simulation.

For lambs challenged with 5,000 larvae per day, the maximum extent of anorexia predicted was similar for foods 2, 4, 5, 6 and 7, but higher for foods with low ME contents (i.e. foods 1 and 3). These effects are attributable to the maximum gastrointestinal capacity constraint predicted throughout the simulation for food 1 and from day 78 for food 3. The duration of anorexia generally decreased with increasing nitrogen content of the diet, the exception being an increase in duration for food 5, due to the impact of a low MP:ME ratio on protein intake described above. A marked increase in duration of anorexia for food 1 can be attributed to the gut fill constraint preventing food intake recovery. The maximum daily egg count predictions followed a similar pattern, with a 15% increase predicted for food 5 and a 56% increase predicted for food 1.

2.4.1.3 Summary of differences between mechanisms

The two mechanisms resulted in different predicted outcomes and implications. For mechanism 1, the energy content of the food had an impact on duration of anorexia, and the maximum extent and duration of anorexia were affected by the combination of a high ME content and low MP:ME ratio (food 5). Other than this, no relationships were observed between anorexia and food composition, except in the presence of the maximum gastrointestinal tract capacity (food 1). The absence of anorexia for food 1 implies that the food intake of the animal is dictated by the first operating constraint, which in this case was the gut fill.

For mechanism 2, the nitrogen content of the food had an impact on the duration of anorexia, and the maximum extent and duration of anorexia were affected by the combination of high ME content and low MP:ME ratio (food 5). Further to

this, the maximum gastrointestinal tract capacity and anorexia constraints were additive, as can be seen in the predictions for infected lambs offered access to food 1.

2.4.2 Comparison of predictions with experimental evidence

Whilst experiments have been performed quantifying the impacts of *T. circumcincta* infection in sheep (Coop *et al.*, 1982; 1985), the experimental data reported did not allow us to draw conclusions about the relationship between anorexia and food composition in parasitised sheep. However, data are available for *Trichostrongylus colubriformis* infections on a variety of different feeds. Although there are many differences between these two nematode species, for example, site of parasitism (abomasum *v.* small intestine), development rate, worm fecundity and acquisition of host immunity (Coop and Kyriazakis, 1999), for purposes of comparison it is assumed that the anorexinogenic components of the immune response involved in *T. colubriformis* infections are similar to those for *T. circumcincta* (Kyriazakis *et al.*, 1998; Kyriazakis, 2010), and consequently may be affected by food composition in a similar manner.

Surprisingly, there are few experiments that have investigated the effects of food energy content on the extent of anorexia; hence it is not possible to draw strong conclusions. However, the effect of protein content on the extent of anorexia has been investigated. First, Greer *et al.* (2009) infected immunologically naïve lambs with 2,000 *T. colubriformis* larvae per day and gave them access to either a high-protein diet (energy = 10.5MJ/kg DM; CP = 175g/kg DM) or a low-protein diet (energy = 11.1MJ/kg DM; CP = 93g/kg DM). The maximum daily egg count at day 42 was 400,000 eggs for lambs fed the high-protein diet, and 700,000 eggs for the low-protein diet. The mean reduction in food intake over the period that anorexia was observed was 0.25 and 0.15 for lambs fed the low- and high-protein food, respectively, in comparison with uninfected control lambs. To compare these results with our model predictions, we ran simulations for both anorexia mechanisms using feed descriptions, live-weight range and level of larval challenge similar to Greer *et al.* (2009); the only difference was that our simulations assumed *T. circumcincta*

infections. Mechanism 1 predicted that the maximum daily egg count remained at about 289,000 eggs for both the high- and low-protein food. The mean reduction in food intake over the period that anorexia was observed was 0.09 and 0.10 for the low- and high-protein-fed lambs, respectively, in comparison with uninfected lambs. On the other hand, mechanism 2 predicted that the maximum daily egg count was about 306,000 eggs for lambs fed the low-protein food, but this decreased to about 289,000 eggs for lambs fed the high-protein food. Further to this, the mean reduction in food intake over the period that anorexia was observed was predicted to be 0.12 and 0.10 for lambs fed the low- and high-protein food, respectively, in comparison with uninfected control lambs. In summary, Greer *et al.* (2009) observed that the maximum daily egg count decreased by 75%, and the mean reduction in food intake also decreased, as the protein content of the food increased. For mechanism 1, the maximum daily egg count remained constant despite the change in protein content, and the mean reduction in food intake increased as the protein content of the food increased. For mechanism 2, the maximum daily egg count increased and the mean reduction in food intake decreased, as the protein content of the food increased. Due to differences in the nematode species the comparisons made here are qualitative rather than quantitative. However, whilst the changes in maximum daily egg count and food intake were smaller than those observed by Greer *et al.* (2009), it was mechanism 2 that resulted in the correct predicted patterns.

Second, Kyriazakis *et al*. (1994) infected immunologically naïve lambs with 2,500 *T. colubriformis* larvae per day and offered them isoenergetic foods (10.4MJ/kg DM) that differed in CP content (90, 164 and 214g/kg DM). The protein content of the food had no impact upon the extent of anorexia, with the reduction in mean food intake being 0.10 in comparison with uninfected lambs for all foods. Again, we simulated these experimental conditions. Mechanism 1 predicted a reduction in mean food intake of 0.07 for lambs offered the low-protein diet in comparison with uninfected lambs, and a reduction in mean food intake of 0.08 for lambs offered the medium- or high-protein diets in comparison with the controls. Mechanism 2 predicted no impact upon the extent of anorexia, with all foods

showing a reduction in mean food intake of 0.10 in comparison with uninfected lambs, the same as that reported by Kyriazakis *et al.* (1994).

Last, Kyriazakis *et al.* (1996b) infected immunologically naïve lambs with 2,500 *T. colubriformis* larvae per day, and offered them access to isoenergetic foods (10.4MJ/kg DM) differing in their CP content (86 or 206g/kg DM). The reduction in mean food intake was 0.18 and 0.11 for lambs offered the low- and high-protein diets, respectively, compared with uninfected lambs. For simulations carried out using the same food descriptions and level of larval challenge, over the same time period, mechanism 1 predicted a reduction in mean food intake of 0.11 and 0.12 for lambs offered the low- and high-protein foods, respectively, in comparison with uninfected lambs. With mechanism 2, reductions in mean food intake were predicted to be 0.14 and 0.10 for lambs offered the low- and high-protein diets, respectively, showing a similar trend to that reported by Kyriazakis *et al.* (1996b). Thus, in all three cases investigated it was mechanism 2 that led to more accurate representations of the trends observed in the experimental data, predicting a relationship between food composition and the extent of anorexia, with impacts on daily egg counts.

2.4.3 Interpretation and implications of model predictions

In terms of the duration of anorexia, there is convincing evidence that food composition has an impact, with the duration being reduced on high-quality foods (Kyriazakis *et al.*, 1996b; Datta *et al.*, 1998; Knox and Steel, 1999). This relationship between food composition and the duration of anorexia has been suggested to be due to food composition affecting the degree of expression of immunity in pathogen-challenged hosts (Coop and Kyriazakis, 1999), subsequently leading to the observed effect on the duration of anorexia via an impact upon immunity and parasite burden. Thus, animals on poor-quality diets (for example, foods of low protein and energy content) may be expected to suffer proportionally more the consequences of infection than animals on good-quality diets (for example, foods of high protein and energy content) (Coop and Holmes, 1996). Unfortunately, mechanism 1 predicted the

opposite of this, with the duration of anorexia tending to increase as the energy content of the food increased.

There is also evidence that the protein content of the feed affects the daily egg count, with lambs offered lower-protein feeds having higher daily egg count than lambs offered a higher-protein feed (Greer *et al.*, 2009). This provides further support to the finding that food composition affects the degree of expression of immunity, with a consequent relationship between food composition and duration of anorexia as suggested above. Once again, these findings are consistent with mechanism 2 in which food composition affected daily egg counts and the duration of anorexia.

Unfortunately, no comparable experimental data could be found for infected lambs on diets of sufficiently low energy content to incur the maximum gastrointestinal tract capacity constraint. In studies that have used foods of sufficiently low quality to cause this constraint, for example, Anindo *et al.* (1998), insufficient detail has been given on food composition and there have been no uninfected control groups. This lack of data may reflect the view and practice that parasitised lambs need to be fed better-quality feeds; whilst this may be a fair conclusion, it may not always be possible in practice.

Although mechanism 2 has been more consistent with the experimental data, the lack of comparable experimental data for foods that impose the maximum gastrointestinal tract capacity constraint does not allow us to draw conclusions on the additivity of signals involved in the regulation of food intake. Mechanism 1 implies that food intake would be determined by the most limiting constraint. It has previously been suggested that the processes regulating appetite are disrupted by cytokine release that accompanies infection (Langhans, 2000; Plata-Salaman, 2001), and thus there is a redundancy of the signals operating to regulate food intake (Kyriazakis, 2003). On the other hand, mechanism 2 implies that there is an additivity in the effects of the signals that control voluntary food intake. It has previously been proposed that various satiety signals act additively to control voluntary food intake (Forbes, 1986; Anil *et al.*, 1993). Thus both mechanisms

present viable approaches to describing the regulation of voluntary food intake, but conclusions cannot be drawn on this topic until the relevant experiments have been carried out.

Further experimental research would help determine the relationship between food composition and parasite-induced anorexia. Whilst several experiments have systematically investigated the effects of food protein content on the extent of anorexia and the impacts of parasitism, further experiments are required to investigate the impacts of food energy and protein content, separately and in conjunction. It would be of particular interest to perform these challenge experiments using *T. circumcintca*, both to obtain *de novo* data for sheep infected by this parasite and to provide data that may be compared with those obtained from sheep challenged with *T. colubriformis*. It would also be of interest to obtain data for foods of sufficiently low energy content as to impose the maximum gastrointestinal tract capacity constraint. This would enable us to determine whether the factors that regulate voluntary food intake act additively, and whether low larval challenges are sufficient to cause anorexia for low-quality foods.

In summary, it would be of great biological interest to better understand the causes and consequences of anorexia. The suggested experiments coupled with predictive models may allow us to achieve this.

2.5 Conclusion

The mechanism by which anorexia is modelled leads to different predicted outcomes from infection. Mechanism 1, reduced intrinsic growth with consequent reductions in food intake, led to predictions that the duration of anorexia increases with increasing energy content of the food, and that food intake is determined by the first operating constraint (maximum gastrointestinal tract capacity). Mechanism 2, a direct reduction in food intake, led to predictions that the duration of anorexia decreases with increasing protein content of the food, and that the impacts of anorexia and the maximum gastrointestinal tract capacity upon voluntary food intake

were possibly additive. Mechanism 2 was more consistent with the theories and experimental data presented for a wide range of food qualities.

2.6 Appendix 1

2.6.1 Intrinsic growth model

Growth was assumed to follow a Gompertz growth curve trajectory. The intrinsic growth rate of the lamb (B (Emmans, 1997)) is estimated as:

$$B = \frac{0.023}{P_m^{0.27}}$$

(1)

where P_m = body protein content at maturity (kg).

The expected (maximum) daily body protein growth ($\Delta PG_{\max}$ (Emmans, 1997)) is estimated as:

$$\Delta PG_{\max} = P \cdot B \cdot \ln\left(\frac{P_m}{P}\right) \text{ (kg/day)}$$

(2)

where P = current body protein mass (kg).

The desired lipid growth (ΔL_{des} (Emmans and Kyriazakis, 1999)) is estimated as:

$$\Delta L_{des} = \Delta PG_{\max} \cdot \left(\frac{L_m}{P_m}\right) \cdot d \cdot \left(\frac{P}{P_m}\right)^{(d-1)} \text{ (kg/day)}$$

(3)

where L_m = body lipid content at maturity (kg), and $d = 1.46 \cdot \left(\frac{L_m}{P_m}\right)^{0.23}$ (Emmans, 1997).

The daily accretion of ash (ΔAsh (Emmans and Kyriazakis, 1997; Emmans and Fisher, 1986)) is estimated as:

$$\Delta Ash = 0.211\Delta PG \text{ (kg/day)}$$

(4)

where ΔPG = protein growth (kg)

The daily accretion of water ($\Delta Water$ (Emmans and Kyriazakis, 1997; Emmans and Fisher, 1986)) is estimated as:

$$\Delta Water = 2.65 \Delta PG \left(\frac{P}{P_m} \right)^{-0.185} \text{ (kg/day)} \tag{5}$$

The expected maximum daily wool growth ($\Delta PWool_{max}$ (Cronje and Smuts, 1994)) is estimated as:

$$\Delta PWool_{max} = \left(\frac{0.0009 \cdot P}{P_m^{0.27}} \right) + \left(0.16 \cdot \Delta PG_{max} \right) \text{ (kg/day)} \tag{6}$$

Gut fill (GF) depends on the properties of the food that the sheep has access to, mainly energy content, and is estimated according to Coffey *et al.* (2001) as:

$$GF = FI \cdot \left(11 - \left(\frac{7 \cdot ME}{15} \right) \right) \text{ (kg/day)} \tag{7}$$

where FI = food intake (kg DM) and ME = metabolisable energy of the feed (MJ/kg DM).

2.6.2 Resource requirements and food intake

The protein required for maintenance (PR_{maint} (Wellock *et al.*, 2003)) is estimated as:

$$PR_{maint} = 0.004 \cdot \left(\frac{P}{P_m^{0.27}} \right) \text{ (kg/day)} \tag{8}$$

The protein required for growth (PR_{Growth} (Wellock *et al.*, 2003)) is estimated as:

$$PR_{Growth} = \frac{\Delta PG_{max}}{ep} \text{ (kg/day)} \tag{9}$$

where ep = efficiency of protein deposition (0.26 (AFRC, 1993)).

The protein required for wool (PR_{Wool} (Vagenas *et al.*, 2007a)) is estimated as:

$$PR_{Wool} = \frac{\Delta PWool_{max}}{ew} \text{ (kg/day)} \tag{10}$$

where ew = efficiency of protein use for wool (0.59 (AFRC, 1993)).

The energy required for maintenance (ER_{maint} (Emmans and Fisher, 1986)) is estimated as:

$$ER_{maint} = 1.63 \cdot \left(\frac{P}{P_m^{0.27}} \right) \text{ (kg/day)} \tag{11}$$

The energy required for growth (ER_{Growth} (Wellock *et al.*, 2003)) is estimated as:

$$ER_{Growth} = \left(bl \cdot \Delta L_{des} \right) + \left(bp \cdot \Delta PG_{max} \right) \text{ (kg/day)} \tag{12}$$

where bl = energetic cost per kg of lipid deposition (56 MJ/kg (Emmans, 1994)) and bp = energetic cost per kg of protein deposition (50 MJ/kg (Emmans, 1994)).

The energy required for wool (ER_{Wool} (Vagenas *et al.*, 2007a)) is estimated as:

$$ER_{Wool} = bp \cdot \Delta PWool_{max} \text{ (kg/day)} \tag{13}$$

The desired food intake for meeting the energy requirements of the lamb (FI_E) is estimated as:

$$FI_E = \frac{ER}{EEC} \text{ (kg DM/day)} \tag{14}$$

where EEC = effective energy content (Emmans, 1994).

The desired food intake for meeting the protein requirements of the lamb (FI_P) is estimated as:

$$FI_P = \frac{PR}{MP} \text{ (kg DM/day)} \tag{15}$$

where MP = feed metabolisable protein content (AFRC, 1993).

The relationship between Effective Energy (*EE*, MJ/kg) and metabolisable energy (*ME*, MJ/kg) is given as:

$$EE = 1.15ME - 3.84 - 4.67DCP \text{ (MJ/kg organic matter)} \qquad \textbf{(16)}$$

where *DCP* = digestible crude protein, $DCP = 0.9CP - 0.032$ (g/kg DM) (Emmans, 1994).

2.6.3 Constrained resources

Constrained food intake (*CFI*) is defined as (Lewis *et al.*, 2004):

$$CFI = \frac{CAP}{0.93 - \left(\dfrac{ME}{15.58}\right)} \text{ (kg/day)} \qquad \textbf{(17)}$$

where *CAP* = capacity of the animal for daily indigestible organic matter (kg) and *ME* = metabolisable energy content of feed (MJ/kg DM).

The capacity of the animal for daily indigestible organic matter (*CAP*, kg) (Lewis *et al.*, 2004) is estimated as the smaller of:

$$CAP = 0.0223 \cdot BW$$

or: **(18)**

$$CAP = 0.0223 \cdot 0.51 \cdot BW_{\text{m}} \text{ (kg/day)}$$

where *BW* = current body weight of lamb (kg) and BW_{m} = body weight of lamb at maturity (kg).

2.6.4 Allocation of nutrients

The daily lipid deposited (Δ*Lipid*) (Vagenas *et al.*, 2007a) is:

$$\Delta Lipid = \frac{\left((FI \cdot EEC) - E_{\text{maint}} - E_{\text{Protein}}\right)}{bl} \text{ (kg/day)} \qquad \textbf{(19)}$$

where E_{maint} = energy for maintenance (MJ/day), $E_{Protein}$ = energy for protein, $E_{Protein} = bp \cdot \Delta PG_{max}$ (MJ/day).

If $\Delta Lipid$ is negative, then lipid will be catabolised to satisfy the animal's energetic needs for other functions as follows:

$$\Delta Lipid = \frac{\left(\left(FI \cdot EEC \right) E_{Maint} - E_{Protein} \right)}{bl_{C}} \text{ (kg/day)} \tag{20}$$

where bl_{C} = heat combustion of lipid (39 MJ/kg (AFRC, 1993)).

Labile protein (P_{Labile} (Houdijk *et al.*, 2001; Sykes, 2000)) is defined by:

$$P_{Labile} = 0.2 \cdot P_{max} \text{ (kg)} \tag{21}$$

where P_{max} = maximum achieved body protein content (kg).

The baseline body lipid level (L_{base} (Vagenas *et al.*, 2007a)) is estimated as:

$$L_{base} = 0.2 \cdot P \text{ (kg)} \tag{22}$$

2.6.5 Protein loss

The potential protein loss (PLI_{Pot}) due to larval intake (LI) when there is no immune response is given as (Yin *et al.*, 2003):

$$PLI_{Pot} = PLoss_{max} \cdot \left(1 + \frac{LI_{max} - LI}{LI_{max} - LI_{infl}} \right) \cdot \left(\frac{LI}{LI_{max}} \right)^{\left(\frac{LI_{max}}{LI_{max} - LI_{infl}} \right)} \text{ (kg/day)} \tag{23}$$

where $PLoss_{max}$ = daily protein loss when LI equals LI_{max} (0.01 kg/day (Steel *et al.*, 1980)), LI_{infl} = inflection point of the relationship between PLI_{Pot} and LI (5,000 larvae per day (Vagenas *et al.*, 2007a; b)) and LI_{max} = maximum of the relationship between LI and PLI_{Pot} (10,000 larvae per day (Steel *et al.*, 1980)).

Protein loss due to larval intake (PLI) (Vagenas *et al.*, 2007a) is given as:

$$PLI = PLI_{Pot} \cdot \left(\frac{PLI_{Pot} \cdot e^{-K_{Imm} \cdot PRQ_{Imm}}}{PLoss_{max}} \right)^{\left(\frac{PAC_{Imm}}{(PAC_{Imm})_{max}} \right)} \quad \text{(kg/day)} \tag{24}$$

where PRQ_{Imm} = protein required for immunity, PAC_{Imm} = protein allocated daily to immunity (kg/day) and $(PAC_{Imm})_{max}$ = maximum protein allocated to immunity ($0.2 \cdot P_{maint}$ (kg/day) (Houdijk *et al.*, 2001)), K_{Imm} = exponent associated with PAC_{Imm} (equation 25).

The exponent associated with PAC_{Imm} (K_{Imm}) (Vagenas *et al.*, 2007a) is given as:

$$K_{Imm} = -\frac{\ln\left(\frac{PLoss_{min}}{PLoss_{max}} \right)}{(PAC_{Imm})_{max}} \tag{25}$$

where $PLoss_{min}$ = value at which the animal stops allocating protein to immunity (0.0001 (Vagenas *et al.*, 2007a; b)).

Fecundity was scaled (F_{Scaled}) (Bishop and Stear, 1997) as:

$$F_{Scaled} = F \cdot \left(\frac{WB}{2500} \right)^{-0.25} \tag{26}$$

Worm mass (WM) (Vagenas *et al.*, 2007a) is estimated as:

$$WM = WB \cdot F_{Scaled} \tag{27}$$

The protein loss caused by worm mass (PWM) is given as (Vagenas *et al.*, 2007a):

$$PWM = PLoss_{max} \cdot \left(1 + \frac{LI_{max} - (0.8 \cdot WM)}{LI_{max} - LI_{infl}} \right) \cdot \left(\frac{0.8 \cdot WM}{LI_{max}} \right)^{\left(\frac{LI_{max}}{LI_{max} - LI_{infl}} \right)} \quad \text{(kg/day)} \tag{28}$$

2.6.6 Immune response

The protein required for immunity for larval intake ($PRQ_{\text{LI\_Imm}}$) (Vagenas *et al.*, 2007a) is estimated as:

$$PRQ_{\text{LI\_Imm}} = \left(PAC_{\text{Imm}}\right)_{\max} \cdot \frac{\ln\left(\dfrac{PLoss_{\min}}{PLI_{\text{Pot}}}\right)}{\ln\left(\dfrac{Ploss_{\min}}{PLoss_{\max}}\right)} \text{ (kg/day)} \tag{29}$$

where $PLoss_{\min}$ = minimum damage for which there is no immune response (0.0001 (Vagenas *et al.*, 2007a; b)).

The protein required for immunity for worm mass ($PRQ_{\text{WM\_Imm}}$) (Vagenas *et al.*, 2007a) is estimated as:

$$PRQ_{\text{WM\_Imm}} = -\frac{\ln\left(\dfrac{PLoss_{\min}}{PWM}\right)}{-K_{\text{Imm}}} \text{(kg/day)} \tag{30}$$

2.6.7 Effect of parasitism on protein partitioning

Protein allocated to production (PAC_{Growth}) (Vagenas *et al.*, 2007a) is given as:

$$PAC_{\text{Growth}} = \frac{PR}{PR + \left(PRQ_{\text{Imm}}\right)_{\text{Tot}}} \text{ (kg/day)} \tag{31}$$

Protein allocated to immunity (PAC_{Imm}) (Vagenas *et al.*, 2007a) is given as:

$$PAC_{\text{Imm}} = \frac{\left(PRQ_{\text{Imm}}\right)_{\text{Tot}}}{PR + \left(PRQ_{\text{Imm}}\right)_{\text{Tot}}} \text{ (kg/day)} \tag{32}$$

Protein associated with the immune function (P_{Imm}) (Vagenas *et al.*, 2007a) is estimated as:

$$P_{\text{Imm}} = 0.59 \cdot PAC_{\text{Imm}} \text{ (kg/day)} \tag{33}$$

Chapter Three

Exploration of the impact of pasture larvae contamination and anthelmintic treatment on genetic parameter estimates for parasite resistance in grazing sheep

3.1 Introduction

Gastrointestinal parasitism in grazing lambs adversely affects animal performance and welfare, and causes significant production losses for the sheep industry (Nieuwhof and Bishop, 2005). Current treatment of parasitism relies heavily on the use of anthelmintics, however the development of anthelmintic resistance is an increasing concern (Jackson, 2008). Breeding for host resistance to nematodes is an alternative control strategy, supported by evidence of heritable variation for faecal egg count (FEC) (Bishop and Morris, 2007) and by results from selection in practice (Kemper *et al.*, 2010).

Commercial breeding programs for resistance require knowledge of genetic parameters for host resistance and production traits, such as heritabilities and genetic correlations between traits. Whilst heritabilities for FEC and BW are relatively consistent (e.g., 0.2 to 0.4), estimates of genetic correlations between FEC and BW are variable, ranging from -0.8 (Bishop *et al.*, 1996) to +0.4 (McEwan *et al.*, 1992; 1995). Variation observed in such correlations may be due to interactions between host genetic resistance and the environment, including parasite epidemiology. Knowledge of how these interactions affect the estimates of genetic parameters is therefore desirable for designing appropriate multi-trait breeding strategies. Gaining this information experimentally is expensive and time consuming. However, exploring such interactions using appropriate mathematical tools may provide

insights into how disease epidemiology and treatment protocols influence estimates of genetic parameters for host resistance and production.

The aim of this study was to use an *in silico* model to explore epidemiological effects (e.g., level of pasture larval contamination) and anthelmintic input on the estimates of genetic parameters for a population of grazing lambs. The hypothesis was that both will strongly influence the direction and magnitude of the correlation between production and resistance traits.

3.2 Materials and Methods

A previously published model (Laurenson *et al.*, 2011) (**Chapter 2**) that accounts for the interactions between host nutrition, genotype and gastrointestinal parasitism in individual lambs was extended to a population of animals, incorporating heritable between-lamb variation in host-parasite interactions. Further to this, a simple epidemiological module was developed to model the free-living stages of the parasite, with host ingestion of infective larvae linked to food intake (FI) to create a grazing scenario.

A brief description of the individual lamb model is given, as well as some necessary modifications to the published model. A more in-depth explanation of how the individual lamb model was extended to a population of animals, the epidemiological module, and the incorporation of anthelmintic drenching protocol is detailed.

3.2.1 Individual lamb model

A schematic diagram describing the structure of the individual lamb model is provided in Figure 3.1. Briefly, we assume the growing lamb attempts to ingest sufficient nutrients to meet requirements for desired growth and maintenance. Infection with gastrointestinal parasites is expected to result in endogenous protein loss (a function of larval challenge and worm burden). Consequently, the lamb

invests in an immune response in order to reduce the impact of parasitism, and this investment itself has a resource (e.g., protein) cost. However, components of the acquisition of immunity (e.g., cytokines) cause a reduction in FI (Greer *et al.*, 2005; Kyriazakis, 2010), commonly known as anorexia. This reduction in FI, which was modelled as a direct function of the rate of acquisition of immunity (Laurenson *et al.*, 2011) (**Chapter 2**), results in the animal ingesting insufficient nutrient resources to fulfil requirements for maintenance and growth. Ingested protein, after the loss due to parasitism, is assumed to be first allocated to meet the maintenance requirements of the animal and remaining protein is allocated towards production and immunity proportional to requirements.

The acquisition of immunity was assumed to be a sigmoidal function of cumulative larval presence in the gastrointestinal tract summed across time, rather than exposure to infective larvae as modelled previously (Laurenson *et al.*, 2011) (**Chapter 2**), as this better captures larval population dynamics particularly with interventions such as anthelmintic treatment. The corresponding functions used to describe the host immune response are given in section 3.2.2.3.
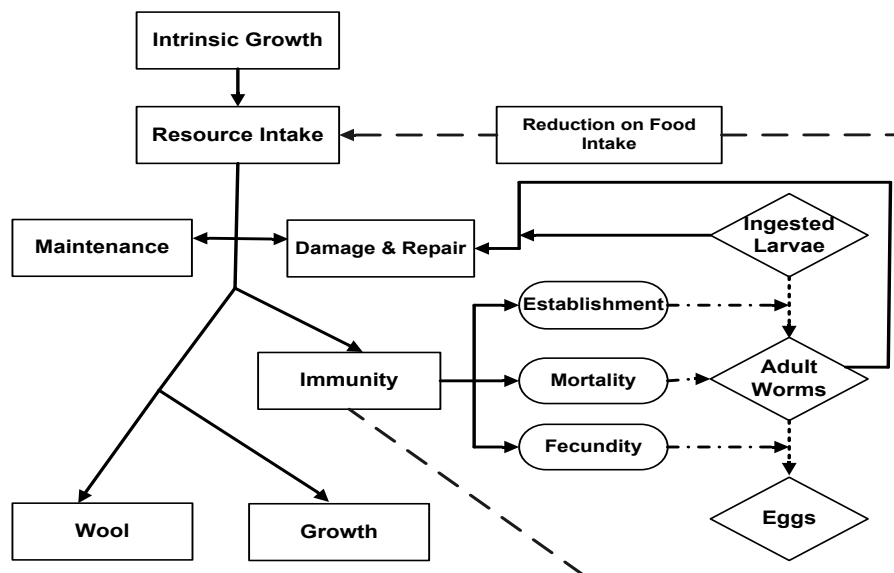
Figure 3.1. A schematic description of the host-parasite interactions in sheep infected with gastrointestinal nematodes. Rectangular boxes indicate the flow of food resources, rounded boxes indicate host-parasite interactions and diamond boxes indicate key quantifiable parasite life-cycle stages.

3.2.2 Population model

Between-animal variation was modelled according to Vagenas *et al.* (2007c), and was assumed to occur for the animal's growth attributes, the maintenance requirements, and in the immune response to gastrointestinal parasites.

3.2.2.1 Variation in growth

The growing lamb was described by the initial fleece-free empty BW (EBW$_0$) and the expected protein and lipid body mass at maturity (P_m and L_m, respectively). Growth was driven by body protein and lipid retention, with expected growth rates being estimated as (Emmans and Kyriazakis, 1997; 1999):

$$dP/dt = P \cdot B \cdot \ln\left(\frac{P_m}{P}\right) \text{ (kg/d)}$$

$$dL/dt = dP/dt \cdot \left(\frac{L_m}{P_m}\right) \cdot d \cdot \left(\frac{P}{P_m}\right)^{(d-1)} \text{ (kg/d)}$$

where *dP/dt* = expected rate of protein retention (kg/d), *dL/dt* = expected rate of lipid retention (kg/d), *P* = current body protein mass (kg), *L* = current body lipid mass (kg), $B = \dfrac{0.023}{P_m^{0.27}}$ (kg/d), and $d = 1.46 \cdot \left(\dfrac{L_m}{P_m}\right)^{0.23}$

Differences in EBW$_0$ at weaning (2 months of age), together with differences in the parameters P_m and L_m, lead to growth rate and final size differences between animals. Thus, these parameters were assumed to be variable and under partial genetic control.

3.2.2.2 Variation in maintenance requirements

The body maintenance requirements for protein (P_{maint}, kg/d) and ME (ME_{maint}, MJ/d) were estimated by Emmans and Kyriazakis (2001) as:

$$P_{\text{maint}} = p_{\text{maint}} \cdot \left(\frac{P}{P_m^{0.27}} \right) \text{ (kg/d)}$$

$$ME_{\text{maint}} = e_{\text{maint}} \cdot \left(\frac{P}{P_m^{0.27}} \right) \text{ (MJ/d)}$$

where p_{maint} = constant associated with protein requirements for maintenance, and e_{maint} = constant associated with energy requirements for maintenance (Emmans, 1994).

Variation in the parameters p_{maint} and e_{maint} signifies differences in the maintenance requirements for protein and energy. Thus, these input parameters were assumed to vary between animals and to be under partial genetic control (Knap and Schrama, 1996).

3.2.2.3 Variation in immune response

The immune response is represented by the host-controlled traits of establishment (ε), mortality (μ) and fecundity (F). The functions used to describe these traits were given as (adapted from Louie *et al.*, 2005):

$$\varepsilon = \left(\frac{\varepsilon_{\max} \cdot K_\varepsilon^3}{K_\varepsilon^3 + \left(\sum_t LP \right)^3} \right) + \varepsilon_{\min} \text{ (proportion of larvae establishing/day)}$$

$$\mu = \left(\frac{\mu_{\max} \cdot \left(\sum_t LP \right)^3}{mi^3 + \left(\sum_t LP \right)^3} \right) + \mu_{\min} \text{ (proportion of adult worms/day)}$$

$$F = \left(\frac{F_{max} \cdot fi^3}{fi^3 + \left(\sum_t LP \right)^3} \right) + F_{min} \quad \text{(eggs/worm/day)}$$

where ε_{max}, μ_{max} and F_{max} = maximum establishment, mortality and fecundity rates, respectively; ε_{min}, μ_{min} and F_{min} = minimum establishment, mortality and fecundity rates, respectively; $\sum_t LP$ = cumulative larval population present in host; K_ε, mi and fi = rate constants for establishment, mortality, and fecundity, respectively.

The lambs were initially naïve to gastrointestinal parasites and acquired immunity as a function of exposure to the infective larval population resident in the host at a rate determined by K_ε, mi, and fi for their respective immunity traits. Subsequently, genetic variation in these rate determining parameters can result in differences in the rate of acquisition of immunity. Further, non-genetic variation in the maxima of traits (ε_{max}, μ_{max}, F_{max}) and the minimum mortality rate (μ_{min}) was also introduced. The minima for fecundity and establishment were set to zero for all animals (Vagenas *et al.*, 2007c).

3.2.2.4 Variation in food intake

In addition to variation in traits related to performance and resistance, random environmental variation in FI (SFI) was assumed to reflect the influence of external factors controlling FI not accounted for explicitly by the model. Due to the correlation between growth and FI tending towards unity in this model, a daily random environmental deviation was added to the expected FI for each animal to achieve a more realistic genetic correlation between FI and growth rate of approximately 0.8 (Cammack *et al.*, 2005).

3.2.2.5 Parameter values and distributions

The model was parameterised such that lamb growth was similar to that of typical breeds grazing British upland and hill pastures (e.g., Blackface), and so that parasitological parameters matched those of Coop et al. (1982). Parameter (mean) values were largely the same as those presented by Laurenson *et al.* (2011) (**Chapter 2**), except that the maximum allowable daily protein loss due to parasitism was increased to 100g, to bring the predicted parasite-induced reductions in growth rate in line with the published values (Coop *et al.*, 1982). The actual average daily protein loss predicted by the model was between 14g and 27g.

The properties of each trait with between-host variation were specified by the population mean, the heritability (h^2) and the coefficient of variance (CV) for each trait (Table 3.1). Estimates for the mean, CV and h^2 of input parameters were chosen to match those of Bishop *et al.* (1996) and Bishop and Stear (1997). It was assumed that all input parameters were normally distributed. As such, predictions for performance traits were normally distributed. However, whilst the immunity related traits were initially normally distributed, as individual lambs developed and expressed immunity, the output traits such as worm burden (WB) and FEC became skewed, matching the over-dispersion described by Bishop and Stear (1997).

All traits, other than the rate-determining parameters associated with immune acquisition, were assumed to be uncorrelated (Doeschl-Wilson *et al.*, 2008). However, the acquisition of immunity was assumed to be a function of overlapping effector mechanisms for establishment, mortality and fecundity (components of Th2 immune responses). Thus, the rate-determining parameters (K^ε, *mi, fi*) were assumed to be strongly genetically and phenotypically correlated (r = 0.5).

Table 3.1. Lamb traits for which variation between animals is assumed within the model and their corresponding parameter values.

Parameter	Description	Category	Mean	CV^1	h^2
P_m, kg	Mature protein mass		9.525	0.10	0.50
L_m, kg	Mature lipid mass	Growth	40.11	0.15	0.50
EBW_0, kg	Initial empty BW		12.73	0.15	0.50
p_{maint}	Coefficient for maintenance protein requirements		0.004	0.15	0.25
e_{maint}	Coefficient for maintenance energy requirements	Maintenance	1.63	0.15	0.25
SFI	Deviation in daily food intake	Growth and maintenance	0.00	0.10	0.00
ε_{max}	Max. establishment rate		0.70	0.20	0.00
μ_{max}	Max. mortality rate		0.11	0.20	0.00
μ_{min}	Min. mortality rate		0.01	0.20	0.00
F_{max}	Max. fecundity rate		20.0	0.20	0.00
K_ε	Rate parameter for larvae establishment	Resistance	190,000	0.60	0.25
mi	Rate parameter for worm mortality		650,000	0.60	0.25
fi	Rate parameter for worm fecundity		210,000	0.60	0.25

[1] Phenotypic variance $\left(\sigma_P^2\right) = \left(\text{mean} \cdot \text{CV}\right)^2$

3.2.2.6 Individual animal phenotypes

As described by Vagenas *et al.* (2007c), animals were simulated within a pre-defined population structure, comprising founder animals, for which breeding values were simulated, and their progeny, for which genotypes and phenotypes were created. Each founder animal had a breeding value A_i for each genetically controlled input trait, sampled from a $N(0, \sigma_A^2)$ distribution, where the genetic variance ($\sigma_A^2 = h^2 \sigma_P^2$) is given by the model inputs for the heritability (h^2) and phenotypic variation (σ_P^2). The breeding values for each trait for each offspring were constructed as $\dfrac{\left(A_{Sire} + A_{Dam}\right)}{2}$ plus a Mendelian sampling term, drawn from a $N(0, 0.5\sigma_A^2)$ distribution (Falconer and

Mackay, 1996). A Cholesky decomposition of the variance-covariance matrix for correlated traits was used to generate the covariances between the breeding values of the animals and also between their residual components (see below). The phenotypic value (P_i) for each of the underlying traits was given by:

$$P_i = \mu + A_i + E_i$$

where μ is the population mean for the trait, A_i is the additive genetic deviation of the ith individual, and E_i is the corresponding environmental deviation sampled from a normal distribution $N(0, \sigma_P^2 (1\text{-}h^2))$.

3.2.3 Epidemiological module

3.2.3.1 Pasture

The pasture was initially defined by the number of hectares (H, ha) that the lamb population was grazing. The grass available (G, kg DM) for grazing was then calculated using a defined initial quantity of grass per hectare (GPH, kg DM/ha). Each day, the grass available was updated to take into account consumption by the lamb population and new grass growth. As such, G was estimated for day t as:

$$G_t = \left(G_{t\text{-}1} - \sum FI_{t\text{-}1}\right) + \left(H \cdot GR\right) \text{ (kg DM)}$$

where $\sum FI$ = total food intake of lamb population (kg DM), GR = grass growth (kg DM/ha, Table 3.2).

Table 3.2. Epidemiological and environmental input values to the model.

Parameter	Description	Value	Source
MR	Mortality rate of larvae	0.035	Gibson and Everett, 1972
PEI	Proportion of eggs to larvae	0.11	Boag and Thomas, 1975
H, ha	Hectares	333	Specified by grazing density
GPH, kg DM/ha	Grass per hectare	1500	Sibbald *et al.*, 2000
GR, kg DM/ha·d	Grass growth	60	Grass Check, 2011[1]

[1]http://www.dardni.gov.uk/ruralni/grcheck270611.pdf (accessed Jun. 30, 2011).

3.2.3.2 Larval contamination of pasture

To create starting conditions, it was assumed that the initial larval contamination of pasture (IL$_0$) arose from a ewe population which was removed from the pasture at lamb weaning (2 months), and was defined by an initial population of eggs and infective larvae (larvae/kg DM). For simplicity, this initial egg contamination of the pasture was modelled such that the number of infective larvae developing on pasture was equal to the number of larvae consumed by the lamb population for the first 7 days, this being the time taken for eggs to develop to infective larvae (TEI, days) (Young *et al.*, 1980). Subsequently, infective larvae arose only from eggs excreted onto pasture by lambs. Infectious larvae, irrespective of their source, were assumed to have a mortality rate (MR; Table 3.2).

Larvae ingested by lambs during grazing, develop into adult worms after 14 days (corresponding to the first day at which eggs are present in the faeces; (Coop *et al.*, 1982)). Adult female worms produce eggs that are excreted in the faeces of the lamb; after developing to infective larvae (TEI, above), these contribute to the larval contamination of the pasture (LC). Larval contamination arising from recontamination by grazing lambs is therefore given as:

$$LC_t = \left(\left(LC_{t\text{-}1} - \sum LI_{t\text{-}1}\right)\cdot\left(1-MR\right)\right)+\left(\sum E_{t-TEI}\cdot PEI\right) \text{ (larvae)}$$

where $\sum LI$ = total larval intake of lamb population, $\sum E$ = total egg output of lamb population, PEI = proportion of eggs developing to infective larvae, Table 3.2.

3.2.3.3 Linking larval intake to food intake

Lambs were assumed to graze randomly across the pasture, thus leading to an equal expected larval intake (LI, larvae) linked to FI (kg DM). The LI of any lamb can be determined from its FI, the grass available for grazing (*G*, kg DM) and the total LC (larvae). Thus, LI for any given lamb was given by:

$$LI_t = \frac{LC_t}{G_t} \cdot FI_t \ \text{(larvae)}$$

3.2.4 Anthelmintic drenching

The ability to give anthelmintic drenches to the lamb population at specified days was included in the model. The anthelmintic drench was specified as having a 95% efficacy against *Teladorsagia circumcincta* (Sargison *et al.*, 2007). Each anthelmintic drench was assumed to equally reduce the WB and larval population resident in the host. Further, the oral administration of anthelmintic was assumed to be effective on the day of administration only, with no residual effects (Borgsteede, 1993).

3.2.5 Simulation procedure and *in silico* experimental design

The simulated flock comprised 10,000 lambs which were assumed to be twins from a non-inbred, unrelated base population of 250 rams, each mated with 20 randomly chosen ewes. The input phenotypes for the lamb population, as described above, are given in Table 3.1. Lambs were grazed on a medium-quality pasture (crude protein = 140g/kg DM, metabolisable energy = 10MJ/kg DM (AFRC, 1993)), at a grazing density of 30 lambs/ha, for a time period of 4 months from weaning to 6 months of age.

The lambs were assumed to be initially naïve, and the initial larval contamination of pasture (IL_0) was set to either control (clean), 1,000, 3,000 or 5,000 *T. circumcincta* larvae/kg DM. Lambs initially ingest around 1kg DM/day and thus these levels correspond to the trickle challenge levels chosen by Coop *et al.* (1982) that led to sub-clinical infections. Further, the lamb population was either given no anthelmintic drench or drenched at 30 day intervals (days 30, 60 and 90) aimed at achieving suppressive nematode control, representing the two extremes of commercial practice (Sargison *et al.*, 2007). Performance traits (empty body weight (EBW, kg) and FI), parasitological traits (FEC and WB), and epidemiological traits (LC, larvae/kg DM) were recorded on a daily basis.

The parasitological traits of WB and FEC were log transformed for the calculation of genetic parameter estimates. Genetic variances and co-variances of the model output traits, and hence heritabilities and genetic/phenotypic correlations, were estimated from a linear mixed model, fitting sire as a random effect. From these outputs it was possible to assess the impact of IL_0 and anthelmintic treatments on trait means, on the genetic parameter estimates for the population of growing lambs, and to observe the evolution of parameters across time.

3.3 Results

3.3.1 Frequency distributions of output traits

Output performance traits were normally distributed at all times. For example, the means (and SD) for EBW were 21.3 (2.5), 31.2 (2.8), 41.5 (3.1) and 51 (3.5) kg at days 30, 60, 90 and 120 post infection, respectively, for lambs grazing on a clean (uncontaminated) pasture.

Although input parameters associated with host resistance were normally distributed, output FEC (Figure 3.2) and WB (results not shown) were skewed. The skewness of the FEC (and WB) distributions changed over time, becoming

progressively right-skewed as the animals became immune, in agreement with field data (Bishop *et al.*, 1996).
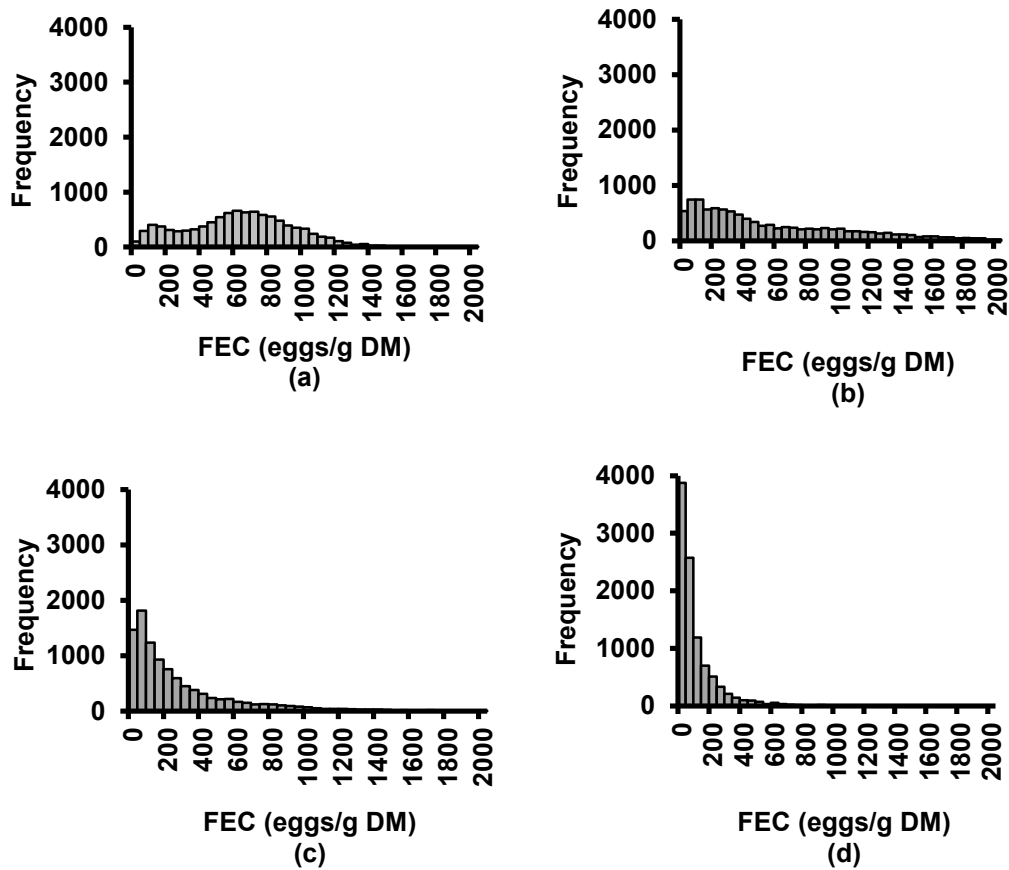
Figure 3.2. Frequency distribution of faecal egg count (FEC) of 10,000 lambs grazing on medium-quality pasture initially contaminated with 3,000 *Teladorsagia circumcincta* larvae/kg DM grass, at a time point of 30 (a), 60 (b), 90 (c) and 120 (d) days post-infection.

3.3.2 Performance traits

Mean population values for EBW and FI are given in Figure 3.3. The average FI over the entire simulated period for the clean pasture was predicted to be 1.96kg/d, whilst the average FI for lambs grazed on pasture with an IL_0 of 1,000, 3,000 and 5,000 larvae/kg DM was predicted to be reduced in comparison with the control group by 13%, 18%, and 21%, respectively, for non-drenched lambs, and 11%, 12%, and 14%,

respectively, for drenched lambs. LC determined LI and the rate of acquisition of immunity, consequently leading to reductions in FI. Thus, increased levels of LC led to increased reductions in FI. The reduction in FI was less for drenched lambs than non-drenched lambs because of the reduction in larval population and subsequently a reduced rate of acquisition of immunity.

Empty BW at the end of the simulation also showed similar predicted reductions for the varying IL_0 level. EBW predicted for lambs grazing on pasture with an IL_0 of 1,000, 3,000 and 5,000 larvae/kg DM were reduced in comparison to the control group by 13%, 19%, and 23%, respectively for non-drenched lambs, and 7%, 9%, and 11%, respectively, for drenched lambs. For non-drenched lambs, reductions in EBW followed reductions in FI, with the added impact of protein loss due to parasitism. For drenched lambs, the reduction in EBW was less than the reductions predicted for FI. This was due to a decrease in the nutrient requirements for the functions associated with parasitism.
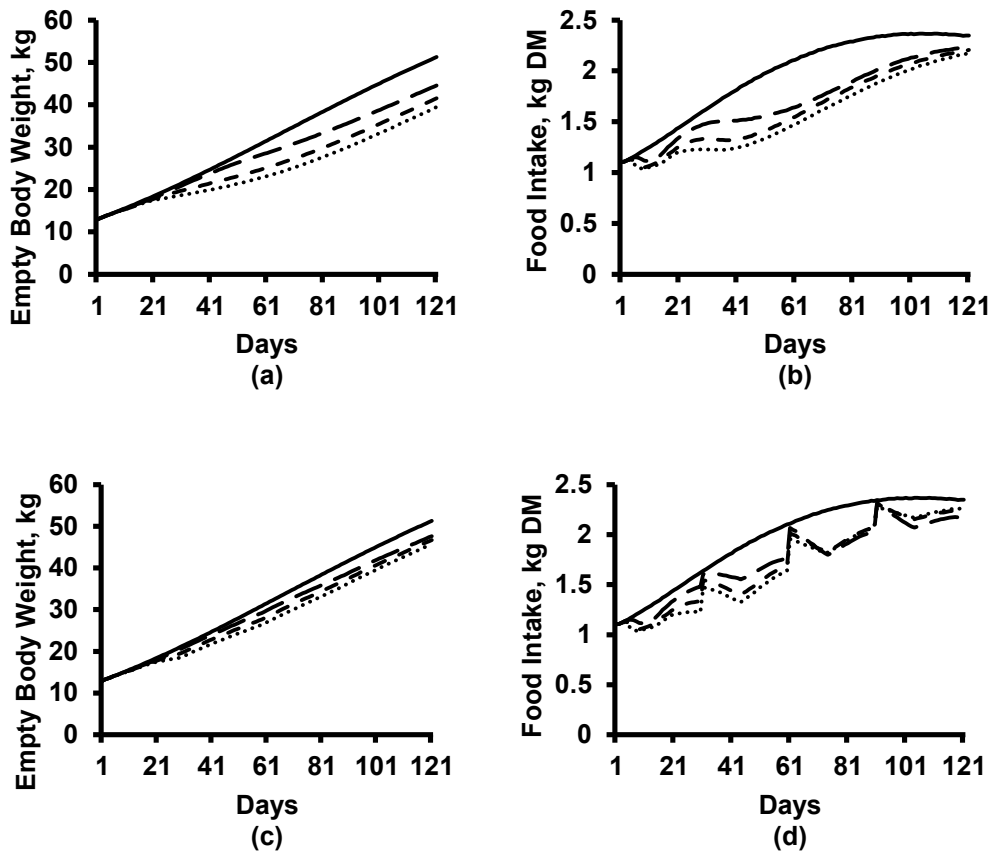
Figure 3.3. Mean production traits of 10,000 lambs grazing on medium-quality pasture initially contaminated with either control (———), 1,000 (— —), 3,000 (– – –) or 5,000 (··········) *Teladorsagia circumcincta* larvae/kg DM grass. Production traits given are empty body weight (a) and food intake (b) for non-drenched lambs, and empty body weight (c) and food intake (d) for drenched lambs.

3.3.3 Parasitological traits

Mean population values for WB and FEC are given in Figure 3.4. For the non-drenched lambs, the maximum predicted WB increased and occurred sooner for increasing IL_0. The maximum average WB (and day of maximum) were 52,645 (84d), 59,878 (70d) and 71,383 (64d) for the IL_0 levels of 1,000, 3,000 and 5,000 larvae/kg DM, respectively. Maximum predicted FEC, and day of maximum, followed a similar pattern. The maximum average FEC (and day of maximum) were 482 (61d), 666 (49d), 937 (30d) eggs/g DM for the IL_0 levels of 1,000, 3,000 and 5,000 larvae/kg DM, respectively. Increased average LC led to increased LI and

subsequently increased WB and FEC; however, the increased rate of acquisition of immunity led to earlier predicted days of maxima for WB and FEC.

For the drenched lambs, the WB and FEC were the same as the non-drenched lambs until first drenching, whereupon WB and FEC dropped dramatically. WB and FEC increased again 14 days after each anthelmintic administration, coinciding with the infective larvae maturation time. For the first 74 days of the simulation, predictions for WB and FEC increased with increasing IL_0. Subsequently, there was a tendency for this pattern to be reversed, as acquisition of immunity was slower on pastures with lower IL_0.
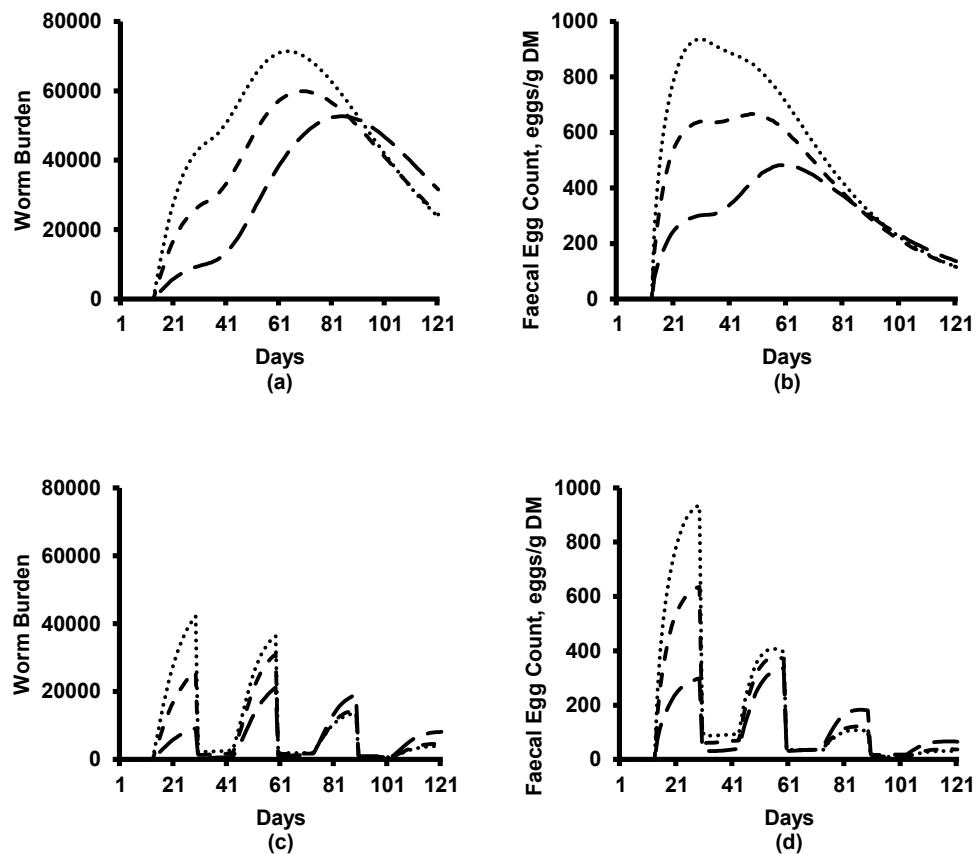
Figure 3.4. Mean parasitological traits of 10,000 lambs grazing on medium-quality pasture initially contaminated with either 1,000 (— —), 3,000 (– – –) or 5,000 (··········) *Teladorsagia circumcincta* larvae/kg DM grass. Parasitological traits given are worm burden (a) and faecal egg count (b) for non-drenched lambs, and worm burden (c) and faecal egg count (d) for drenched lambs.

3.3.4 Pasture larval contamination

Predictions for LC (larvae/kg DM) are given in Figure 3.5. As modelled, LC fell slowly for the first 7 days whilst larvae deposited by the ewes were still developing, then decreased more rapidly until new larvae were observed on pasture from day 21 onwards, whereupon LC increased rapidly. The maximum predicted LC (and day of maximum) for non-drenched lambs were 4978 (83d), 5762 (67d) and 6977 (57d) larvae/kg DM for IL_0 levels of 1,000, 3,000 and 5,000 larvae/kg DM, respectively. Lambs grazing on pasture with lower levels of IL_0 took longer to acquire immunity than lambs grazing on higher levels, and hence had a delay before LC decreased. Ultimately, LC levels were becoming rather similar at the end of the simulation period regardless of IL_0.

For drenched lambs, LC was reduced 7 days after the administration of each anthelmintic drench, this being the time taken for eggs to mature to infective larvae, and continued to fall for a period of 14 days.
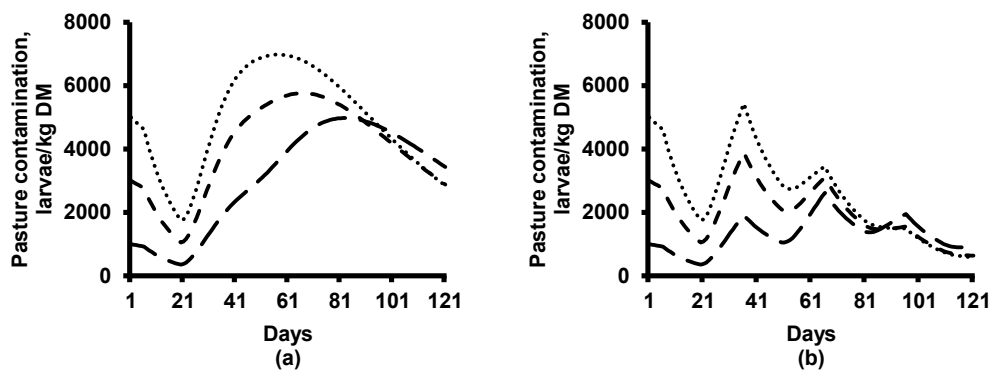
Figure 3.5. Pasture larval contamination (larvae/kg DM) for medium-quality pasture initially contaminated with either 1,000 (— —), 3,000 (– – –) or 5,000 (·········) *Teladorsagia circumcincta* larvae/kg DM grass, for non-drenched lambs (a), and drenched lambs (b).

3.3.5 Heritabilities

The heritabilities (h^2) for EBW and log transformed WB and FEC are given in Figure 3.6. The h^2 of EBW for the control group remained relatively constant at around 0.55 throughout the simulation, with the initial value (0.53) differing from the input value of 0.5 simply as a function of sampling from random distributions. However, h^2 of EBW for non-drenched lambs grazing contaminated pasture tended to fall with age, reaching minimum values of 0.36, 0.29, and 0.27 for IL_0 of 1,000, 3,000 and 5,000 larvae/kg DM, respectively. For drenched lambs the h^2 of EBW remained high, never dropping below 0.47 for all levels of IL_0. The h^2 of EBW is a function of both the intrinsic genotype for EBW and the host genotype associated with resistance to parasites. The latter affects EBW through the acquisition of immunity leading to the realised WB levels and the associated production penalties. For the control group, the host resistance genotype has no impact on the heritability of EBW and consequently this parameter remained high (0.55). However, for non-drenched lambs, the host resistance genotype had a large impact on the h^2 of EBW and led to the reduced values reported above. For drenched lambs, due to the decreased impact of parasitism, the host resistance genotype had a lesser impact on the h^2 of EBW, and thus this parameter remained high (0.47).

Estimates of h^2 for log transformed WB and FEC were initially close to zero, but increased over time as genetic differences in immunity became more apparent. Final estimates for WB and FEC were around 0.25, with increasing levels of IL_0 resulting in this value being reached at slightly faster rates. For drenched lambs, a rapid increase in h^2 is predicted at time intervals approximating the anthelmintic drenching protocol. The reduced WB following drenching causes a reduction in the impact of host resistance genotype on the h^2 of both WB and FEC. The new WB following drenching is more strongly influenced by FI which, as a function of EBW, is highly heritable. Thus the heritabilities of WB and FEC tend to rise abruptly following anthelmintic drenching.
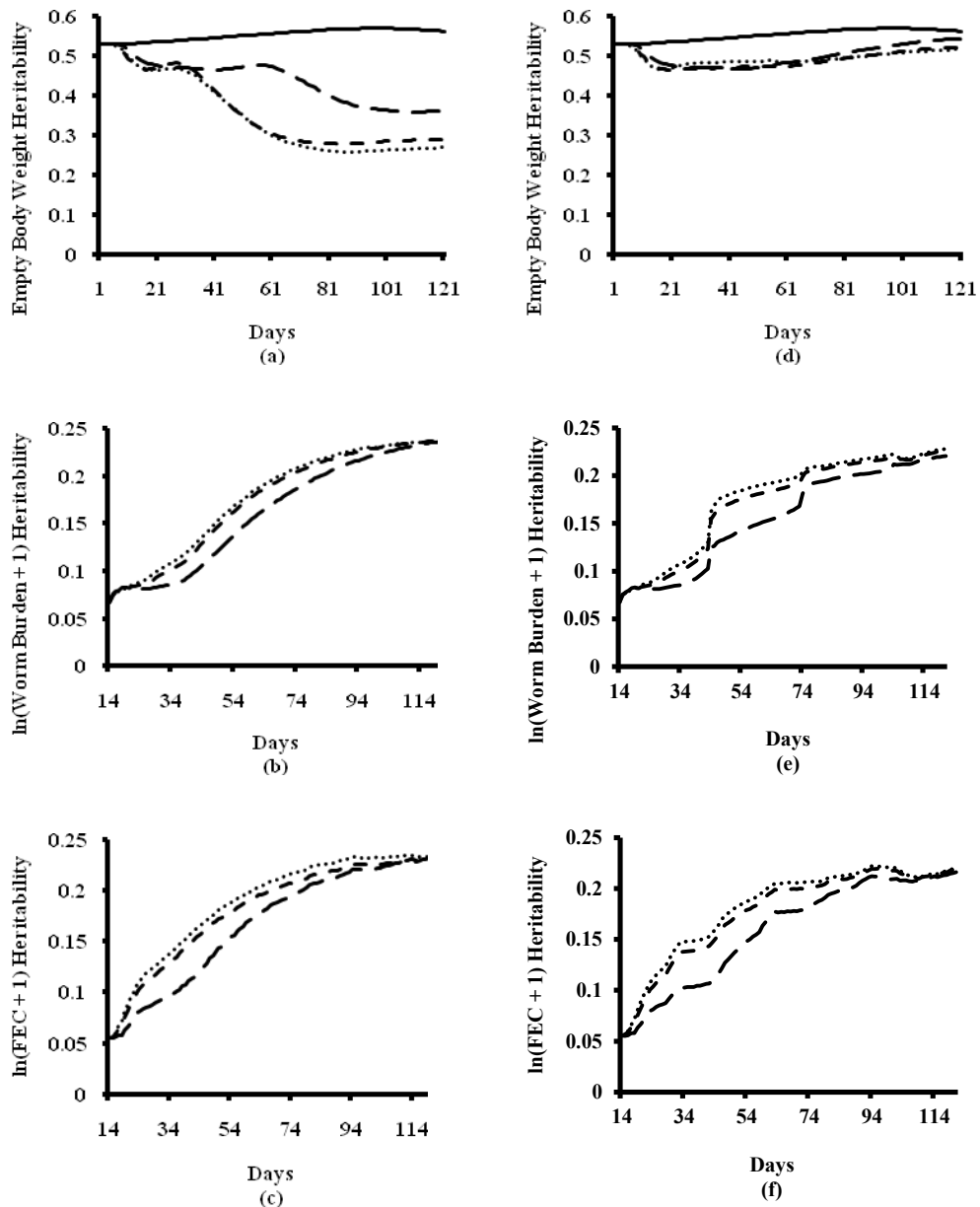
Figure 3.6. Heritabilities of empty body weight (a), ln(worm burden + 1) (b) and ln(faecal egg count + 1) (c) for non-drenched lambs, and empty body weight (d), ln(worm burden + 1) (e) and ln(faecal egg count + 1) (f) for drenched lambs grazed on medium-quality pasture initially contaminated with either control (———), 1,000 (— —), 3,000 (– – – ·) or 5,000 (··········) *Teladorsagia circumcincta* larvae/kg DM grass.

3.3.6 Genetic correlations

Estimated genetic correlations between EBW and log transformed WB and FEC are given in Figure 3.7. For non-drenched lambs, the estimated genetic correlation between EBW and log transformed WB was initially close to 1; however, this is essentially an artefact of the starting conditions of the simulation with larger animals having a greater initial FI and LI, leading to a greater WB. As lambs acquired immunity, the estimated genetic correlations fell and ultimately became negative, leading to ultimate correlations of -0.28, -0.47, and -0.55, respectively, for the non-drenched lambs grazing pastures with an IL_0 of 1,000, 3,000, and 5,000 larvae/kg DM, respectively.

The use of anthelmintics to control WB reduced the impact of parasitism and consequently broke the relationship between EBW and WB. Thus, at the end of the simulation, the estimated genetic correlations between EBW and log transformed WB was close to zero (Figure 3.7).

Patterns of genetic correlation of log transformed FEC with EBW differed from those for WB, as FEC is scaled by faecal output, and is hence partially corrected for the effect of FI and body size. In these results, the correlations were initially between -0.3 and -0.4 for all levels of IL_0. As WB and FEC rose, so did the protein loss due to parasitism, and due to the initial link between EBW and WB mentioned above, this led to larger reductions in the growth rate for lambs with higher EBW than those with smaller EBW. Thus, the genetic correlation between EBW and FEC during this period tended towards zero. As the acquisition of immunity progressed, the immune responses of the genetically more resistant hosts were increasingly able to reduce WB, FEC, and protein loss due to parasitism, therefore increasing the growth rate and causing the genetic correlations between EBW and FEC to become increasingly negative. For non-drenched lambs the genetic correlations reached values of -0.44, -0.65, and -0.73 for IL_0 of 1,000, 3,000 and 5,000 larvae/kg DM, respectively, at the end of the simulation, suggesting apparently beneficial consequences of host resistance under the scenarios simulated.

The impact of WB on EBW through protein loss due to parasitism is well illustrated in the genetic correlations predicted between EBW and FEC for drenched lambs. In this case, the suppression of WB (and FEC) using anthelmintic drenching reduced protein loss due to parasitism, and therefore removed the beneficial consequences of resistance. Thus, for the drenched lambs, the estimated genetic correlations remained closer to zero.
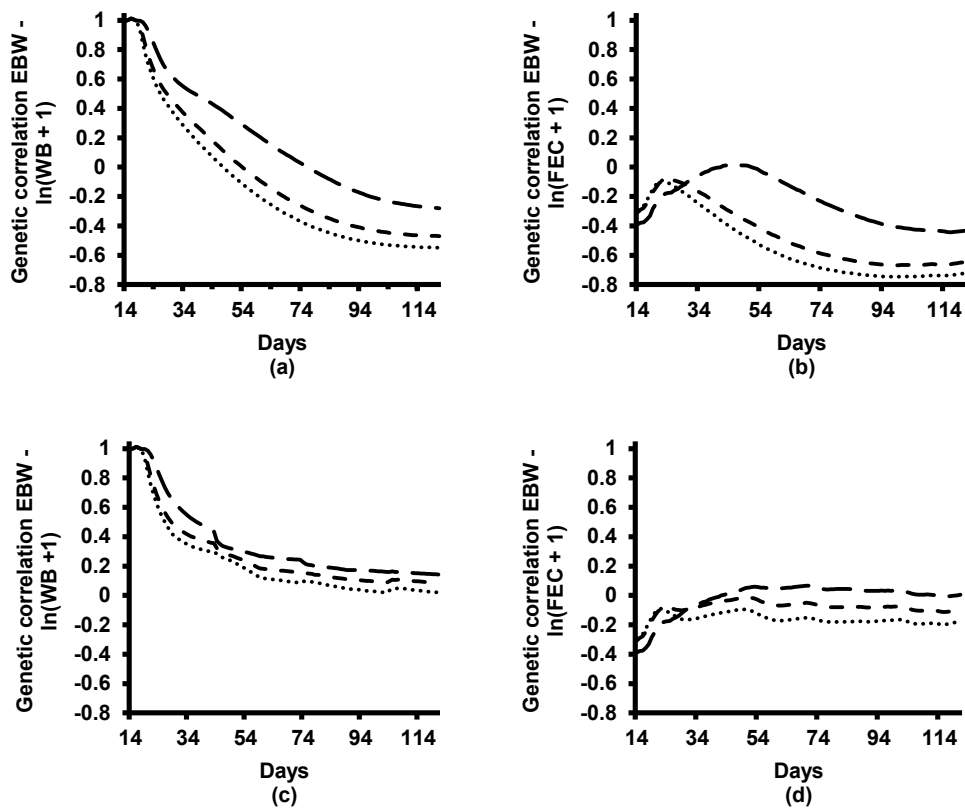
Figure 3.7. Estimated genetic correlations between empty BW and ln(worm burden + 1) (a), and empty BW and ln(faecal egg count + 1) (b) for non-drenched lambs, and empty body weight and ln(worm burden + 1) (c), and empty BW and ln(faecal egg count + 1) (d) for non-drenched lambs. Lambs were grazed on medium-quality pasture initially contaminated with either 1,000 (──), 3,000 (‐ ‐ ‐ ‐)   or 5,000 (··········) *Teladorsagia circumcincta* larvae/kg DM grass. EBW = empty BW (kg), WB = worm burden (number of adult worms), FEC = faecal egg count (eggs/g DM).

3.4 Discussion

Due to the reduced efficacy of anthelmintics in controlling gastrointestinal parasitism, breeding for host resistance to nematode parasites has become an attractive alternative (Bishop *et al.*, 2011). However, commercial sheep breeding programs are likely to be complex, including production goals (e.g., increased BW at slaughter) in the selection objectives as well as host resistance to nematodes. To design appropriate breeding strategies, genetic parameter estimates are required for the individual traits; this includes heritabilities as well as the correlations between traits. Knowledge of such genetic parameters would enable selection indices to be created, combining different traits to find the optimal strategy for improving both performance and resistance traits.

Current genetic parameter estimates for traits describing host resistance are variable due to many factors which may include host resistance genotype, nutritional environment, the intensity of infection and environmental factors affecting the prevalence of parasites (Coop and Kyriazakis, 1999). Heritability estimates for the BW of parasitised sheep are generally moderate in magnitude and range from 0.15 to 0.40 (Safari *et al.*, 2005). Similarly, h^2 estimates for FEC range between 0.2 and 0.4 (Safari *et al.*, 2005; Bishop and Morris, 2007), with a mean h^2 for FEC of 0.27 (Safari *et al.*, 2005). However, greater variability is seen for the genetic correlation between FEC and BW, and this parameter appears to vary according to production environment, as well as level and species of parasite challenge. Published values from European studies have tended to be negative and strong, for example up to -0.8 in Scottish Blackface lambs (Bishop *et al.*, 1996) and -0.6 in Polish lambs (Bouix *et al.*, 1998). However, under New Zealand and Australian conditions, strong correlations are seldom seen, with values generally between 0 and -0.3 (Bisset *et al.*, 1992; Douch *et al.*, 1995; Eady *et al.*, 1998; Pollott and Greeff, 2004). However, slightly positive (i.e. unfavourable), correlations have also been reported (McEwan *et al.*, 1992; McEwan *et al.*, 1995; Greeff and Karlsson, 1998; Morris *et al.*, 2000). These genetic parameter estimates have differing implications for the predicted direction and rate of genetic progress. Negative correlations describe the situation

where lower FEC is associated with higher BW (i.e., a favourable relationship), whilst positive correlations imply an unfavourable relationship between FEC and BW. As such, for effective use within breeding strategies, it is important to understand how differing environmental factors affect genetic parameter estimates and predict the consequences of selection. In the present chapter, we used a novel *in silico* mathematical model to investigate the impact of both the level of LC and anthelmintic input on the estimates of genetic parameters, with the expectation that both will strongly influence the direction and magnitude of the correlation between production and host resistance traits.

Previously, Bishop and Stear (1999) used a mathematical model to investigate the impact of level of infection on genetic parameter estimates for host resistance and performance traits. However, this model did not fully account for interactions between host resistance and the development of immunity, and did not include parasite-induced anorexia. A recently published parasite-host interaction model of individual lambs (Laurenson *et al.*, 2011) (**Chapter 2**) provided the basis for the current model that accounted for the above interactions (i.e. for animal genotype characteristics for growth and resistance, the acquisition of immunity, and the impacts of parasitism on production traits). Further, this model was flexible enough to allow for the necessary alterations needed to explore the impact of level of LC and anthelmintic drenching on genetic parameter estimates for a population of growing lambs. Additions made to the model included the extension from the individual lamb to a population model (using the coding from Vagenas *et al.*, 2007c; Doeschl-Wilson *et al.*, 2008), incorporating heritable between-lamb variation, the inclusion of a simple epidemiological module including the linking of FI to LI to allow for the modelling of a grazing scenario, and the incorporation of an anthelmintic protocol to explore the impacts of treatment. Together, these changes allow prediction of genetic parameters for performance and host resistance traits, and investigation of the impact of external factors such as differing levels of LC or treatment protocols. Thus the current model, presented here, accounts for many

factors omitted from previous models and, as such, is expected to shed further light on the causes of variability in genetic parameter estimates.

The predictions reported here for the h^2 of EBW were higher than previously reported values for BW-related traits (see above). However, it should be noted that these published values are generally for the h^2 of BW, whereas predictions made here are for EBW. Heritability estimates for BW in field studies are subject to measurement sampling error and variation caused by gut fill. Such factors would be expected to add noise to measurements, and thus it is not surprising that published values for the h^2 of BW are lower than the predictions for h^2 of EBW made here. Predictions for the h^2 of FEC at the end of the simulation fell within the range of published values (see above). Further, Vagenas *et al.* (2007d) estimated the h^2 of FEC for Blackface lambs from 2 to 6 months of age with an anthelmintic drenching protocol similar to that applied here, with values rising from 0.1 at 2 months of age to 0.4 at 6 months of age. Whilst the predicted values do not reach the same value at 6 months, the pattern of rising h^2 over time remains similar.

The comparison of predicted h^2 estimates for WB against published values is constrained by the number of publications reporting this value. Davies et al. (2005), building on analyses first presented by Stear et al. (1997), reported the h^2 of WB to be 0.13, whilst Gauly et al. (2002) reported a value of 0.54. With so few published values, and the likelihood of large sampling errors, it is not possible to give an objective comparison with the predicted value from this model of 0.24.

As far as we are aware, no published values are available with which to compare the predicted genetic correlation between EBW and WB. However, there is a plethora of published values for the genetic correlation between BW and FEC. As described above, these estimates range from -0.8 (Bishop *et al.*, 1996) to +0.4 (McEwan *et al.*, 1992; 1995); however, these estimates are often subject to large standard errors, associated with small population size. Final estimates of the genetic correlation between EBW and FEC were predicted in our study to fall within the range of -0.75 (non-drenched lambs) and 0.07 (drenched lambs). These predicted

values fall within the range of the published estimates; however, positive values similar to McEwan *et al.* (1992; 1995) estimated for sheep with predominantly *Trichostrongylus* spp. infections were not predicted. In a previous modelling study Doeschl-Wilson *et al.* (2008) addressed the variation in genetic correlations between EBW and FEC by exploring the impact of host nutrition, gene pleiotropy and variation in resource allocation. The conclusion was that the relatively high positive correlations between BW and FEC could only be produced by pleiotropic effects. However, this study was carried out using the intrinsic growth reduction mechanism for parasite-induced anorexia (detailed in **Chapter 2**) and with a maximum protein loss due to parasitism of 10g per day. Thus, the investigation into the impact of variation in resource allocation may be expected to have little impact on the genetic correlations between EBW and FEC due to a maximum reduction of 10g in available protein in comparison to the desired requirements. Using the direct reduction in food intake mechanism for parasite-induced anorexia (detailed in **Chapter 2**) along with the increased maximum protein loss due to parasitism (100g) implemented in the current model the available protein may be reduced as much as 635g in comparison to the desired requirements. Therefore, with the current model the relatively high positive correlations between BW and FEC may also be caused by variation in resource allocation which may arise from breed differences.

The level of IL_0 had a significant impact on the predictions for the genetic correlation between EBW and FEC. Previously, Bishop and Stear (1999) predicted that the estimated genetic correlation became stronger as the IL_0 increased, ranging from -0.16 for drenched lambs grazing on a pasture with an IL_0 of 250 larvae/kg DM to -0.40 for drenched lambs grazing on a pasture with an IL_0 of 4,000 larvae/kg DM. Similarly, for the predictions made here, genetic correlations became stronger with increasing IL_0, with predictions for drenched lambs being weaker and predictions for non-drenched lambs being stronger than the values predicted by Bishop and Stear (1999). Further, anthelmintic drenching led predictions for the genetic correlation between EBW and FEC to become weaker (i.e. close to zero), in comparison with the non-drenched lambs, because of reduced production penalties caused by parasitism.

Similarly Bishop and Stear (1999) modelling a 4-weekly drenching protocol, predicted that increasing the production penalty led to increasingly negative genetic correlations, with reduced production penalties leading to correlations which tended towards zero. Thus, anthelmintic treatment protocol can potentially have a marked impact on the interpretation of resistance and performance traits within a breeding program, as well as the recommended breeding goal.

Comparisons of predictions between drenched and non-drenched lambs highlight the impact of acquisition of immunity in reducing WB, FEC, and altering genetic parameters for these traits, and also go towards explaining the predictions made for pasture contamination. For example, it was predicted that increasing levels of LC led to increasing rates of the acquisition of immunity and consequently faster control of the parasite population. Thus, at the end of the simulation, pasture contaminations were similar for all levels of IL_0, although the net impact of parasitism differed, being greater with increasing initial parasite challenge. However, it should be noted that the epidemiological aspects of our model were deliberately simple with static parameters unaffected by factors such as light, humidity, temperature, sward/grazing height, and larval migration onto herbage. Including these factors into the epidemiological module will increase its complexity, but it is doubtful that it will affect the magnitude and direction of the predictions.

Whilst differing levels of IL_0 and differing anthelmintic drenching protocols are shown to be a possible cause for variation in genetic parameter estimates, they do not fully account for the variability observed in published values. Most notable among these are the large variability observed for the genetic correlation between EBW and FEC. However, the extreme values reported within the literature may be subject to small population sizes and hence associated with large standard errors. Increasing population size within field experiments would lead to smaller standard errors and hence more robust genetic estimates. Thus, it may not be surprising that the extreme values reported within literature were not predicted by our model. However, it should be noted that our model assumed that traits underlying resistance

and growth were uncorrelated; altering this assumption can lead to more extreme relationships between EBW and FEC (Doeschl-Wilson *et al.*, 2008).

The predicted genetic parameter estimates changed over time, generally as a consequence of the accumulated production penalties caused by parasitism and the factors that affect this. As such, reported genetic parameter estimates may be influenced by the time/age at which the parameters are reported. For example, positive genetic correlations between EBW and FEC may be a result of reporting the correlations at a time when the acquisition of immunity has not progressed sufficiently to lead to large between-animal differences in the reduction of performance penalties caused by parasitism. As FEC is recorded as an indicator of sheep resistance, between-animal differences in FEC become a better indicator of genetic differences in acquired immunity as time and cumulative exposure to larvae progress.

Lastly, these results give insight into optimal recording and breeding strategies. For example, for the estimation of breeding values (*EBV*s) for production (BW) and host resistance (FEC) traits, these results suggests that it may be better to make these assessments under a drenching scenario. This is due to anthelmintic drenching breaking the link between BW and FEC, as shown in the genetic correlations reported here, allowing for each individual trait to be assessed independently. In other words, under these circumstances the EBV for BW of an animal is less likely to be influenced by its resistance genotype. However, the use of such EBVs for animal selection decisions will then depend entirely on the husbandry protocol used by the breeder. In situations where the use of anthelmintics is minimal (e.g. organic systems or situations with extensive anthelmintic resistance), then the breeder would select on both the production and resistance EBV, using an appropriate selection index, knowing that improved resistance will also increase productivity. Conversely, if extensive anthelmintic usage is appropriate, then the breeder may base selection predominantly on production EBV.

In summary, the aim of this study was to explore the impacts of level of LC and anthelmintic input on the estimates of genetic parameters for a population of grazing lambs. Both were found to substantially influence the magnitude of the correlation between production and host resistance traits, with increasing levels of IL_0 leading to increasingly negative (i.e. favourable) correlations, and anthelmintic drenching causing weaker correlations to be predicted. Further, other factors affecting genetic parameter estimates were also noted, including factors affecting production penalties caused by parasitism. These findings combined, shed light on the possible causes of variability in the genetic parameter estimates reported within the relevant literature. Further, the results help to clarify optimal protocols for assessing genotypes for host resistance and production traits, and also assist in defining appropriate selection strategies for different environments and production systems.

Chapter Four

Exploration of the epidemiological consequences of resistance to gastrointestinal parasitism and grazing management of sheep

4.1 Introduction

Gastrointestinal parasitism is one of the most pervasive challenges to the health and welfare of ruminants, causing significant production losses for the sheep industry (Nieuwhof and Bishop, 2005). Effective control of nematode infections relies heavily on the use of antiparasitic drugs; however, reduced efficacy due to the evolution of drug resistance by parasite populations (Jackson and Coop, 2000; Bartley *et al.*, 2003) has stimulated the search for alternative sustainable control methods. Selection for increased host resistance to gastrointestinal parasitism has previously been proposed as an alternative or complementary control strategy, and is supported by evidence of heritable variation in faecal egg count (FEC) (Bishop and Morris, 2007) and by results of selection in practice (Kemper *et al.*, 2010). However, the benefits of such a strategy seem to depend on management practices (Vagenas *et al.*, 2007c).

Although selection for resistance may be viewed as beneficial, predicting the actual benefits of selection for resistance in grazing ruminants can be difficult, due to complex interactions between parasite epidemiology (including host population infection dynamics), management practices including grazing, and host resistance to nematode infections (Bishop and Stear, 1997). Further, the combination of selection for resistance and other control methods, such as grazing management, may provide further complementary benefits and lead to reduced anthelmintic use (Coop and Kyriazakis, 2001). For example, a mixed population may benefit from grazing with animals selected for resistance, whilst grazing selected animals separately may be

used to provide pastures with reduced larval contamination. However, resistance of parasites to pharmaceuticals has reduced the effectiveness of grazing management, which is further complicated by the availability of truly clean pasture (Waller, 2006b).

Exploring these complex interactions experimentally and determining the benefits of control methods separately or in combination can be costly and time consuming. When using an appropriate mathematical model such constraints can be considerably reduced. As such, the mathematical model of Laurenson *et al*. (2012a) (**Chapter 3**) which includes heritable between-animal variation in host-parasite interactions, the epidemiology of *Teladorsagia circumcincta* and anthelmintic drenching protocols, is a tool which may be used to gain insight into the interactions between host resistance, grazing management and parasite epidemiology. Here we use this mathematical model to quantify these interactions between host genotype, control interventions and disease epidemiology. Specifically, we explore the epidemiological consequences of grazing animals with differing genotype for resistance under contrasting drenching protocols. The question being addressed is to what extent does pasture contamination change when grazed by sheep of contrasting genotypes for resistance, as assessed by grazing susceptible and resistant sheep together and apart, and are these differences influenced by anthelmintic treatments. Further, we explore the impact of alternative grazing strategies, aimed at reducing host exposure to infective larvae, on the genetic and epidemiological effects. Specifically, we explore the impact of set grazing *vs*. movement onto lightly contaminated or clean pastures, and the impact that such movements have on parasite burden and growth rate.

4.2 Materials and Methods

The mathematical model of Laurenson *et al*. (2011; 2012a) (**Chapters 2** and **3**) which includes heritable between-lamb variation in host-parasite interactions, the epidemiology of *Teladorsagia circumcincta* and anthelmintic drenching protocols,

was used as the basis of addressing the interaction between host resistance genotype and parasite epidemiology. A brief description of this model is given below.

4.2.1 Individual lamb model

In the model a growing lamb is assumed to attempt to ingest sufficient nutrients to meet requirements for growth and maintenance, as defined by its genotype. However, infection with gastrointestinal parasites is expected to result in endogenous protein loss (a function of larval challenge and worm burden), and consequently the lamb invests resources in an immune response to counteract this. Further, components of the host response (e.g., cytokines, gastrin) are associated with a reduction in food intake (Fox *et al.*, 1989; Greer *et al.*, 2005; Kyriazakis, 2010), commonly known as (parasite-induced) anorexia. The combination of protein loss due to parasitism, resource investment in immunity and anorexia results in a lamb acquiring insufficient nutrient resources to fulfil all its requirements for maintenance and optimal growth. Thus, ingested resources, after losses due to parasitism, are assumed to be first allocated to meet the maintenance requirements and remaining resources to be allocated towards growth and immunity proportional to requirements (Kahn *et al.*, 2000; Doeschl-Wilson *et al.*, 2008).

4.2.2 Population model

Individual animals are simulated within a pre-defined population structure, with between-animal variation assumed to occur for the animal growth attributes, such as maximum growth rate and body composition, maintenance requirements, and immune response to gastrointestinal parasites. Initial input parameters involved in these functions are assumed to be normally distributed, thus distributions such as the over-dispersion of faecal egg count (FEC) described by Bishop and Stear (1997) occur as a consequence of the functions that underlie the model rather than as a result of direct input. All traits, other than those associated with immune acquisition, are assumed to be uncorrelated (Doeschl-Wilson *et al.*, 2008). Traits associated with immune acquisition are assumed to be a function of overlapping effector mechanisms

(components of the Th2 immune response) (Jenkins and Allen, 2010), and as such strongly genetically and phenotypically correlated (r = +0.5). Further, random environmental variation in daily food intake is assumed to reflect the influence of external factors controlling food intake not accounted for explicitly by the model, and are added to achieve a genetic correlation between food intake and growth rate of approximately 0.8 (Cammack *et al.*, 2005).

The model has been parameterised such that lamb growth characteristics were similar to those of the Scottish Blackface, the most numerous British breed, and that parasitological parameters matched those of Coop et al. (1982; 1985). The properties of each trait with between-host variation were specified by the population mean, the heritability and the coefficient of variance for each trait, and have been chosen to match those of Bishop et al. (1996) and Bishop and Stear (1997). A detailed description of the population model and parameter input values can be found in Laurenson *et al*. (2012a) (**Chapter 3**).

4.2.3 Epidemiological module

The grazing pasture is defined by the number of hectares and grass available for grazing, taking into account grass growth and grass consumed. In the main scenario modelled below the pasture was assumed to be initially contaminated with a population of eggs and larvae arising from a ewe population removed from pasture at lamb weaning. Subsequent larval contamination of pasture was assumed to arise from eggs excreted by lambs, taking into account egg to infective larvae development time, a mortality rate for infective larvae and the removal of infective larvae from pasture due to grazing. Lambs are assumed to graze randomly across the pasture, leading to an expected larval intake directly proportional to food intake.

4.2.4 Anthelmintic drenching

The anthelmintic drench was specified as having a 95% efficacy against *Teladorsagia circumcincta* (Sargison *et al.*, 2007). Each anthelmintic drench is assumed to equally reduce the adult and larval population resident in the host. Further, the oral administration of anthelmintic is assumed to be effective on the day of administration only, with no residual effects (Borgsteede, 1993).

4.2.5 Simulation procedure and *in silico* experimental design

The mathematical model was used to explore the relationship between host resistance to nematodes and the epidemiology of *Teladorsagia circumcincta* (Experiment 1), and the impact of differing grazing scenarios on FEC (eggs/g) and host resistance on pasture contamination levels (PC, larvae/kg DM) (Experiment 2).

4.2.5.1 Experiment 1 – Effect of host resistance on nematode epidemiology

To give a description of the impact of differing levels of initial larval contamination of pasture on host performance and parasitological traits, a population of 10,000 lambs was simulated grazing on a medium-quality pasture (crude protein = 140g/kg DM, metabolisable energy = 10MJ/kg DM (AFRC, 1993)), at a density of 30 lambs/ha for a period of 4 months from weaning to 6 months of age. The lambs were assumed to be initially parasitologically naïve and the initial larval contamination of pasture (IL_0) was set to either control (clean), 1,000, 3,000 or 5,000 *T. circumcincta* larvae/kg DM. Lambs initially ingest around 1kg DM/day and thus these levels correspond to the trickle challenge levels chosen by Coop *et al.* (1982) that led to sub-clinical infections. Further, the lamb population was either given no anthelmintic treatment or drenched at 30 days intervals (days 30, 60 and 90), representing the two extremes of commercial practice (Sargison *et al.*, 2007).

In order to explore the impact of host resistance genotype on the model predictions the individual lambs were assessed according to their FEC at the end of

the simulation, as predicted by the model. The 1,000 lambs (10%) with the smallest FEC were deemed resistant (*R*) and the 1,000 lambs (10%) with the largest FEC were deemed susceptible (*S*). This equates to approximately 15 years of selection based on FEC, as outlined in the Discussion. These specific groups of lambs were then re-simulated to be grazing at the same density on separate pastures (**R**$_{sep}$ & **S**$_{sep}$, where bold font indicates grazing separately) to assess the impact of host resistance to nematodes on the epidemiology of *T. circumcincta*.

The hypothesis investigated was that host resistance category would lead to differences in pasture contamination, which would build up over time, exaggerating the apparent differences in resistance. Therefore, this procedure was repeated over 3 consecutive grazing seasons to allow time for differences between the two groups in model predictions to increase and then stabilise. All groups (population, **R**$_{sep}$ & **S**$_{sep}$) were subjected to the same IL_0 in year 1. For the following grazing seasons, IL_0 was assumed to be a function of the final pasture contamination predicted for each group from the previous year. As such IL_0 for years 2 and 3 for the population, **R**$_{sep}$ and **S**$_{sep}$ groups were calculated as:

Population: $IL_0 = IL_0(year1)$ (larvae/kg DM)

R$_{sep}$ group: $IL_0 = \left(\dfrac{PC_R}{\left(\dfrac{PC_R + PC_S}{2} \right)} \right) \cdot IL_0(year1)$ (larvae/kg DM)

S$_{sep}$ group: $IL_0 = \left(\dfrac{PC_S}{\left(\dfrac{PC_R + PC_S}{2} \right)} \right) \cdot IL_0(year1)$ (larvae/kg DM)

where, PC_R and PC_S are the final pasture contamination predictions of the previous year for the **R**$_{sep}$ and **S**$_{sep}$ groups, respectively.

4.2.5.2 Experiment 2 – Effect of grazing management on nematode epidemiology

The scenarios simulated in Experiment 1 assumed that lambs remained on same pasture throughout the grazing season, whereas in reality lambs are often moved between pastures either in an attempt to control nematode infections or simply because of limited pasture availability. Thus, a second set of experiments was devised to take into account more realistic scenarios in which differing grazing strategies were implemented during the grazing season.

The hypothesis investigated was that the conclusions from experiment 1 may change with different grazing management strategies. Four scenarios were simulated in order to explore the impact of grazing strategies on live weight (LW, kg), FEC and PC predictions. Scenario 1 assumed that lambs remained on the same pasture throughout the grazing season, as before. Scenario 2 assumed that lambs were moved to a pasture with a larval contamination the same as the IL_0 and no egg contamination, representing a pasture in which ewes or other infected animals had been grazing post lamb weaning and removed at least 1 week before lamb placement; one week corresponding to the time taken for eggs to develop to infective larvae (Young *et al.*, 1980). Scenario 3 assumed that lambs were moved to a pasture with the same larval contamination as the pasture that lambs are being moved from but with no egg contamination, representing a pasture on which a similar population or group of lambs had been grazing and removed at least 1 week before lamb placement. Scenario 4 assumed that lambs were moved to a clean pasture, and thus represents the extremity of any grazing scenario. In reality, any grazing practice will have consequences similar to at least one of these scenarios.

As with Experiment 1, a population of 10,000 lambs, an **R**$_{sep}$ group (1,000 lambs) and an **S**$_{sep}$ group (1,000 lambs) were simulated grazing on a medium-quality pasture, at a grazing density of 30 lambs/ha for 4 months from weaning. IL_0 was set to either control, 1,000, 3,000 or 5,000 larvae/kg DM. Each group or population were simulated for a single grazing season for the above 4 scenarios and moved at 40 day

intervals (days 40 and 80), and were given either no anthelmintic treatment or drenched on the same days.

4.2.6 Model outputs

Lamb performance, parasitological and epidemiological traits were generated by the model on a daily basis. Traits reported here are live weight (LW, kg), faecal egg count (FEC, eggs/g DM faeces) and pasture contamination (PC, larvae/kg DM pasture). Worm and larval burdens in the host were also captured, but not reported in detail here.

4.3 Results

4.3.1 Experiment 1 – Effect of host resistance on nematode epidemiology

Figure 4.1 provides an overview of PC and FEC predictions for the population of 10,000 lambs grazed on pastures of differing IL_0 and given either no anthelmintic treatment or drenched at 30 day intervals. Predictions differed in accordance with the initial larval contamination of pasture. Increased ingestion of larvae led to increased parasitic burden and subsequently increased FEC; however, this also led to increased rates of acquisition of immunity and hence earlier predicted maxima for both PC and mean FEC. At the end of the simulated grazing season PC and mean FEC predictions were similar for all levels of IL_0. The final LW (day 121) for animals grazed on clean pasture was predicted to be 67.6kg, whilst final LW for lambs grazed on pasture with an IL_0 of 1,000, 3,000 and 5,000 larvae/kg DM were predicted to be reduced in comparison to the control group by 11%, 16% and 20%, respectively, for lambs given no anthelmintic treatment, and by 7%, 8% and 9%, respectively, for drenched lambs. Thus, increasing IL_0 led to increasing reductions in LW, with drenched animals performing better than lambs given no anthelmintic treatment. However, LW losses were still substantial in drenched lambs.
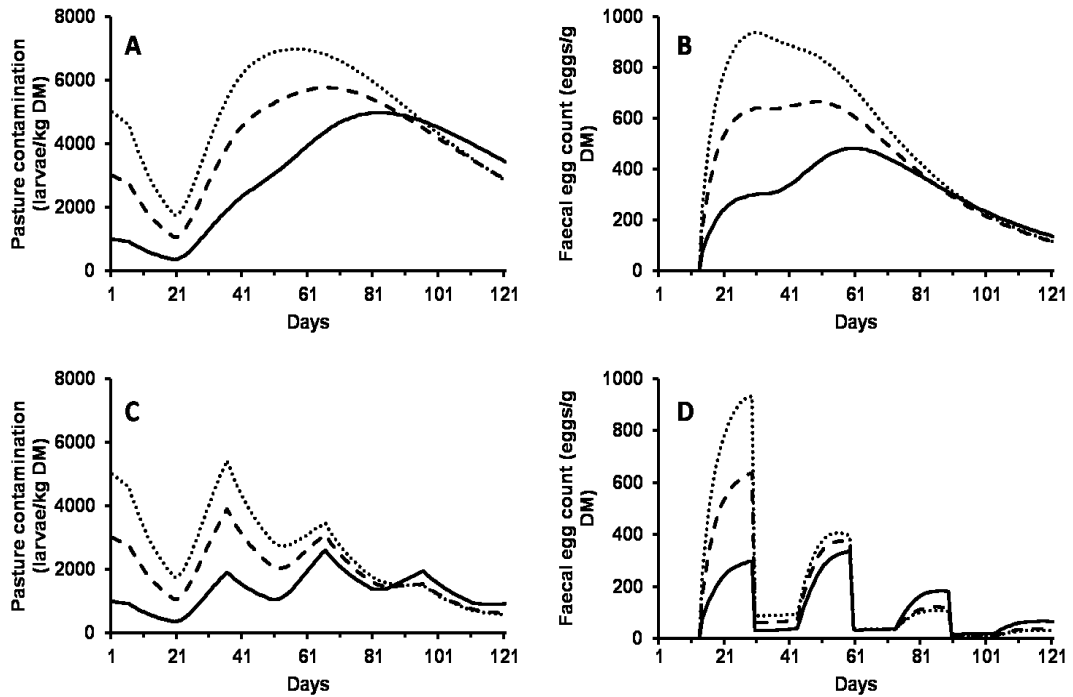
Figure 4.1. Pasture contamination (larvae/kg DM pasture) (A) and faecal egg count (eggs/g DM faeces) (B) for lambs given no anthelmintic treatment, and pasture contamination (C) and faecal egg count (D) for lambs drenched at 30 day intervals. The population of 10,000 lambs were grazed on pastures with an initial larvae contamination of 1,000 (———), 3,000 (– – –) or 5,000 (·········) *T. circumcincta* larvae/kg DM.

Figure 4.2 provides the PC and FEC for the population of 10,000 lambs, the R_{sep} group and the S_{sep} group grazed on pasture with an IL_0 of 3,000 larvae/kg DM for three grazing seasons and given no anthelmintic treatment, and is given as an example of similar trends predicted for pastures with an IL_0 of 1,000 and 5,000 larvae/kg DM. During the first grazing season, despite all groups having the same IL_0, the FEC predictions for the S_{sep} group were larger and the day of maximum occurred later than for the population mean, whilst for the R_{sep} group FEC predictions were reduced and the day of maximum occurred sooner. These differences in FEC predictions resulted in a similar divergence of PC predictions from day 21, after the first eggs excreted by the host matured to infective larvae. The maximum PC (and day of maximum) for the population, R_{sep} and S_{sep} groups were 5,762 (69d), 1,650 (35d) and 13,027 (83d) larvae/kg DM, respectively. Large

differences in FEC were still apparent at day 121. These differences are also evident in the PC predictions on day 121 with values of 2,880, 190 and 9,426 larvae/kg DM being predicted for the population, R_{sep} and S_{sep} groups, respectively. These final PC predictions for the first grazing season therefore led to proportional differences in the initial larval contamination of pasture for the second grazing season.
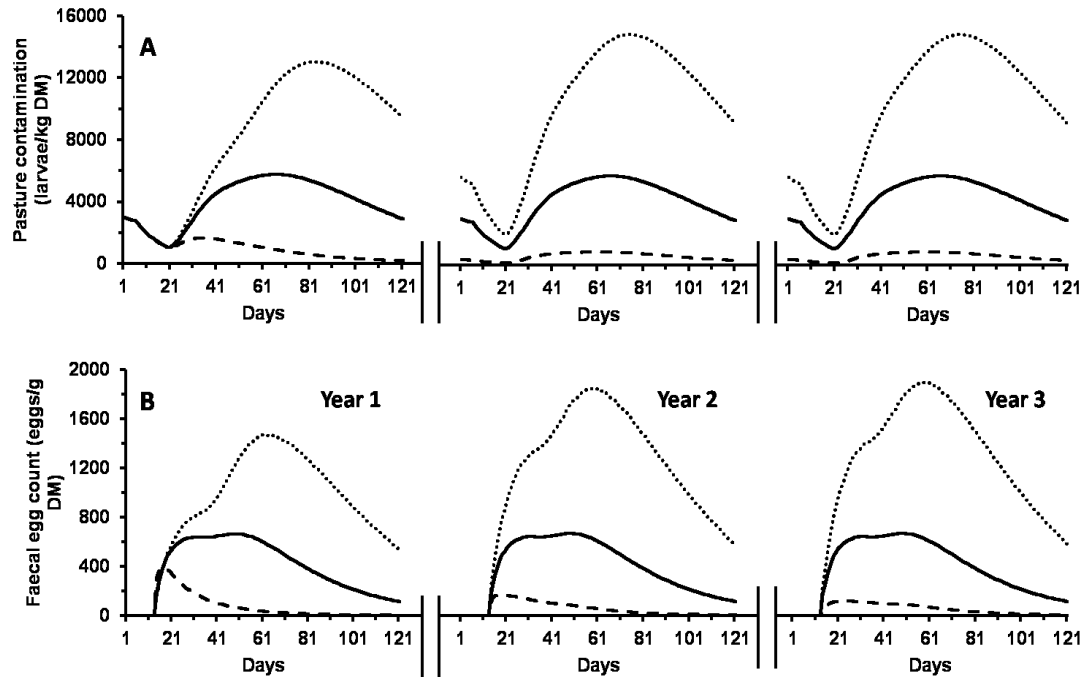
Figure 4.2. Pasture contamination (larvae/kg DM pasture) (A) and faecal egg count (eggs/g DM faeces) (B) for a population of 10,000 lambs (———), a resistant (R_{sep}) group of 1,000 lambs (– – – ·) and a susceptible (S_{sep}) group of 1,000 lambs (··········) grazing separately over 3 grazing seasons. Lambs were grazed on a pasture initially contaminated with 3,000 *T. circumcincta* larvae for the first season, and given no anthelmintic treatment.

The increased IL_0 for the S_{sep} group in year 2 led to an increased FEC and hence an increased maximum PC (14,611 larvae/kg DM) compared to year 1. The day of maximum PC for year 2 was predicted to be earlier than year 1 (75 *vs*. 81 days). However, despite the initially higher PC during year 2, predicted PC at day 121 was similar to predictions for year 1. As a consequence, year 3 predictions for PC and FEC remained very similar to year 2 for the S_{sep} group.

The R_{sep} group showed the opposite trend. The decreased IL_0 in year 2 for the R_{sep} group led to a decreased FEC and subsequently a reduced maximum PC (931 larvae/kg DM) and this maximum occurred later than in year 1 (i.e., 69 *vs*. 45 days). The PC predicted on day 121 slightly increased from year 1 and was 250 larvae/kg DM; and a very similar pattern was predicted for year 3.

PC averaged across the third grazing season for the population, R_{sep} and S_{sep} groups with an IL_0 on year 1 of 1,000, 3,000 or 5,000 larvae/kg DM and either given no anthelmintic treatment or drenched at 30 day intervals are provided in Table 4.1. For all groups (population, R_{sep} and S_{sep}), average PC increased with increasing IL_0 for both non treated and drenched lambs. Further, when comparing drenched lambs with those given no anthelmintic treatment, reductions in average PC were greater for the S_{sep} group than for the R_{sep} group: the average PC predictions for the drenched S_{sep} group were reduced by 56%, 61% and 55% for an IL_0 in year 1 of 1,000, 3,000 and 5,000 larvae/kg DM, respectively, in comparison to the same group given no anthelmintic treatment. Whilst, the average PC predictions for the drenched R_{sep} group were reduced by 44%, 32% and 17% for an IL_0 on year 1 of 1,000, 3,000 and 5,000 larvae/kg DM, respectively, in comparison to the same group given no anthelmintic treatment. Whilst these R_{sep} group values are substantial in percentage terms, in absolute values there was little difference between PC for drenched and un-drenched lambs as shown in Table 4.1.

Table 4.1. Average pasture contamination (days 1-121, larvae/kg DM pasture) predictions of year 3 for the population of 10,000 lambs (**Pop**), the 1,000 resistant (**R_{sep}**) and 1,000 susceptible (**S_{sep}**) lambs grazed separately for 3 years. Lambs were grazed on pasture with an initial pasture contamination (IL_0, larvae/kg DM pasture) of either 1,000, 3,000 or 5,000, and either undrenched or drenched with an anthelmintic at 30 day intervals.

IL_0 (larvae/kg DM)	Undrenched			Drenched		
	Pop	**R_{sep}**	**S_{sep}**	**Pop**	**R_{sep}**	**S_{sep}**
1000	3,085	496	7,574	1,319	280	2,948
3000	4,005	547	10,087	1,951	376	4,391
5000	4,893	585	12,895	2,545	484	5,785

To fully quantify the interaction between host resistance and nematode epidemiology it is necessary to disentangle the immediate effect of host resistance on FEC from the impact it has through altering pasture contamination and hence the subsequent larval challenge. To do this, predictions for the R_{sep} and S_{sep} groups were compared to their counterparts (*R* and *S*) grazed within the population of 10,000 lambs during the third year of grazing (Table 4.2).

Table 4.2. Average faecal egg count (days 14-121, eggs/g DM faeces) predictions of year 3 for 1,000 resistant and 1,000 susceptible lambs grazed separately (R_{sep} and S_{sep}) or within a population (R and S) of 10,000 lambs for 3 years. Lambs were grazed on pasture with an initial pasture contamination (IL_0, larvae/kg DM pasture) of either 1,000, 3,000 or 5,000, and either undrenched or drenched with an anthelmintic at 30 day intervals.

IL_0 (larvae/kg DM)	Undrenched				Drenched			
	R	*S*	R_{sep}	S_{sep}	*R*	*S*	R_{sep}	S_{sep}
1000	79	609	54	836	45	181	27	251
3000	98	862	58	1305	62	240	35	360
5000	118	1080	61	1674	77	297	41	461

For *S* and *R* lambs grazed within the population and given no anthelmintic treatment, the average FEC of *S* lambs was increased by 771%, 880% and 915% in comparison to *R* lambs for the IL_0 levels of 1,000, 3,000 and 5,000 larvae/kg DM, respectively. For lambs drenched at 30 day intervals, the average FEC of *S* lambs was increased by ~382% in comparison to *R* lambs for all IL_0 levels.

For lambs grazed separately and given no anthelmintic treatment, the average FEC of the R_{sep} group was decreased by 32%, 41% and 48% in comparison to their counterparts (*R*) grazed with the population, and the average FEC of the S_{sep} group was increased by 37%, 51% and 55% in comparison to their counterparts (*S*) grazed with the population, for the IL_0 levels in year 1 of 1,000, 3,000 and 5,000 larvae/kg DM, respectively. These differences in average FEC for lambs grazed separately led to the average FEC predictions for the S_{sep} group being increased by 1548%, 2240% and 2744% in comparison to the R_{sep} group for the IL_0 levels in year 1 of 1,000, 3,000 and 5,000 larvae/kg DM, respectively. These values are two to three-fold

greater than the equivalent values reported above for lambs grazed within the population. When treated with anthelmintics at 30 day intervals, the relative comparisons of R_{sep} *vs*. *R* and S_{sep} *vs*. *S* were almost identical to those of untreated lambs, albeit with lower absolute values. Further, the ratio of S_{sep} to R_{sep} was once again two to three times greater than *S* to *R*.

LW predictions for day 121 in the third year of grazing for the R_{sep} and S_{sep} groups grazed separately and the *R* and *S* groups grazed with the population are given in Table 4.3. These predictions show that the LW at day 121 remains similar for both resistant groups, whether grazed separately (R_{sep}) or within the population (*R*) and that anthelmintic drenching has little to no impact on these predictions. However, for susceptible lambs the S_{sep} group lambs grazed separately were predicted to have a lower LW at day 121 in comparison to those grazed with the population (*S*). Further, anthelmintic drenching had a significant impact on LW for both grazing separately (S_{sep}) and grazed with the population (*S*), with anthelmintic drenching causing a greater recovery of LW in lambs grazed separately (S_{sep}) than in those grazed with the population (*S*).

Table 4.3. Final live weight (day 121, kg) predictions of year 3 for 1,000 resistant and 1,000 susceptible lambs grazed separately (R_{sep} and S_{sep}) or within a population of 10,000 lambs (*R* and *S*) for 3 years. Lambs were grazed on pasture with an initial pasture contamination (IL_0, larvae/kg DM pasture) of either 1,000, 3,000 or 5,000, and either undrenched or drenched with an anthelmintic at 30 day intervals. Lambs grazed on a control pasture (0 larvae/kg DM pasture) had a final live weight of 67.6kg at day 121.

IL_0 (larvae/kg DM)	Undrenched				Drenched			
	R	*S*	R_{sep}	S_{sep}	*R*	*S*	R_{sep}	S_{sep}
1000	62.5	54.6	62.6	49.3	62.5	63.6	63.0	61.6
3000	62.5	46.5	62.6	38.6	62.6	62.2	62.9	59.2
5000	62.4	42.2	62.6	35.6	62.6	60.9	62.9	58.1

4.3.2 Experiment 2 – Effect of grazing management on nematode epidemiology

Similar patterns in PC, FEC and LW were predicted for the 4 grazing strategies for all levels of IL_0, with the differences arising from IL_0 occurring in accordance with the differences predicted in Experiment 1. Thus, to highlight the effects of grazing scenario, only results for the IL_0 of 3,000 larvae/kg DM are presented. Figure 4.3 provides the PC and FEC for the population of 10,000 lambs grazed on pasture with an IL_0 of 3,000 larvae/kg DM for one grazing season and moved according to scenarios 1 to 4 at 40 day intervals. Lambs were given either no anthelmintic treatment or drenched on days 40 and 80 (coinciding with moves).
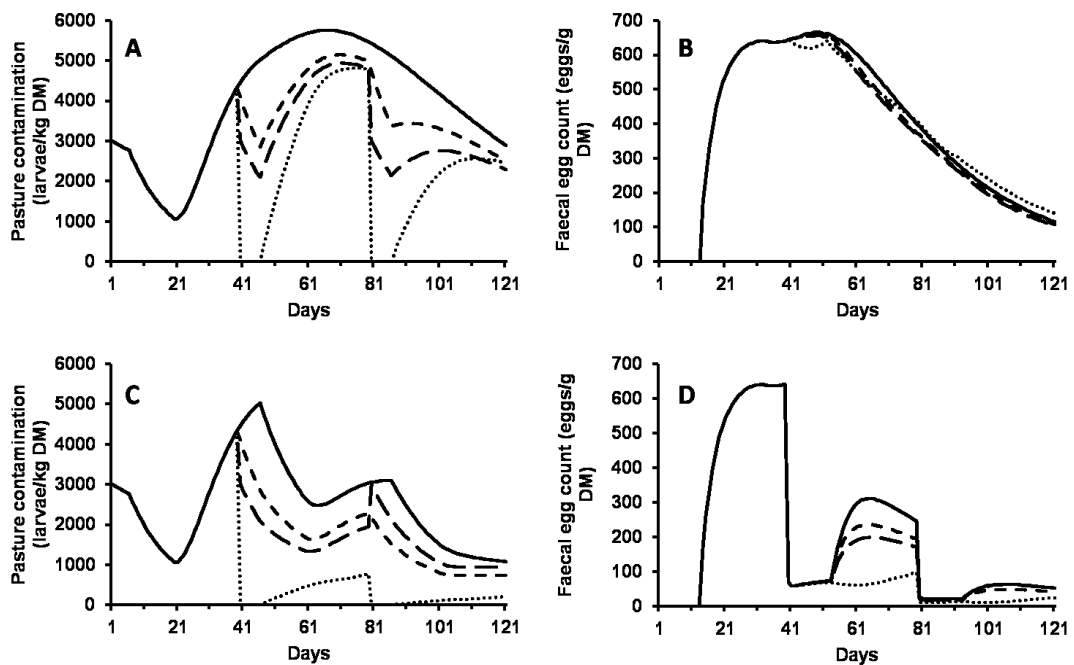
Figure 4.3. Pasture contamination (larvae/kg DM pasture) (A) and faecal egg count (eggs/g DM faeces) (B) for lambs given no anthelmintic treatment, and pasture contamination (C) and faecal egg count (D) for lambs drenched at 40 day intervals, whilst subjected to different grazing management scenarios. The population of 10,000 lambs were grazed on pastures with an initial larvae contamination of 3,000 *T. circumcincta* larvae/kg DM. Grazing scenarios carried out on days 40 and 80 were either no move (Scenario 1, ———), move to a pasture with a pasture contamination of 3,000 larvae/kg DM and no egg contamination (Scenario 2, — —), move to a pasture with equal current pasture contamination and no egg contamination (Scenario 3, – – –) or move to a clean pasture (Scenario 4, ··········).

For lambs given no anthelmintic treatment the impact of each grazing strategy on PC depended on when the impacts were evaluated. Differences between strategies were large immediately after moves, but declined over time. By days 79 and 121, PC predictions were similar for all grazing strategies simulated. However, despite some differences between scenarios in PC at various times during the grazing season, almost no impacts were predicted on FEC. Whilst small differences were seen between scenarios in parasite burden (results not shown), differences in rates of acquisition of immunity as a function of larval challenge, coupled with the differences food intake which arise as a consequence of larval challenge differences, resulted in trivial predicted differences in FEC across all 4 grazing strategies.

For lambs given anthelmintic drenches on the day of the pasture move (days 40 and 80), PC was markedly reduced after the day 40 treatment (and move). For scenarios 1 to 3, the proportional differences in PC generally decreased over time, with PC being similar by day 121. Only scenario 4 was successful in substantially reducing PC, with PC remaining at zero for 7 days, this being the time taken for eggs from the worms that survived the anthelmintic treatment to mature into infective larvae. Differences between scenarios in FEC were observed following the pre-patent period of *T. circumcincta* after each pasture move and drench, with FEC never reaching zero due to the assumed inefficacy of the anthelmintic. In general, differences between grazing scenarios in FEC mirror the differences seen in PC. Crucially, however, the results for the anthelmintic scenarios contrast with those seen for the no treatment scenarios; with no treatment the predicted FEC values were essentially identical across scenarios, whereas when anthelmintics were used some differences (albeit small) were predicted between scenarios.

LW predictions on day 121 for the population, R_{sep} and S_{sep} groups for all scenarios investigated, assuming an IL_0 of 3,000 larvae/kg DM, are given in Table 4.4. For the groups given no anthelmintic treatment, the 4 grazing scenarios simulated led to a maximum difference of only 0.5kg in LW predictions for the population, 0.2kg for the R_{sep} group and 1.6kg for the S_{sep} group. For groups treated with anthelmintics, LW predictions at day 121 were increased by ~10% for the

population and by ~35% for the S_{sep} group, compared to groups given no anthelmintic treatment, whilst anthelmintic treatment showed little to no impact on LW predictions for the R_{sep} group. For the population and S_{sep} groups, scenario 4 resulted in the greatest LW when drenched, and scenario 3 the greatest LW when undrenched. Across all scenarios R_{sep} lambs were always heavier, on average, at day 121 than S_{sep} lambs.

Table 4.4. Final live weight(days 121, kg) predictions for a population of 10,000 lambs (**Pop**), the 1,000 resistant (**R_{sep}**) and 1,000 susceptible (**S_{sep}**) lambs grazed separately for 3 years. Lambs were grazed on pasture with an initial pasture contamination (IL_0, larvae/kg DM pasture) of 3,000, and either undrenched or drenched with an anthelmintic at 40 day intervals. Lambs either remained on the same pasture during the entire grazing season (Scenario 1), moved to a pasture with the same larvae contamination but no egg contamination (Scenario 2) at days 40 and 80, moved to a pasture with a larvae contamination of 3000 larvae/kg DM but no egg contamination (Scenario 3) at days 40 and 80, or moved to a clean pasture (no larvae or egg contamination) at days 40 and 80.

Scenario	Undrenched			Drenched		
	Pop	**R_{sep}**	**S_{sep}**	**Pop**	**R_{sep}**	**S_{sep}**
1	56.7	62.6	44.0	62.3	62.8	60.7
2	57.0	62.7	44.9	61.5	62.8	58.9
3	57.1	62.6	45.6	61.5	62.6	60.4
4	56.6	62.8	45.1	63.2	63.6	62.7

4.4 Discussion

Host genetic variation in FEC within populations of sheep has led to the proposition that selection for increased host resistance to gastro-intestinal parasitism using FEC as an indicator of host resistance may be used as an alternative or complementary strategy to anthelmintic use (Woolaston and Baker, 1996). Further, additional benefits may also arise from the impact of selection on pasture contamination, which may provide further opportunities aimed at controlling nematode infections through the use of differing flock grazing strategies. Thus, this chapter addressed the extent of likely epidemiological effects attributable to host genotype, the rate at which these effects accumulate, the impact of anthelmintic treatment on such effects and on the

comparison between susceptible and resistant animals, and the impact of different grazing strategies which aim to reduce exposure of lambs to larvae.

4.4.1 Host resistance and nematode epidemiology

Previous studies aimed at investigating the impact of selection for host resistance have reported on the divergence of FEC in lines selected for resistance and susceptibility grazing on the same pasture (Woolaston and Piper, 1996; Morris *et al.*, 1997). However, an additional benefit may arise from the impact of selection on pasture contamination, and thus by grazing selected or genetically distinct lines on the same pasture the impact of host genotype on FEC and LW may have been underestimated. As a point of comparison, our genetic differences between selected groups and the population average correspond to approximately 15 years of selection on FEC, using standard quantitative genetics theory applied to a log-transformed trait (Falconer and Mackay, 1996) (assuming a standardised selection intensity of 1, male and female generation intervals of 1 and 3 years, respectively, and a heritability of 0.25). The ca. 5-fold difference in FEC between the population average and the resistant group in year 1 agrees almost exactly with the 80% reduction in FEC seen after 15 years of selective breeding for FEC in Australian merino sheep (Kemper *et al.*, 2010). In practice, selective breeding is unlikely to be carried out for increased susceptibility, and the *S* group simply represents susceptible animals within the wider population or a breed difference.

Our model predicts that there would be substantial differences between the FEC, PC and LW for grazing separately as opposed to grazing sheep of different resistance to nematodes together. The question is whether these predictions are consistent with the literature. Three experiments into the impact of such grazing management on resistant and susceptible lambs were identified from available literature for this purpose. In the first, Gruner *et al.* (2002) selected 30 resistant and 30 susceptible lambs from a population of 200 lambs grazed together, such that the susceptible group lambs had an FEC 3.4-fold higher than the resistant group for *Teladorsagia circumcincta*. These lambs were subsequently given an anthelmintic

drench and moved to separate pastures with equal pasture contamination until 4 years of age. By the end of the experiment pasture contamination with *T. circumcincta* larvae was 2-fold higher for the pasture grazed by the susceptible group in comparison with the pasture grazed by the resistant group. Such experimental results clearly display the impact of host resistance genotype on pasture contamination levels.

In a second experiment, Bisset *et al.* (1997) compared resistant and susceptible lambs grazing together and separately. The resistant and susceptible groups were established through divergent breeding lines of Romney sheep, selected for low or high FEC, respectively. Further, a minimal anthelmintic drenching strategy was used and administered to both flocks at the same time. When resistant and susceptible lambs were grazed together for a period of 8 months the average FEC of the susceptible line was 5-fold higher than the resistant line, whilst when the resistant and susceptible lines were grazed separately the average FEC of the susceptible line was around 10-fold higher than the resistant line corresponding to a 6-fold difference in pasture contamination.

In the final experiment, Leathwick *et al.* (1998) assessed the impact of host resistance genotype on pasture contamination, FEC and LW, with a similar experimental design to the simulation study reported here. Resistant and susceptible lambs were produced from divergent lines of Perendale sheep. Prior to the trial the average divergence in average FEC between the lines when grazed together was 4.5-fold (Morris *et al.*, 1997). For 3 grazing seasons resistant and susceptible lambs produced from the divergent lines were grazed on separate pastures and given regular anthelmintic treatment. Each year the same pastures were used for the grazing of resistant and susceptible lambs. By the end of the third grazing season pasture contamination on the pasture grazed by susceptible lambs was 3.6-fold higher than the pasture grazed by resistant lambs and the average FEC of susceptible lambs was 13-fold higher than resistant lambs. Further, the reduction in pasture contamination caused by grazing resistant and susceptible lambs together in comparison to the

susceptible lambs grazed separately was estimated to result in a 1.7kg increase in LW.

Such experiments suggest that grazing resistant and susceptible lambs on separate pastures leads to pasture contamination differences that result in observed differences between average FEC around 2 to 3-fold greater than when resistant and susceptible lambs are grazed together. The simulations carried out in this chapter predict effects of similar magnitude on PC and FEC. However, this divergence of FEC and PC predictions for resistant and susceptible lambs grazed separately is predicted to take 2 grazing seasons to fully emerge, after which differences stabilise as the relationship between the host resistance genotype effect on FEC and the parasite epidemiology reaches equilibrium. Whilst, this stabilisation of FEC and PC predictions following grazing seasons clarifies results presented by Bishop and Stear (1997), in which selection was a continuous process, some factors that may affect PC and FEC predictions were not taken into account in the current model, because their effects were expected to be small. Environmental effects on parasite epidemiology such as the effect of temperature and humidity on the rate of development and mortality of free-living stages and the migration of infective larvae onto herbage, as described in the model of Leathwick *et al*. (1992), may be expected to have some impact on pasture contamination predictions. However, as perturbations in PC through pasture moves were predicted to have little impact on the FEC and LW predictions made here, environmental factors that may affect PC predictions may be of little relevance when investigating the impact of host resistance genotype. Further, genetically resistant sheep have been reported to avoid parasites through grazing behaviour to a greater extent than susceptible sheep (Hutchings *et al.*, 2007). Such differences in grazing behaviour were not taken into account within the current model. However, their impacts, as reported by Hutchings *et al*. (2007), would only amount to an 8% difference in larval exposure and thus would not account for the larger differences in FEC and PC predictions between resistant and susceptible animals reported here.

Whilst substantial differences in FEC were predicted between resistant lambs grazed separately or together with the population, no differences were predicted for final LW whether given anthelmintic treatment or not, due to the relatively small parasite burdens involved and an adequate period for recovery of differences between LW predictions before the end of the grazing season. In contrast, susceptible lambs grazed within the population had a final LW around 6.6kg larger than when grazed separately for un-drenched lambs and around 2.6kg for drenched lambs, similar to those reported by Leathwick *et al.* (1998). Reduced pasture contamination faced by susceptible lambs grazing within the population led to reduced production losses in comparison to susceptible lambs grazed separately. These predictions for LW suggest that anthelmintic drenching may be better targeted towards susceptible lambs that would benefit from treatment, and it may therefore be possible to reduce the use of anthelmintics in more resistant animals where no benefit to treatment was predicted. Further, susceptible lambs were predicted to benefit from grazing together with a more resistant population due to the reduction this would cause on pasture contamination. This may equate to a situation where two breeds (one more susceptible than the other) are run on a farm; the susceptible breed would benefit from grazing with a resistant breed without any significant deleterious impact on the resistant breed. This recommendation may be further extended to mixed grazing scenarios where different host species, such as sheep and goats, show differences in susceptibility to the same nematode species.

4.4.2 Grazing management and nematode epidemiology

Grazing management, involving pasture moves aimed at reduced host exposure to infective larvae have been proposed as a possible control strategy for nematode infection in numerous review articles (Cabaret *et al.*, 2002; Sayers and Sweeney, 2005; Waller, 2006b; Stear *et al.*, 2007). The argument behind this is that reduced exposure to infective larvae would lead to reduced larval intake causing a reduced worm burden and hence a reduced FEC. However, factors such as the effect of density dependence on the fecundity of nematodes (Bishop and Stear, 2000), or the

impact of reduced larval intake on the development of immunity (Dobson *et al.*, 1990) may well decrease the benefits that are actually seen.

Previously, the combination of anthelmintic treatment with evasive strategies has been highly recommended (Michel, 1969; Boag and Thomas, 1973; Michel, 1976). This strategy assumed 100% efficacy of anthelmintics such that 'clean animals' were moved to clean pasture, causing very low re-infection rates prolonging the suppressive effect of anthelmintic treatment on FEC (Waller *et al.*, 1995). However, such a strategy may cause high selective pressure for anthelmintic resistance (Waghorn *et al.*, 2009) and following the initial recommendations for this strategy, anthelmintic resistance was reported in a number of cases (Britt, 1982; Cawthorne and Whitehead, 1983; Taylor and Hunt, 1988). Since then, the reported incidences of resistance to various anthelmintic compounds has increased (Jackson and Coop, 2000), and as such the combination of anthelmintic treatment and evasive grazing strategies as a control strategy for nematode infection needs to be considered carefully in order to optimise parasite control and pasture management and to minimise selection pressure for anthelmintic resistance, for example through targeted selective treatment (TST) (Kenyon *et al.*, 2009).

In more recent times, two experiments were carried out to evaluate whether the evasive grazing strategies were still an appropriate method for the control of nematode infections. In the first, Githigia *et al*. (2001) reported that moving lambs to a clean pasture reduced FEC to less than a third for lambs given no anthelmintic treatment, and to zero for lambs drenched at the time of the pasture move. However, this experiment contained no control group in which lambs remained on infected pasture and thus the reduction observed in FEC cannot be unambiguously attributed to the move to a clean pasture, as the observed reduction in FEC may as equally likely arise from the development of immunity, irrespective of grazing strategy. In the second, Boa *et al*. (2001) reported an experiment in which populations of lambs either drenched or un-drenched remained on the same pasture or were moved to a clean pasture. Whilst anthelmintic treatment was found to reduce FEC, moving the lambs to a clean pasture was reported to have no significant impact on FEC. These

two experiments suggest that there is some confusion over the expected effects of such grazing management on host performance and parasite epidemiology.

The simulations carried out here, which include density-dependence effects and the impact of reduced larval intake on the rate of acquisition of immunity, predict that FEC would be affected little by the pasture move scenarios investigated when lambs are given no anthelmintic treatment. Further, peak PC predictions for scenarios including a pasture move were reduced by 10% in comparison to the scenario with no pasture move, echoing similar findings reported by Boa *et al.* (2001) for lambs given no anthelmintic treatment. The differing grazing management scenarios investigated also predicted minimal impact on the predicted final LW regardless of the host resistance genotype. These predictions suggest that without anthelmintic treatment, little benefit is seen from strategies involving pasture moves, due to the worm burden present within the host population causing rapid contamination of new pastures, irrespective of the host resistance genotype of the sheep population.

Whilst differing grazing strategies had minimal impact on lambs given no anthelmintic treatment, for both resistant and susceptible lambs given a drench and moved to a clean pasture both PC and FEC predictions were much reduced and final LW slightly increased in comparison to all other scenarios. This finding suggests that both resistant and susceptible breeds may benefit from a drench and move strategy, and that in this case the pasture move may have prolonged the beneficial impact of anthelmintic treatment. However, PC arising from such a scenario may result in the emergence of anthelmintic resistance due to the selective pressure exerted on the within-host nematode population and the lack of refugia (Barnes *et al.*, 1995). Thus drenching and moving to a clean pasture may be considered a high-risk practice for the selection for anthelmintic resistance (Waghorn *et al.*, 2009). Further, whilst anthelmintic efficacy was assumed to be 95% in these simulations (whether due to anthelmintic resistance or issues arising from administration), the issue of the emergence of anthelmintic resistance was not accounted for in this model, and as such the efficacy of a second anthelmintic drench in such a drench and move

scenario may expected to be reduced. Therefore it is possible that the benefits of anthelmintic drenching on LW predictions may be reduced in such a scenario, and the slight increase in final LW predicted here may also be reduced.

4.4.3 Conclusions and implications

Host resistance genotype is predicted to have large impacts on parasite epidemiology, such that resistant lambs reduce pasture contamination and thus provide an additional benefit in reducing the grazing population's exposure to infective larvae. However, grazing strategies aimed at reducing host exposure to infective larvae using pasture relocation were predicted to have little impact of FEC and LW. Such findings suggest that control strategies aimed at impacting the propagation of nematode infections are of much greater benefit than those that aim to control free-living stages.

Anthelmintic treatment was predicted to have little impact on LW predictions for resistant lambs, whilst causing improvements in LW gain for susceptible lambs suggesting that anthelmintic use may be reduced by targeting susceptible lambs without a reduction in LW gain across a population. Susceptible lambs were also predicted to benefit from grazing with more resistant populations, thus leading to the possible recommendation that sheep breeds known to be susceptible to nematode infections may benefit from grazing the same pasture as breeds known to be more resistant.

Chapter Five

Modelling the short- and long-term impacts of targeted selective treatment on the performance of grazing lambs and the emergence of anthelmintic resistance

5.1 Introduction

The control of gastrointestinal parasitism using chemotherapeutic strategies is under threat due to the emergence of anthelmintic resistance (Kaplan, 2004; Wolstenholme *et al.*, 2004; Jabbar *et al.*, 2006; Papadopoulos *et al.*, 2012), and thus threatens the sustainability of livestock systems (Waller, 2006a; Besier, 2007; Papadopoulos, 2008). Numerous alternative strategies, including the maintenance of refugia (van Wyk, 2001; Soulsby, 2007; Jackson and Waller, 2008; Leathwick *et al.*, 2009) and targeted selective treatment (TST) (van Wyk *et al.*, 2006; Kenyon *et al.*, 2009; Besier, 2012; Kenyon and Jackson, 2012) have been proposed to reduce the rate of development of anthelmintic resistance and maintain sustainable parasite control.

Maintaining a proportion of the nematode population *in refugia* (unexposed to anthelmintic) preserves susceptible parasite genotypes, thus slowing the development of anthelmintic resistance (van Wyk, 2001; Nielsen *et al.*, 2007; Soulsby, 2007; Torres-Acosta and Hoste, 2008). In temperate climates, up to 95% of the total nematode population may be found on pasture (Barnes *et al.*, 1988), thereby providing a relatively large reservoir of anthelmintic susceptibility in the namtode population. However, various factors affect parasite epidemiology and hence the parasite population *in refugia*, including environmental conditions (Morgan and van Dijk, 2012), host immune response (Laurenson *et al.*, 2012b) (**Chapter 4**) and management practices (Leathwick *et al.*, 2009). Thus, the proportion of the total

nematode population on pasture may be expected to vary within a grazing season. Further, treatment frequency has also been associated with the emergence of anthelmintic resistance (Jackson and Coop, 2000; Coles, 2005; van Wyk *et al.*, 2006). As such, refugia based treatment strategies aim to reduce treatment frequency and administer anthelmintics at appropriate times when the proportion of the total nematode population on pasture is high.

TST strategies reduce the number of anthelmintics treatments administered, and thus increase the nematode population *in refugia*, by selective treatment of only those animals that will benefit most from treatment whilst leaving the rest of the flock untreated (van Wyk *et al.*, 2006; Kenyon *et al.*, 2009). Thus, a TST approach provides a strategy capable of exploiting the over-dispersion of parasitic nematodes within the host population (Sreter *et al.*, 1994; Bishop and Stear, 1997; Hoste *et al.*, 2001). However, the implementation of TST regimes requires determinant criteria for the identification of animals susceptible to parasitism. The optimal determinant criterion would be the trait that allows for the greatest reduction in the number of anthelmintics administered whilst retaining the highest level of flock performance in terms of weight gain. Various phenotypic traits have previously been proposed, including parasitological traits such as faecal egg count (FEC) as an indicator of host resistance (Cringoli *et al.*, 2009; Gallidis *et al.*, 2009), and performance traits such as live weight (Leathwick *et al.*, 2006a; b) and weight gain (Waghorn *et al.*, 2008; Stafford *et al.*, 2009; Gaba *et al.*, 2010) as indicators of host resilience. However, whilst each trait has been independently evaluated for use as the determinant criteria in a TST regime (Kenyon and Jackson, 2012), a direct comparison of the effectiveness of the different traits in identifying susceptible animals for treatment has not as yet been carried out.

Field studies investigating refugia-based strategies and TST regimes have focused on the short-term impacts upon flock performance and parasitic burdens due to the difficulty of measuring changes in the frequency of resistance genotypes within nematode populations (Gilleard, 2006). On the other hand, simulation studies have previously been used to provide insights into the long-term relationship

between such control strategies and the emergence of anthelmintic resistance (Barnes *et al.*, 1995; Leathwick *et al.*, 1995; Learmount *et al.*, 2006; Dobson *et al.*, 2011). However, these simulation studies have been restricted to modelling nematode epidemiology (Gaba *et al.*, 2010) making comparison with performance based field studies difficult. Thus, the aim of this study was to use an appropriate mathematical model to make a comparison of traits previously proposed for use as determinant criteria for TST regimes and to investigate the impacts of TST regimes and drenching frequency on both sheep performance and the emergence of anthelmintic resistance. By doing so, we will quantify the short-term impacts of various TST strategies and make a link between short- and long-term consequences of the method.

5.2 Materials and Methods

The mathematical model of Laurenson *et al.* (2011; 2012a) (**Chapters 2** and **3**) which describes the epidemiology of *Teladorsagia circumcincta* and the impact of host nutrition, genotype and gastro-intestinal parasitism on a population of growing lambs, was used as the basis of this study. Several modifications were made to the model, including the addition of a new module to provide a description of anthelmintic resistance genotypes within the nematode population and the differing phenotypic susceptibility of such genotypes to anthelmintic treatment. A brief overview of the existing model (host-parasite interaction model and epidemiological model), as well as a more in depth description of the new module (anthelmintic resistance model), are given below.

5.2.1 Host-parasite interaction model

In the model of Laurenson *et al.* (2011) (**Chapter 2**), gastrointestinal parasitism of a growing lamb was assumed to result in endogenous protein loss (Yakoob *et al.*, 1983), modelled as a function of parasitic burden (Vagenas *et al.*, 2007a). To counteract this protein loss, each animal invests in an immune response which affects the rates of nematode establishment, fecundity and mortality (Louie *et al.*, 2005), and thus reduces parasitic burden. However, components of the immune response (e.g.

cytokines) are associated with a reduction in food intake (Greer *et al.*, 2008; Kyriazakis, 2010), commonly known as (parasite-induced) anorexia. The combination of protein loss due to parasitism, investment in immunity and anorexia, result in the lamb acquiring insufficient nutrient resources to fulfil requirements for optimal growth, and thus lamb growth rate reduces.

The individual lamb model was extended to a population level (Laurenson *et al.*, 2012a) (**Chapter 3**) by including between-animal variation in optimal growth rate, body composition (expected protein and lipid content at maturity), maintenance requirements (protein and energy), and immune response (rate of acquisition, as well as initial and final rates for establishment, mortality and fecundity). Initial input parameters involved in these functions were assumed to be normally distributed and all traits, other than those associated with host resistance, were assumed to be uncorrelated (Doeschl-Wilson *et al.*, 2008). Resistance traits, as a function of overlapping effector mechanisms (components of the Th2 immune response) (Jenkins and Allen, 2010), were assumed to be strongly correlated (r = +0.5). Further, random environmental variation in daily food intake was included to achieve a genetic correlation between food intake and growth rate of approximately 0.8 (Cammack *et al.*, 2005).

The model was parameterised such that lamb growth characteristics were similar to those of the Scottish Blackface, parasitological parameters matched those of Coop *et al.* (1982; 1985) for lambs infected with *T. circumcincta*, and the between-animal variance of each trait was chosen to match those of Bishop *et al.* (1996) and Bishop and Stear (1997).

5.2.2 Epidemiological model

In the epidemiological module of Laurenson *et al.* (2012a) (**Chapter 3**), the grazing pasture was defined by the number of hectares and grass available for grazing (Sibbald *et al.*, 2000), taking into account grass growth and grass consumed on a daily basis. This pasture was assumed to be initially contaminated with a number of eggs and larvae arising from a ewe population removed from pasture at lamb

weaning. Subsequent larval contamination of pasture was assumed to arise from eggs excreted by lambs, taking into account egg to infective larvae development time (Young *et al.*, 1980), a mortality rate for infective larvae (Gibson and Everett, 1972) and the removal of infective larvae from pasture through grazing. Lambs were assumed to graze randomly across the pasture, leading to an expected larval intake directly proportional to food intake. Thus, the epidemiological model was linked to the host-parasite interaction model through food intake and eggs excreted by the population of lambs.

5.2.3 Anthelmintic resistance model

Anthelmintic resistance was assumed to be conferred by two alleles, resistant (R) and susceptible (S) (Barnes *et al.*, 1995; Leathwick *et al.*, 1995; Learmount *et al.*, 2006). This is in agreement with the monogenic mechanism for benzimidazole resistance (Elard and Humbert, 1999). The resistance genotypes of the initial population of infective *T. circumcincta* larvae on pasture were assumed to arise from random mating setting the initial frequency of the R allele at 0.01 (Barnes *et al.*, 1995), assuming Hardy-Weinberg equilibrium. The frequency of R in the worm burden of each host was used for new larvae arising from eggs excreted by each lamb, with genotype proportions again calculated assuming Hardy-Weinberg equilibria. All genotypes were assumed to be equally fit (Barrett *et al.*, 1998; Elard *et al.*, 1998), such that in the absence of anthelmintic drenching the frequency of R remains the same throughout the simulated grazing season. The allele conferring anthelmintic resistance (R) was modelled to be recessive (Elard and Humbert, 1999; Silvestre and Cabaret, 2002). Anthelmintic drenching was assumed to reduce the population of infective larvae and adult nematodes resident within a host by 99% for heterozygous (RS) and homozygous susceptible genotypes (SS), and by 1% for homozygous resistant genotypes (RR). Further, the oral administration of anthelmintic was assumed to be effective on the day of administration, with no residual effects (Borgsteede, 1993). Thus in the first instance, anthelmintic drenching causes a 99% reduction in parasitic burden and, with imposition of density-dependent effects on parasite fecundity, a 96.9% reduction in FEC, similar to the post-treatment efficacies

for *T. circumcincta* reported by Sargison *et al*. (2007). The total population of each resistance genotype was tracked on a daily basis in hosts and on pasture, along with the frequency of R. Further, the nematode population *in refugia* (unexposed to anthelmintic) was calculated daily.

5.2.4 Simulation procedure and *in silico* experimental design

A population of 10,000 lambs were simulated to be grazing on a medium-quality pasture (crude protein = 140g/kg DM, metabolisable energy = 10MJ/kg DM (AFRC, 1993)), at a density of 30 lambs/ha for a period of 4 months from weaning to 6 months of age. The lambs were assumed to be initially parasitologically naïve and the initial larval contamination of pasture was set to 3,000 *T. circumcincta* larvae/kg DM pasture. Lambs initially ingest around 1kg DM/day and thus this level corresponds to a trickle challenge level shown by Coop *et al*. (1982) to lead to sub-clinical infections.

To determine the most appropriate drenching occasions during the grazing season, a simulation was run to determine the total population of nematodes (eggs, larvae and adult worms) and the parasitic population (larvae and adult worms) resident within a host population given no anthelmintic treatment (Figure 5.1). The initial proportion of the nematode population resident within the host population rose rapidly until day 13 as larvae were ingested from pasture, and decreased again after ingested larvae matured to adult worms and started contributing eggs to the nematode population on pasture on day 14. Following this period, the proportion of nematodes within host rose steadily for the remainder of the simulated grazing season. As a consequence, day 30 was chosen as the first drenching occasion due to the low proportion of the nematode population resident within host and a high total population of nematodes, thus representing a high refugia pool. Day 60 was chosen as the second drenching occasion due to this being the time point at which the highest total parasite population was predicted. Finally, day 90 was chosen as the third drenching occasion, such that drenching would occur at 30 day intervals and thus represent a suppressive control strategy (Sargison *et al*., 2007). Hence, four

scenarios were created to represent a range of drenching frequencies, including no treatment (no drenches), drenching at day 30 (1 drench), drenching on days 30 and 60 (2 drenches) and drenching at 30 day intervals (3 drenches).
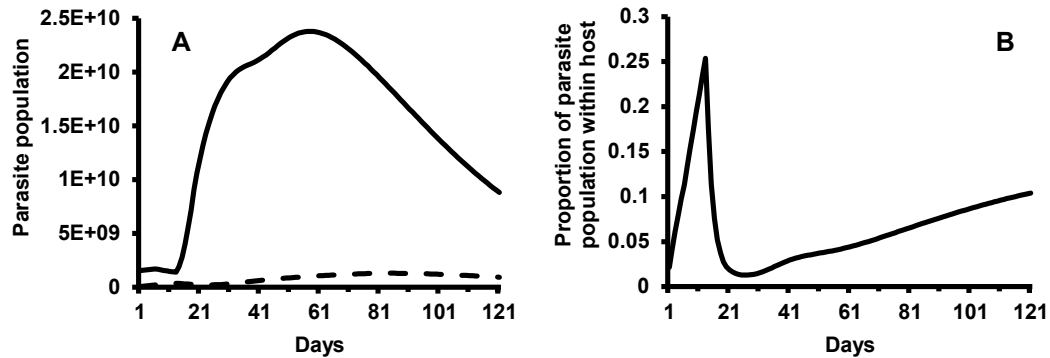
Figure 5.1. A) Total parasite population (———) and parasite population within host (‐ ‐ ‐ ‐), B) proportion of parasite population within host in comparison to the total parasite population; for a population of 10,000 lambs grazing on a medium-quality pasture with an initial pasture contamination of 3,000 *T. circumcincta* larvae/kg DM pasture.

Targeted selective treatment (TST) was achieved by drenching a percentage of the lamb population according to a given determinant criterion. The determinant criteria evaluated for use in a TST regime included live weight (LW, kg), growth rate (kg d^{-1}), FEC (eggs/g DM faeces), a combination of LW and FEC, and random selection. LW was assumed to be measured the day prior to treatment with lighter lambs preferentially drenched. Growth rate was calculated assuming lambs were weighed at the start of the grazing season and on the day prior to each treatment with lambs preferentially drenched according to the lowest weight gain since the last weighing. FEC measurements were assumed to be taken 5 days prior to treatment to allow time for the samples to be processed and analysed, with a random sampling error with a variance of 0.2 (Bishop *et al.*, 1996; Stear *et al.*, 2009) added to recorded values. Subsequently, lambs with the highest FEC were preferentially drenched. The combination of LW and FEC was achieved by using sampled FEC and LW measured as described above. Log-transformed FEC (ln(FEC+1)) and LW were weighted according to their respective standard deviations, and the traits combined by adding

negative LW to log-transformed FEC. Lambs were then preferentially drenched according to the highest values. Finally, random selection was achieved using a random number generator to identify the lamb IDs to be drenched. In order to investigate a range of TST scenarios, treatment was assumed to occur for each of the 10th percentiles (10%, 20%, …, 90%, 100%) of the host population as indicated by each of these determinant criterion.

5.2.4.1 'Short-term' effects

To investigate the short-term impact of drenching frequency and TST strategies on sheep production and the emergence of anthelmintic resistance, the population of lambs was simulated over a single grazing season for each of the drenching frequencies and TST scenarios, initially using FEC as the determinant criterion due to this trait being the most direct indicator of parasitic burden. Daily output traits recorded included performance traits such as average empty body weight (EBW, kg), epidemiological traits such as pasture contamination (PC, larvae/kg DM grass), parasitological traits such as average worm burden (WB) and average FEC (eggs/g DM faeces), and anthelmintic-resistance traits such as the proportion of worms *in refugia* and the frequency of R in the nematode population on pasture.

Subsequently, to compare the various determinant criteria described above, the population of lambs were grazed over a single grazing season for each of the TST scenarios and each of the determinant criterion for a single drenching occasion on day 30. Results for additional drenches are not reported here, as they give a similar pattern of results when comparing determinant criteria. Output traits recorded included average EBW, average FEC and the frequency of R in the nematode population on pasture at the end of the grazing season (day 121).

5.2.4.2 'Long-term' effects

To investigate the long-term effects of drenching frequency and TST regimes on sheep production and the emergence of anthelmintic resistance, the population of

lambs was simulated over several years under each of the drenching frequencies and for each of the TST scenarios using FEC as the determinant criterion for anthelmintic treatment, as our results showed FEC to be the most effective criterion (see below). Each year, all input parameters remained the same except that the initial frequency of R was set to the final value from the previous year, thus making the assumption that little selective pressure was placed on the nematode population between the simulated seasons. Anthelmintic treatment was abandoned once resistance was determined to be present within the parasite population according to a FEC reduction test as described by Coles *et al*. (1992). As such, drenching was stopped if the reduction in FEC, in comparison to an appropriate control population, was less than 95% ten days post anthelmintic treatment and the lower bound of the 95% confidence level (as calculated using the variance of reduction) was less than 0.9.

Output traits recorded included average EBW, the frequency of R in the nematode population on pasture and the total number of anthelmintics administered before resistance was reported. Further, in order to determine the net benefit of anthelmintic treatment on sheep performance, the increase in EBW attributable to anthelmintic treatment was calculated by subtracting the EBW of an undrenched population, and the total EBW gain summed across the period of time until anthelmintic treatment was halted.

5.3 Results

5.3.1 'Short-term' effects with FEC as the determinant criterion

Figure 5.2 provides a description of the impact of drenching differing proportions of the sheep population, as determined by FEC for a single anthelmintic treatment administered on day 30, over a single grazing season. In brief, the higher the percentage of the population drenched, the greater the reductions in PC, average WB and average FEC. These reductions in WB and PC resulted in reductions in the impacts of parasitism on average weight gain and consequently increased final average EBW at day 121. Following anthelmintic treatment, the percentage refugia on pasture reduced as the eggs produced from nematodes exposed to anthelmintic

treatment developed to infectious larvae. Thus, the higher the percentage of the lamb population drenched, the greater the reduction in refugia. The reduction in refugia peaked around 20 days after the anthelmintic was administered, coinciding with the maximum reduction in PC. Following this, and in the absence of a further anthelmintic treatment, the percentage refugia increased again as the population of infective larvae exposed to anthelmintic treatment was reduced via the mortality rate of infectious larvae on pasture and the ingestion of infective larvae by the grazing sheep population. By the end of the simulated grazing season the PC, average WB, average FEC, and percentage refugia were similar for all percentages of the lamb population drenched.

However, the same trend was not predicted for the frequency of R within the nematode population on pasture. Following anthelmintic treatment, the frequency of R on pasture rapidly increased due to the high frequency of R within the surviving parasitic burdens of the host population. The eggs produced by this population took 7 days to develop to infective larvae on pasture (Young *et al.*, 1980), and thus there was a period following anthelmintic treatment in which lambs ingested larvae from pasture where the frequency of R was unaffected by the anthelmintic treatment. Thus the frequency of R on pasture, after peaking around 20 days post anthelmintic treatment, reduced as the frequency of R within the host population was diluted by new adult worms establishing. The frequency of R on pasture stabilized around 40 days following anthelmintic treatment with increasing drenching percentages of the host population leading to increasing final frequencies of R on pasture at the end of the grazing season.
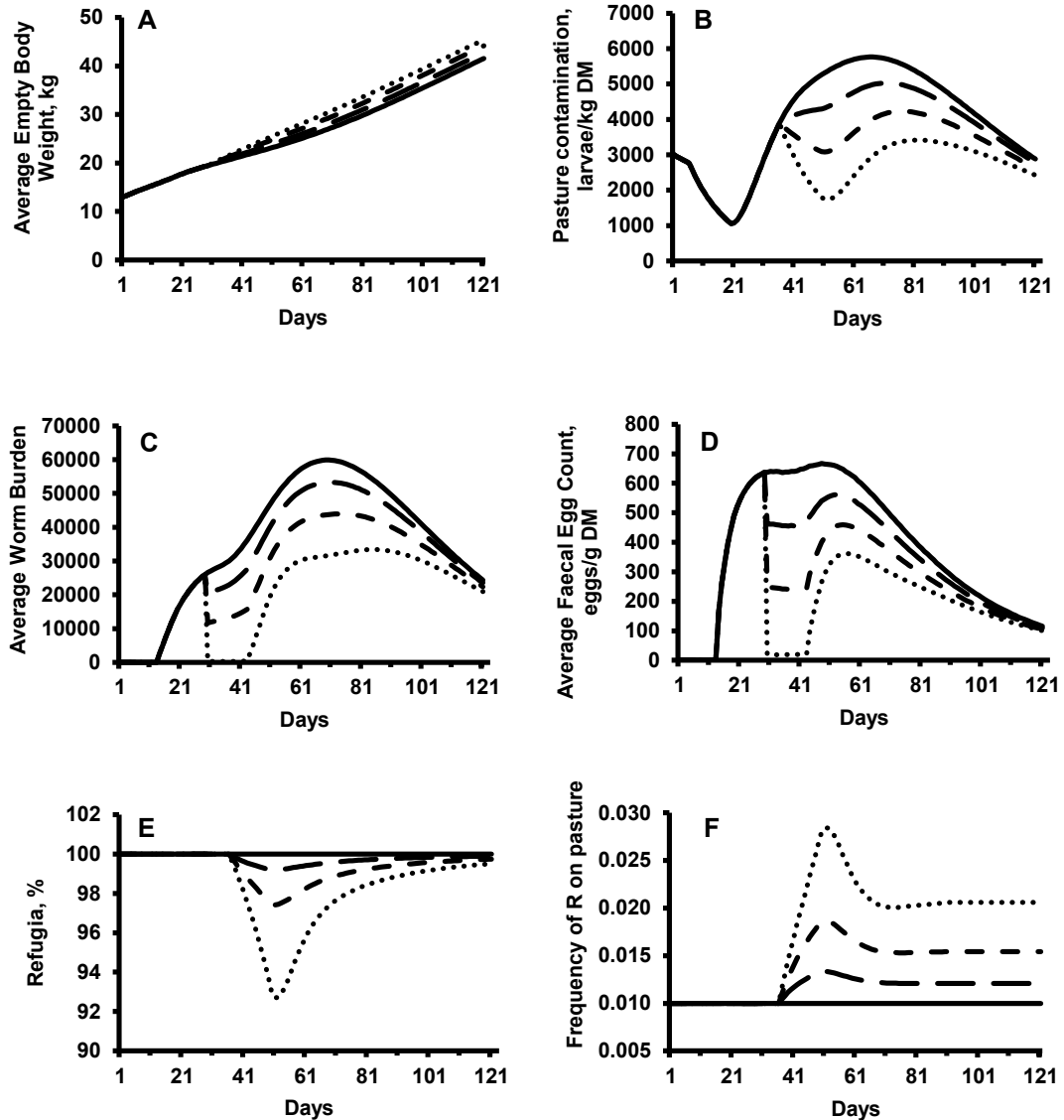
Figure 5.2. A) Average empty body weight (kg), B) Pasture contamination (larvae/kg DM pasture), C) Average worm burden, D) Average faecal egg count (eggs/g DM), E) Refugia, %, F) Frequency of R on pasture; for 10,000 lambs grazing on pasture initially contaminated with 3,000 *T.circumcincta* larvae/kg DM and either given no anthelmintic drench (———), 20% of lambs drenched (— — ·), 50% of lambs drenched (– – – ·), or all lambs drenched (··········). Anthelmintic treatment was administered on day 30, and the percentage of lambs drenched was determined using FEC.

Figure 5.3 gives the final average EBW, average FEC and final frequency of R on pasture for populations of lambs drenched at differing percentiles as determined by FEC for the differing drenching frequencies. For all drenching frequencies, the final average EBW increased, and the average FEC decreased, with increased percentage of the population drenched. The relationship between final average EBW (or average FEC) and percentage of the population drenched was curvi-linear with anthelmintic drenching having a decreasing impact on final average EBW and average FEC as the percentage of the population given anthelmintic treatment increased.

However, each drenching occasion did not have equal impact on the final average EBW and average FEC. Whilst a single anthelmintic treatment on day 30 had an impact on both final average EBW and average FEC for all of the differing percentages of the population drenched, the administration of a second drench on day 60 only increased this impact by ~58% (rather than doubling it), whilst the administration of a third drench at day 90 only added a further 6%. Thus, the third drench on day 90 had only a minimal impact on the final average EBW and average FEC.

The final frequency of R on pasture increased with increasing drenching frequency and proportion of the host population drenched. The increase in the frequency of R for the 2 drench scenario was around 3-fold that predicted for the 1 drench scenario, whilst the same values for the 3 drench scenario were around 7-fold under the same comparison. Thus, increasing the drenching frequency reduced the benefits derived from each anthelmintic treatment whilst increasing the rate of emergence of anthelmintic resistance.
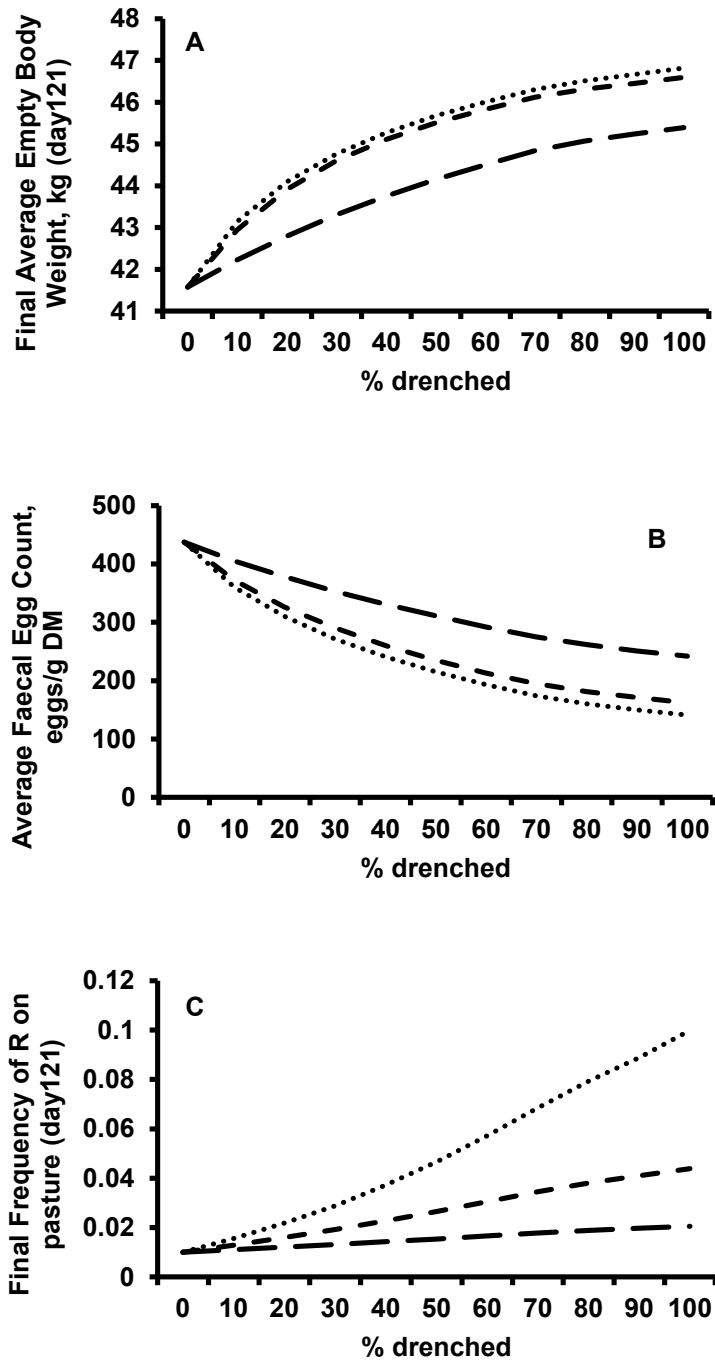
Figure 5.3. A) Final (day 121) average empty body weight (kg), B) Average (days 14-121) faecal egg count (eggs/g DM), C) Frequency of R on pasture; for 10,000 lambs grazing on a pasture initially contaminated with 3,000 *T.circumcincta* larvae/kg DM and drenched by percentage of population according to FEC either once (on day 30, — — ·), twice (on days 30 and 60, – – – –), or three times (days 30, 60 and 90, ··········).

5.3.2 Comparison of determinant criteria

Figure 5.4 gives the final average EBW, average FEC and final frequency of R for the populations of lambs drenched at differing percentiles as determined either randomly or by LW, growth rate, FEC or the combination of LW and FEC, and given a single drench on day 30. As previously mentioned, the optimal determinant criterion for use in a TST regime may be considered to be the trait which allows for the smallest percentage of the host population to be drenched whilst maintaining a high average EBW. As such, a good determinant criterion would produce a convex curvi-linear relationship between final average EBW (or average FEC) and percentage drenched. Random selection of lambs, included as a control against which the determinant criteria could be evaluated, was predicted to result in a linear relationship between final average EBW and percentage of the lamb population drenched, similarly for average FEC.

Using FEC as the determinant criterion was predicted to result in the best relationship between EBW (or FEC) and percentage drenched, with ever-reduced percentages of the population drenched being predicted to result in slightly greater marginal increases in EBW and decreases in FEC. Using the combination of LW and FEC was predicted to be the second best determinant criterion, with the beneficial impacts of using this marker being predicted to be half-way between those predicted for FEC alone and drenching randomly. However, using LW as the determinant criterion was predicted to give little to no improvement in comparison to randomly selecting animals. Further, using growth rate as the determinant criterion was predicted to give concave relationships between output traits and the proportion drenched, and hence was worse than selecting animals randomly for treatment.

The predictions for final frequency of R on pasture for each of the determinant criteria being assessed are shown in Figure 5.4c. Criteria that had more favourable impacts on average FEC, such as using FEC as the determinant criterion, resulted in higher predicted frequencies of R in comparison to random selection of lambs. However, for all determinant criteria, the final frequency of R reduced with reducing percentiles of the population drenched.
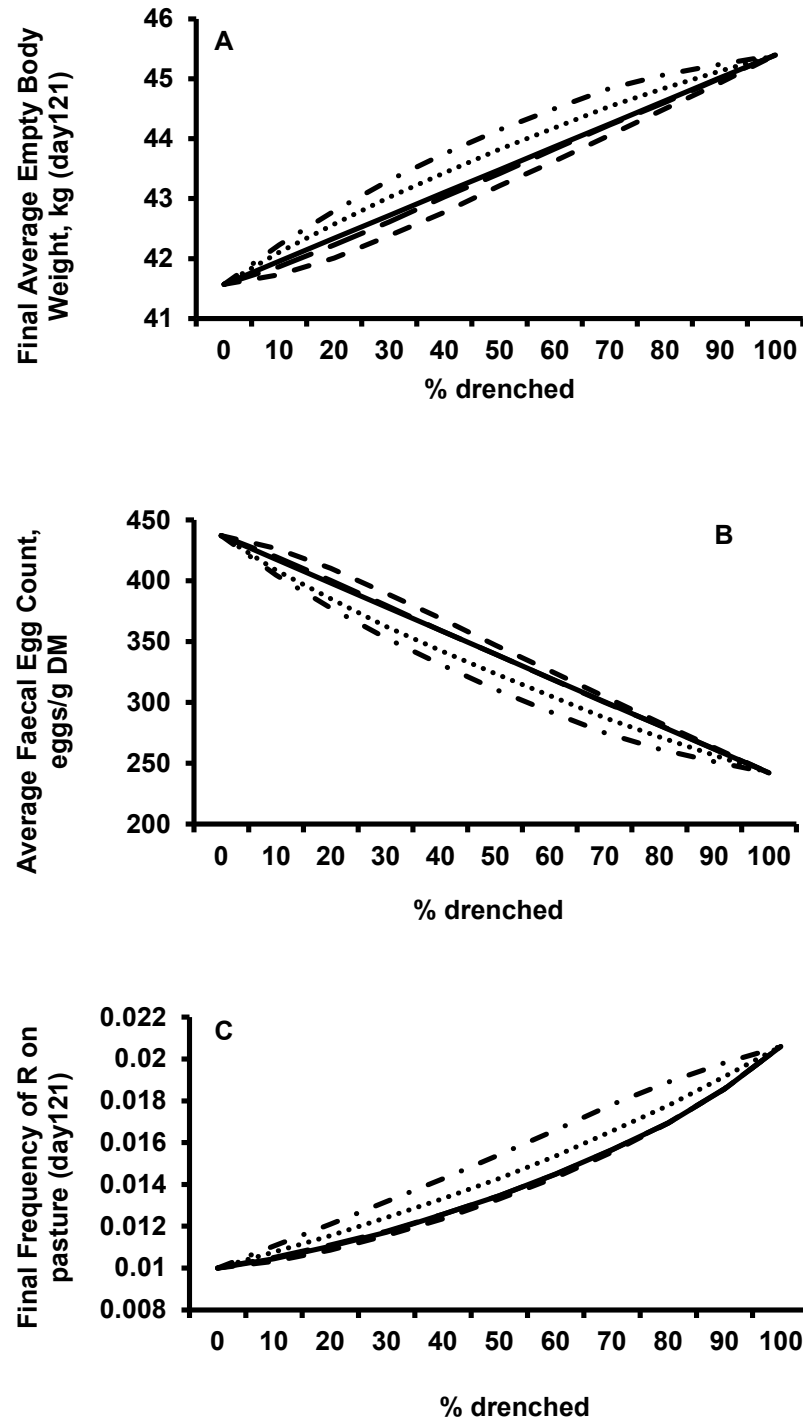
Figure 5.4. A) Final (day 121) average empty body weight (kg), B) Average (days 14-121) faecal egg count (eggs/g DM), C) Frequency of R on pasture; for 10,000 lambs grazing on a pasture initially contaminated with 3,000 *T.circumcincta* larvae/kg DM and drenched on day 30 by percentage of population either randomly (———) or according to faecal egg count (– · – · –), live weight (— — —), growth rate (– – – –) or the combination of faecal egg count and live weight (··········).

5.3.3 'Long-term' effects

Figure 5.5 gives an example of how the frequency of R and the average EBW gain attributable to anthelmintic treatment change over time (years) for differing drenching percentiles for a single drench at day 30, using FEC as the determinant criterion. In these examples the simulation was allowed to continue past the point at which the anthelmintic failed the FEC reduction test. Drenching higher percentages of the lamb population was initially predicted to result in the highest average EBW gain. However, increasing the percentage of the population drenched also caused an increase in rate at which anthelmintic resistance emerged in the parasite population, with the frequency of R increasing earlier for higher drenching percentages. This increase in the frequency of R caused decreased anthelmintic efficacy and therefore the average EBW gain decreased as the frequency of R in the nematode population on pasture increased. Thus, drenching higher proportions of the host population led initially to increased average EBW gain but a short duration of anthelmintic efficacy.
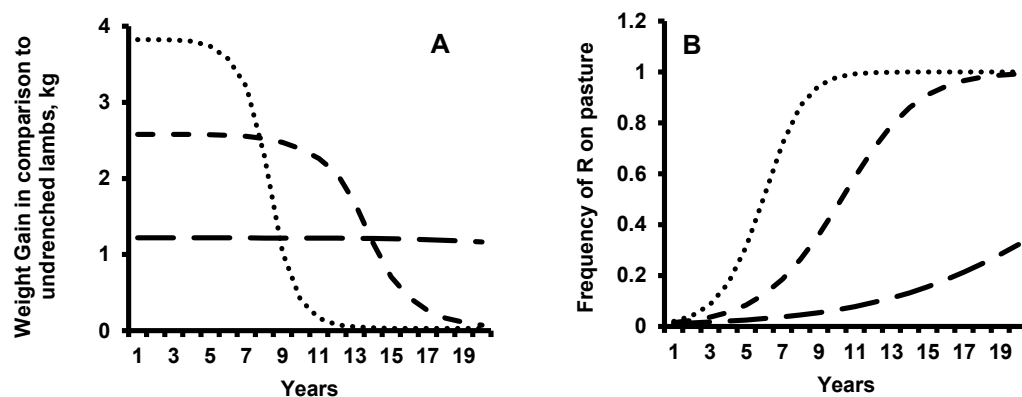
Figure 5.5. A) Average weight gain in comparison to undrenched lambs (kg), B) Frequency of R on pasture; for 10,000 lambs grazing on pasture per year and initially contaminated with 3,000 *T.circumcincta* larvae/kg DM . Either 20% of lambs were drenched (― ― ―), 50% of lambs drenched (‐ ‐ ‐ ‐), or all lambs given anthelmintic treatment on day 30 (··········) using FEC as the marker for TST over a period of 20 years.

Figure 5.6a gives the total benefits of differing drenching frequencies for all drenching percentiles, using a FEC reduction test as a determinant of when to abandon the anthelmintic drenching. The FEC reduction test (Coles *et al.*, 1992) was found to result in an anthelmintic drench failing if the frequency of R on pasture was equal to or greater than 0.2 prior to the anthelmintic treatment being administered, representing a frequency of R just prior to the large and rapid reductions in efficacy as predicted in Figure 5.5. The total EBW gain attributed to anthelmintic treatment until resistance was reported was predicted to increase with decreasing drenching frequency, as may be expected from the predictions given in Figure 5.3. However, the total EBW gain attributed to anthelmintic treatment was predicted to be largely independent of the percentage of the host population drenched. Whilst total additional weight gain (i.e. the benefits of the anthelmintic summed across all animals and all years) was largely unaffected, decreasing the drenching frequency and percentage of the population drenched was predicted to result in increased duration of efficacy (Figure 5.6b) and number of drenching occasions (Figure 5.6c), whilst decreasing the total number of anthelmintic treatments administered (Figure 5.6d).
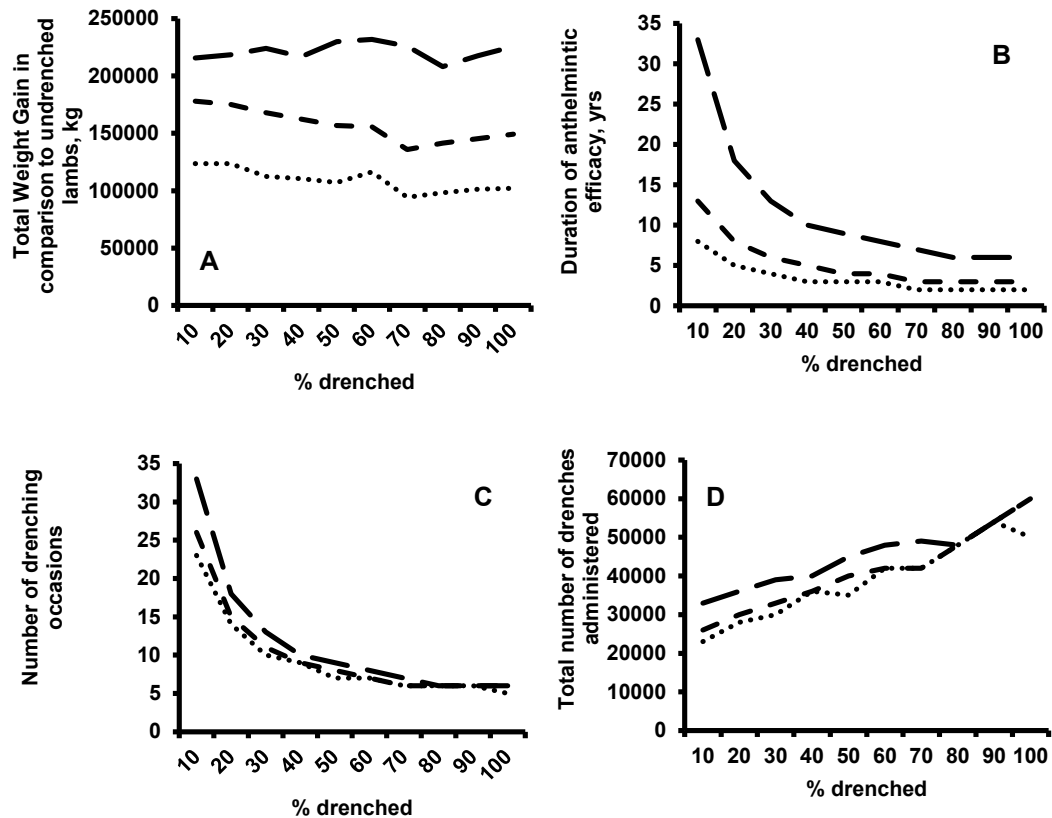
Figure 5.6. A) Total increase weight gain for drenched lambs in comparison to undrenched lambs across the lifetime of anthelmintic (i.e. until anthelmintic resistance was determined to be present according to a FEC reduction test (Coles *et al.*, 1992)), B) Duration of efficacy (years), C) Number of drenching occasions, D) Total number of drenches administered; for 10,000 lambs grazing on a pasture initially contaminated 3,000 *T.circumcincta* larvae/kg DM each year up to 33 years and given an anthelmintic drench at day 30 of each grazing season (— — -), days 30 and 60 of each grazing season (– – – ·), or days 30, 60 and 90 of each grazing season (··········).

5.4 Discussion

Refugia based strategies, including TST, have previously been suggested as a method of reducing the rate of development of anthelmintic resistance whilst retaining sustainable levels of parasitic control. Most field studies investigating these strategies have focussed on their short-term impacts on performance traits (Kenyon and Jackson, 2012), due to the difficulty in assessing changes in anthelmintic resistance over short time scales (Besier, 2012), although Leathwick *et al*. (2006a) have

reported a field study to investigate the short-term impact of drenching adult ewes on the development of anthelmintic resistance. Conversely, modelling studies have tended to focus on the long-term impact of such strategies on the emergence of anthelmintic resistance (Leathwick, 2012; Barnes *et al.*, 1995), although shorter-term impacts of drenching on the development of anthelmintic resistance have also been reported (Leathwick *et al.*, 1995; Learmount *et al.*, 2006). Here we used modelling to link performance traits and anthelmintic resistance, over both short and long time horizons, when evaluating various TST strategies.

In principle, three factors influence the effectiveness of TST, viz., (i) the proportion of animals drenched at each treatment, (ii) the number and timing of treatments and (iii) the determinant criterion used to rank animals for treatment. In terms of the first factor and for all determinant criteria, decreasing the proportion of the lamb population drenched was predicted to reduce the benefits of anthelmintic treatment on average EBW, but decrease the rate at which anthelmintic resistance develops (reduce the frequency of the R allele). Further, the short-term impact of anthelmintic drenching on the frequency of R was predicted to be similar to the profile described by Leathwick *et al.* (1995), where a simulation model for nematodiasis in lambs was used to explore the impact of anthelmintic drenching on the development of anthelmintic resistance. These results reflect the trade-off between resistance management and the effective control of parasitism (Besier, 2012).

Refugia-based strategies usually involve alterations to the timing and frequency of anthelmintic drenches such that treatment is administered at a time when a high refugia pool is present on pasture. The investigation into drenching frequency, the second factor identified above, predicted that with each consecutive drench the impact on EBW reduced such that the third drenching occasion had minimal impact on EBW. However, each consecutive drench was predicted to have an increasing impact on the frequency of resistance alleles, with this change coinciding with reductions in refugia. Further, increasing proportions of the nematode population *in refugia* at the time of drenching were predicted to lead to a

decreased impact of drenching on the frequency of R. Thus, these predictions provide support for the use of refugia based strategies, with reduced drenching frequencies leading to increasing performance benefits and decreased rate of emergence of anthelmintic resistance.

In terms of the third factor, numerous determinant criteria have previously been proposed for use in TST regimes, including indicators of host resistance (FEC) and host resilience (LW and weight gain). However, FEC has only been evaluated as a determinant criterion for TST by assessing the impact on milk production in adult ewes (Cringoli *et al.*, 2009; Gallidis *et al.*, 2009), and thus no comparison could be made between experimental studies and the predictions for impact on lamb growth. In the comparison reported here, using FEC as the determinant criterion was predicted to allow the greatest reduction in the number of anthelmintics treatments administered whilst retaining the highest average EBW, whereas LW and growth rate were predicted to give little to no improvement in comparison with selecting animals at random. In fact, the use of growth rate resulted in a worse relationship between the number of anthelmintics administered and the average EBW than selecting animals at random. FEC may be considered as the best determinant criterion due to being an indicator of parasitic burden, whilst performance traits such as LW and growth rate are both affected by many factors other than gastrointestinal parasitism. Such factors may include the link between food intake and growth rate (Cammack *et al.*, 2005), such that lambs with a faster growth rate ingest more grass and are exposed to a greater parasitic challenge than lambs with a slower growth rate. Currently all experimental studies investigating TST regimes have only compared the selectively treated groups to a suppressive drenching scenario, whilst failing to evaluate the determinant criteria against a group where an equal number of animals are randomly selected to remain untreated.

Leathwick et al. (2006b) used LW to identify the 10% heaviest lambs which then remained untreated for flock drenches administered at 28 day intervals over a period of 8 months. The average LW gain of this group was then compared to a group where all lambs received treatment on each drenching occasion. Whilst no

significant differences were reported between groups for mean monthly LW gain, our analysis of the reported results shows that the total LW gain across the entire experimental period for the selectively drenched group was reduced by 12% in comparison to the group where all animals received treatment. However, under the same scenario, our simulations predicted only a 2% reduction in total weight gain. In a field study investigating the impact of TST regimes on the development of anthelmintic resistance, Leathwick et al. (2006a) used LW to identify the 15% heaviest lambs which then remained untreated for flock drenches administered on 6 occasions per year for a period of 5 years. The mean concentration of albendazole required to prevent 50% of parasite eggs developing to the third larval stage for *T. circumcincta*, estimated using a larval development assay, for the selectively treated group was reported to be reduced by 20% in comparison to a group where all animals were treated, corresponding to a 22% reduction in the frequency of R predicted by simulating this scenario. Finally, Gaba et al. (2010) used a LW and FEC index, similar to that outlined here, to selectively treat 10% of lambs within a group for a monthly drenching regime. The average LW gain and FEC of this group were then compared to a group where all lambs received a monthly drench. Whilst no significance differences were attained, the selectively treated group were reported to have a 7.7% reduction in total LW gain, and a 35% increase in FEC, in comparison to the group where all lambs were treated. Similarly, simulating this scenario with our model predicted a 10% reduction in total weight gain and a 40% increase in FEC.

It should be noted that validating model predictions by comparison to field studies may be constrained by differences in climatic conditions, population sizes, species compositions, management practices and levels of drug resistance. For example, in the studies of Leathwick et al. (2006b) a mixed species infection was identified including parasitism with *H. contortus* and, as such, the impacts of parasitism on weight gains may be expected to differ from simulations where only *T. circumcincta* infections were modelled. In the study performed by Gaba et al. (2010) only 12 lambs were included in each group, thus despite the apparent agreement of their results with our simulation, their study size may be too small to make an objective comparison. Currently, there are insufficient field studies investigating TST

regimes and differing determinant criteria to make an adequate comparison to the predictions reported here, however our model predictions may provide a starting point from which expectations may be developed.

The long-term impact of drenching frequency and TST regimes on the frequency of the R allele reported here are very similar to those reported in previous simulation studies (Barnes *et al.*, 1995; Gaba *et al.*, 2010; Leathwick, 2012). However, the long-term impacts of drenching frequency and TST regimes on sheep performance have not previously been investigated. Whilst no major differences were predicted in total weight gain benefits (summed across all animals and years) across the differing TST percentiles for the lifetime of the anthelmintic, reducing the proportion of the lamb population drenched increased the duration of anthelmintic efficacy and reduced the total number of drenches administered before resistance was reported. Whilst the latter result may appear counter-intuitive to some, it reflects a reduction in the number of unnecessary drenches, and hence less wasted money. Drenches which are effectively targeted towards lambs with a high parasitic burden may, as a consequence, be expected to have a greater impact on the emergence of anthelmintic resistance, creating a catch-22 situation. Lastly, reducing the frequency of anthelmintic treatment was predicted to lead to increased total gain in EBW and a prolonged duration of anthelmintic efficacy. Thus, in the long-term, reducing the drenching frequency increased total sheep productivity, whilst reducing the proportion of animals treated increased the duration of efficacy and reduced unnecessary drenches.

The description of anthelmintic resistance used in this model was such that resistance was under monogenic control (Elard and Humbert, 1999), conferred by a recessive allele (Silvestre and Cabaret, 2002), with all genotypes being assumed to be equally fit (Barrett *et al.*, 1998; Elard *et al.*, 1998). However, resistance to anthelmintics such as avermectins and levamisole appear to be multigenic (Gilleard, 2006), the mode of inheritance of anthelmintic resistance may differ between anthelmintic drugs, and the fitness of resistant and susceptible genotypes may also differ (Leignel and Cabaret, 2001). Alterations in any of these assumptions may be

expected to alter the rate at which anthelmintic resistance develops (Barnes *et al.*, 1995). In particular, modeling resistance as multigenic or including decreased fitness for genotypes with greater resistance will reduce the rate of emergence of anthelmintic resistance. Hence, the scenarios investigated here may represent greater risk situations than sometimes seen under field conditions.

In summary, this simulation study has provided insights into the short- and long-term impacts of TST regimes and drenching frequency on both sheep production and the emergence of anthelmintic resistance, providing valuable information previously absent from published literature. Specifically, FEC was identified as an appropriate trait for use as a determinant criterion in TST strategies; however, the optimal proportion of the lamb population to be treated is a trade-off between short- and long-term evolution risks for anthelmintic resistance. Paradoxically, the TST strategies most likely to give good short-term parasite control are those that are most efficient at exerting selective pressure on the parasite population.

Chapter Six

The use of estimated breeding values for host resistance, based on phenotypes or genetic markers, as determinant criteria for targeted selective treatment regimes

6.1 Introduction

The previous chapter compared the use of different phenotypic traits as determinant criteria for a targeted selective treatment (TST) regime. The phenotypic traits evaluated for their potential to identify individuals for treatment included parasitological traits such as faecal egg count (FEC) as an indicator of host resistance (Cringoli *et al.*, 2009; Gallidis *et al.*, 2009) and performance traits such as live weight (Leathwick *et al.*, 2006b) and weight gain (Waghorn *et al.*, 2008; Stafford *et al.*, 2009; Gaba *et al.*, 2010) as indicators of host resilience. Out of these phenotypic traits, using FEC as the determinant criterion was predicted to allow for the greatest reduction in the number of anthelmintic drenches administered whilst maintaining the highest level of flock performance in terms of weight gain. However, as a concentration measurement, FEC is affected by faecal output and thus food intake (Niezen *et al.*, 1998; Athanasiadou *et al.*, 2005), and is therefore prone to variation as a result of environmental factors and differences in the genetic growth attributes of the host population that may affect desired and actual food intake (Stear *et al.*, 1996). Further, FEC measurements are subject to sampling errors which add variation to the recorded values (Stear *et al.*, 2009) and may therefore further obscure the identification of susceptible animals. As such, a determinant criterion that reduces environmental variation and sampling errors from FEC measurements may further improve the identification of susceptible animals for a TST regime.

The ability to identify resistant and resilient animals has previously been an area of interest for use in selective breeding programs (Bisset and Morris, 1996; Stear *et al.*, 2001). Traditionally this has been achieved using different phenotypic traits (Hunt *et al.*, 2008; Kemper *et al.*, 2010), however it is possible to obtain an estimated breeding values (EBV) for individual animals based upon individual and relatives' faecal egg count data (Morris *et al.*, 1998; Bisset *et al.*, 2001; Woolaston and Windon, 2001). Using the EBV for FEC may provide a method of reducing the noise due to the factors described above and give a better indication of the underlying host resistance. Further, with DNA tests now available for supposed parasite resistance (http://www.pfizeranimalgenetics.co.uk/default.aspx) and promising advances in quantitative trait loci mapping and whole genome selection (Hunt *et al.*, 2008), in the near future it may be possible to use these techniques to identify animals for both selective breeding programs and TST regimes. The aim of this simulation study was to evaluate whether using an EBV based on FEC or genetic markers for host resistance as the determinant criteriaprovide an improvement in the identification of susceptible animals for a TST regime in comparison to using FEC as the determinant criterion.

6.2 Materials and Methods

The mathematical model previously detailed in **Chapter 5**, which describes the epidemiology *Teladorsagia circumcincta*, anthelmintic resistance genotypes within the nematode population, and the impact of host nutrition, genotype and gastrointestinal parasitism on a population of grazing lambs, was used to evaluate the potential of using EBVs based on FEC and genetic markers for host resistance as determinant criteria for TST regimes in comparison to FEC.

6.2.1 Simulation procedure and *in silico* experimental design

A population of 10,000 parasitologically naïve lambs, generated from mating 250 sires to 5000 dams according to a pre-determined mating structure, was simulated to

be grazing post weaning on a medium-quality pasture (crude protein = 140g/kg DM, metabolisable energy = 10MJ/kg DM (AFRC, 1993)), at a grazing density of 30 lambs/ha for a period of 4 months from weaning to 6 months of age. The initial larval contamination of pasture was set to 3,000 *T. circumcincta* larvae/kg DM (Coop *et al.*, 1982), and the frequency of the recessive allele that confers anthelmintic resistance (R) to the parasite was initially set to 0.01 (Barnes *et al.*, 1995).

Basing TST on lamb EBV for FEC poses a specific problem as these two approaches may be considered to be incompatible. This issue is best illustrated in the circumstance where only a proportion of animals are treated, in this instance anthelmintic intervention only impacts upon recorded FEC of treated animals yet affects the estimation of breeding values for the entire population. Therefore, we propose three solutions to this problem; Firstly, the EBV for FEC may be based on the parental average EBV for the entire growth period, where the parental EBVs for FEC were estimated in a system where all animals were treated the same. Thus, this approach assumes that the lamb population arose from a breeding program for which parental EBVs were available. Secondly, the EBV for FEC may be based on only the first FEC sampling occasion before animals are selectively treated. Finally, it may be assumed that we already know the lamb EBV for FEC, this approach may be considered to equate to perfect genomic selection.

The parental average EBV for FEC (pEBV) was calculated by simulating the population of lambs over a single grazing season in the absence of any anthelmintic treatment. The FEC of each lamb (including a sampling error with a variance of 0.2 (Bishop *et al.*, 1996; Stear *et al.*, 2009)) was recorded on days 30, 60, 90 and 120. The EBVs for FEC were then calculated for the sires, dams and lamb population for the log-transformed FEC (ln(FEC+1)) data using an animal model in ASReml (Gilmour *et al.*, 2009), setting day of sampling as a fixed effect. Individual lambs were then assigned an EBV for FEC by taking the average of the EBVs calculated for their parents (pEBV). This serves as an approximation of the likely accuracy of parental EBVs, where parents arise from breeding programs. The model was then rerun, as described below, using pEBV as the determinant criterion.

The EBV for FEC of each lamb based on the first sampling occasion (fEBV) was calculated from log-transformed FEC data (including sampling error) recorded prior to the first anthelmintic treatment using an animal model in ASReml (Gilmour *et al.*, 2009). Phenotypic FEC measurements were assumed to be taken 5 days prior to anthelmintic treatment to allow time for the samples to be processed and analysed.

The individual lambs true EBV for FEC (akin to perfect genomic selection) was calculated using the FEC (without sampling error) recorded on days 30, 60, 90 and 120, in the absence of anthelmintic treatment. The log-transformed FEC data was then used to calculate the true EBV for FEC (tEBV) for each lamb using an animal model in ASReml (Gilmour et al., 2009), setting the day of sampling as a fixed effect. The model was then rerun, as described below, using tEBV as the determinant criterion.

To investigate the potential of using these differing EBVs for FEC to identify susceptible animals for treatments, two further determinant criteria were included in this simulation study as a basis of comparison. These included the phenotypic FEC (pFEC) investigated in the previous chapter and a random selection process (RAN) achieved using a random number generator to identify the lamb IDs to be drenched.

These various determinant criteria were then evaluated for use in a TST regime. The population of lambs were simulated for a single grazing season with anthelmintic treatment administered only on day 30. This day and treatment frequency were previously identified as having the highest impact on weight gain in both the short (i.e. a single grazing season) and long-term (i.e. across grazing seasons) whilst presenting a time at when the parasite population *in refugia* is at its highest (**Chapter 5**). The proportion of the lamb population given anthelmintic treatment was investigated at each of the 10th percentiles (0%, 10%, 20%....90%, 100%) and was chosen by each of the determinant criteria investigated. Outputs from these simulations, such as empty body weight (EBW, kg) and FEC (eggs/g DM) were recorded for each lamb on each day of the simulated grazing season. Further, the frequency of the allele that confers anthelmintic resistance (R) was tracked on a

daily basis to give an indication of the impact of differing determinant criteria on the emergence of anthelmintic resistance. The optimal determinant criterion for TST may be considered as either the criterion which results in the highest level of flock performance (in terms of average weight gain) or the criterion which enables the treatment of the fewest animals.

6.3 Results

Figure 6.1 gives the final average EBW, the average FEC and the final frequency of R for the populations of lambs drenched at differing percentiles as determined by either pEBV, fEBV, tEBV, pFEC or RAN. Random selection (RAN) was predicted to result in a linear relationship between percentage of the lamb population drenched and final average EBW or average FEC, implying that no specific drenched percentile was predicted to be any more beneficial than any other.

Using the tEBV as the determinant criterion for TST was predicted to result in the best relationship between EBW (or FEC) and percentage drenched, allowing for the greatest reduction in the number of anthelmintics administered for any given achieved performance criterion or, alternatively, having the greatest impact on average FEC and maintaining the highest average EBW for any given number of anthelmintic drenches administered. For example, when 50% of the lamb population were drenched using the EBV for true FEC as the determinant criteria, an increase of 1kg in the average EBW and a decrease of 11% in the average FEC, were predicted in comparison to the random selection of animals (RAN). In comparison, using pEBV, fEBV or pFEC as the determinant criteria were predicted to increase the average EBW by 0.55, 0.46 and 0.67kg, respectively, and decrease the average FEC by 6.4%, 5.7% and 8.5%, respectively, when the same comparison is made to the randomly selected animals (RAN). Thus, in terms of EBW and FEC outputs, using pFEC as the determinant criterion was more effective than EBVs based on partial information. In particular, the FEC phenotype at the 1st sampling occasion was marginally more effective than the EBV based on the same information.
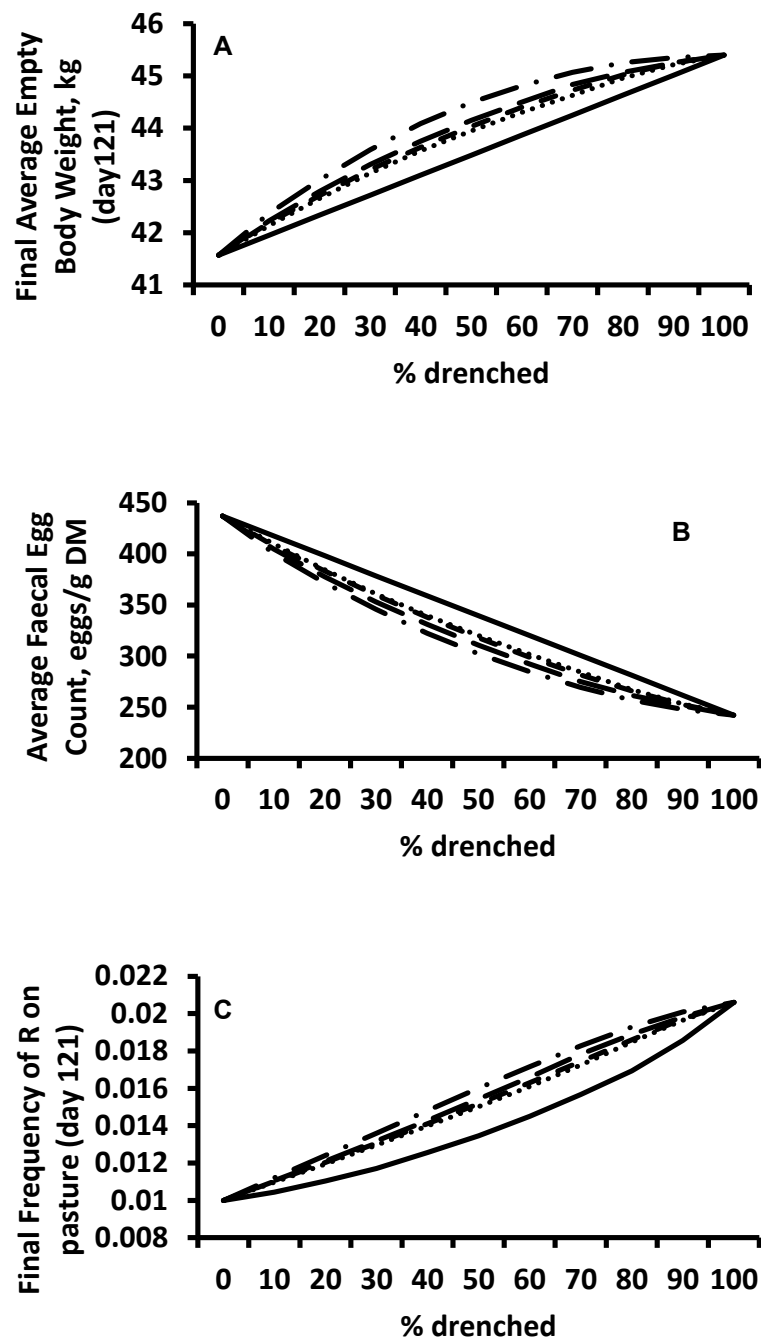
Figure 6.1. A) Final (day 121) average empty body weight (kg), B) Average (days 14 to 121) faecal egg count (eggs/g DM), C) Frequency of R on pasture; for 10,000 lambs grazing on a pasture initially contaminated with 3,000 *T. circumcincta* larvae/kg DM and drenched on day 30 by percentage of population either randomly (———) or according to tEBV (— · —), pEBV (– – – ·), fEBV (··········) or pFEC (— —). For explanation of determinant criteria see section 6.2.1.

The final frequency of R on pasture for each of the determinant criteria investigated increased when the proportion of the host population drenched was increased. Further, determinant criteria that had the greatest impact in reducing the average FEC also resulted in higher frequencies of R being predicted in comparison to random selection of lambs. Thus, determinant criteria that allowed for better identification of susceptible animals for anthelmintic treatment also produced a greater selection pressure for anthelmintic resistant nematode genotypes, and hence resulted in an increased rate of development of anthelmintic resistance.

6.4 Discussion

The EBV for individual animals based upon FEC data (Morris *et al.*, 1998; Bisset *et al.*, 2001; Woolaston and Windon, 2001), and genetic markers for host resistance to parasites (Kemper *et al.*, 2011) have been previously identified as potential methods for the identification of resistant animals for selective breeding programs. In a (future) situation where a farmer/breeder is using EBVs or genomic selection for a wide range of traits, then such information may also be used to identify susceptible animals for TST at no extra cost and thereby reduce the need to take FEC samples. Whilst it is not currently possible to assess the use of determinant criteria that may arise from these methods, using a mathematical modelling approach may provide an indication of the future potential of using EBVs based on genetic markers for host resistance to parasites in a TST regime, and establish the upper bounds for what may be useful in such a strategy.

Using tEBV (akin to perfect genomic selection) was predicted to be the best determinant criterion for use in a TST regime, of those that we considered. In principle, this may always have been expected to be the case as variation in FEC measurements caused by sampling errors are removed, allowing for more precise identification of susceptible animals. Thus, reducing the sampling error during FEC measurement was identified as a key factor in optimising determinant criteria for use in a TST regime.

Using genetic markers for host resistance as the determinant criteria for a TST regime, and thereby removing sampling error, requires a significant improvement in our knowledge of the genetic control of host resistance to parasites and a number of technological advances. For such a trait to be used, specific genetic markers need to be identified for host resistance to different nematode species and different host breeds (Miller *et al.*, 2006). Numerous loci have previously been identified to be involved in host resistance (Stear *et al.*, 1995; Dominik, 2005; Crawford *et al.*, 2006; Davies *et al.*, 2006; Beraldi *et al.*, 2007; Bishop and Morris, 2007; Keane *et al.*, 2007). However, the identification of these loci was based on microsatellite linkage, and thus may be considered to be population specific. Even with 50k SNP chips, the ability to predict the host resistance of an individual sheep is poor (Kemper *et al.*, 2011). High density SNP chips or genotyping by genome sequencing might provide the tools which may enable us to obtain a good predictive power, however, the financial cost of these techniques would need to be considerably reduced before they became affordable to sheep breeders. Further, even though the financial cost of this method may be expected to decrease over time as high throughput screening systems and diagnostic tools continue to be developed, the short-term marginal gains of TST are quite small and thus may never be expected to cover the cost of using these techniques to identify susceptible animals for treatment. However, if the identification of host resistance is incorporated into a wider genomic selection indices combining favourable alleles at many loci for many traits (Meuwissen *et al.*, 2001), then this method may be plausible for use in a TST regime.

Reducing the sampling error during the measurement of FEC may also be achieved through taking duplicate FEC samples for each lamb (Stear *et al.*, 1995). Whilst sampling, processing and analysing the FECs of a population of sheep may be labour intensive and financially costly, this method is considerably cheaper than genomic screening for host resistance. Further, all the technology required for this is already available and EBVs based on FEC data can easily and quickly be calculated. The requirement to sample FEC may be reduced if the lambs are the progeny of sheep with known EBV for parasite resistance. Whilst environmental differences

across differing years may cause genotype by environment interactions (McEwan *et al.*, 1997) and thus have a small impact upon the estimation of lamb EBVs based upon their parental average, generally the estimation of EBVs in this manner would be expected to be quite consistent. For the calculation of the pEBV made here, environmental conditions remained identical, and the pEBV was highly correlated with the fEBV (r = 0.9). Here, we explored the potential of using pEBV as the determinant criterion for a TST regime, and the beneficial impact on average EBW and average FEC was only slightly reduced when using this determinant criterion in comparison to using pFEC, and this slight reduction in average EBW predictions may be easily offset by the cost of FEC sampling.

Making a comparison of the predictions for fEBV and pFEC allows for a direct evaluation of the use of EBVs for TST regimes. For the predictions obtained from this simulation study, pFEC provided a better determinant criterion than fEBV in terms of the impacts of anthelmintic treatment on average EBW and FEC. Whilst phenotypic FEC measurements (including sampling error) may be affected by permanent environmental effects, they provide a description of the state of parasitism and egg contribution at a specific point. Whereas, EBVs are a weighted average of the individual and its family members therefore giving a better indication of the animal's genotype, however, this does not necessarily accurately indicate the phenotypic experience of the individual lamb at that particular time point (e.g. it may be dis-similar to family members due to a large permanent environmental effect). Further, pEBV was predicted to be a better determinant criterion than fEBV, although this criterion was still predicted to lead to reduced average EBW predictions in comparison to pFEC. However, it should be noted that the model used here does not contain all sources of environmental fluctuation and are thus likely to be over-optimistic. The improvement in using pEBV as the determinant criterion in comparison to using fEBV, provides an example of the impact of reducing the sampling error through the duplication of FEC measurements (i.e. calculations for EBV were based on four time points across the grazing season rather than a single

sampling occasion). By utilising information captured at older ages, it may also give a marginally better description of the lamb genotype later in the season than fEBV.

In summary, it was predicted that whilst EBVs for FEC provided a better indicator of the resistance genotype of individual lambs, phenotypic traits provided a better description of the current status of parasitism and thus were more appropriate as determinant criteria for TST regimes, ignoring cost implications. The key factor involved in the optimisation of determinant criteria was identified as being the reduction of sampling errors during the measurement FEC which may be easily reduced through the duplication of samples. Ultimately, the choice of determinant criterion may depend on who bears the cost of trait recording, i.e. whether it is the commercial farmer (as in pFEC and fEBV) or the pedigree breeder (as in pEBV). The tEBV approach would require costs to be offset against efforts to improve other traits.

Chapter Seven

General Discussion

7.1 Introduction

The control of gastrointestinal parasitism using chemotherapeutic strategies is under threat due to the emergence of anthelmintic resistance (Kaplan, 2004; Wolstenholme *et al.*, 2004; Jabbar *et al.*, 2006; Papadopoulos *et al.*, 2012) and thus threatens the sustainability of livestock systems (Waller, 2006b; Besier, 2007; Papadopoulos, 2008). Therefore, there is a desire to move towards production systems less reliant upon pharmaceutical intervention to maintain sustainable nematode control in the future. Numerous alternative control strategies have previously been proposed as a means of reducing reliance on anthelmintic drugs, including host nutrition (Coop and Kyriazakis, 1999), selective breeding for host resistance (Bishop *et al.*, 1996; Stear *et al.*, 2001), grazing management (Githigia *et al.*, 2001; Niven *et al.*, 2002) and refugia-based strategies such as targeted selective treatment (Kenyon *et al.*, 2009).

Experimental investigations exploring the impact of alternative control strategies can be costly, time consuming and technically difficult (Barnes *et al.*, 1995). However, using a mathematical modelling approach may provide a way to explore the potential of such strategies whilst considerably reducing the financial and diuternal costs associated with experimental investigation. Previously developed mathematical models have focussed on either the impact of parasitism on host performance (Vagenas *et al.*, 2007a) or the epidemiology of parasites (Barnes *et al.*, 1995; Leathwick *et al.*, 1995). These approaches have not been combined in order to assess the impact of control strategies on both host performance and the epidemiology of nematodes, and importantly the development of parasite resistance to drugs. Hence, the overall aim of this thesis was to evaluate and modify an existing mathematical model of nematode infections (Vagenas *et al.*, 2007a), and thus use a

mathematical modelling approach to investigate the relationship between host-parasite interactions and parasite control strategies. This approach was expected to provide a description of the potential impacts of different control strategies, give insights into some of the causes of variation currently reported within literature for experimental studies, and identify gaps in our knowledge which may aid in the implementation of alternative control strategies or provide further opportunities for parasite control that aim to reduce the requirement to use anthelmintic treatments and thus prolong their efficacy.

7.2 Evaluation and modification of the mathematical model

A previously developed mathematical model (Vagenas *et al.*, 2007a) that describes the impact of host nutrition, genotype and gastrointestinal parasitism in a growing lamb, provided an appropriate starting point for this study. This model was evaluated to determine its appropriateness for use in the exploration of host-parasite interactions and parasite control strategies. A number of issues were identified where parameterisation could be improved, including the functions that describe the acquisition of host immune response upon exposure to infective larvae, the allocation of constrained nutrient resource intake towards protein and lipid retention, and the parameterisation of host-parasite interactions including the relationship between food intake and nematodes. Thus, the existing model was extended to address these issues and input parameter values altered such that the simulated outputs matched those of Coop *et al.* (1982; 1985) for Scottish Blackface lambs given a trickle challenge of *Teladorsagia circumcincta*. The modified model was then validated by comparing simulated outputs against experimental studies including Greer *et al.* (2008) and Valderrábano *et al.* (2002). In this validation, whilst model predictions compared favourably to the reported experiments, the model consistently under-predicted the impacts of parasitism on growth rate and food intake.

In addition to updating the model and refining its parameterisation, specific focus was directed towards the differing functional and causal hypotheses proposed to explain the occurrence of parasite-induced anorexia. Thus, two differing mechanisms proposed to account for the reduction in the food intake of growing

animals during exposure to gastro-intestinal parasitism were evaluated (**Chapter 2**). These were either a reduction in the intrinsic growth rate of animals (Wellock *et al.*, 2003; Vagenas *et al.*, 2007a) or a direct reduction in the relative food intake of the parasitized animals (Sandberg *et al.*, 2006). A lack of comparable data for *T. circumcincta* infection in sheep under differing nutritional regimes, necessitated the use of experimental studies into other nematode infections, e.g. *Trichostrongylus colubriformis* infections, to draw conclusions about which mechanism provided the better description of parasite-induced anorexia. Whilst there are many differences between these two nematode species, for the purpose of comparison it was assumed that the mechanisms involved in parasite-induced anorexia were similar for the two species (Sandberg *et al.*, 2006). Experiments chosen for comparison to the simulated outputs of each mechanism included Greer *et al.* (2009), Kyriazakis *et al.* (1994), and Kyriazakis *et al.* (1996b). In these studies, increasing the protein content of food was reported to lead to reductions in observed anorexia and daily egg counts of parasitized lambs. In comparison, the mechanism involving a reduction in intrinsic growth rate predicted that increasing the protein content of food resulted in increased anorexia with no impact on daily egg count, whilst the mechanism involving a direct reduction in food intake predicted similar patterns to those reported for the experimental studies. Thus, the mechanism involving a direct reduction in the relative food intake of parasitized animals was concluded to provide a better description of parasite-induced anorexia, and may be considered to be consistent with the hypothesis that anorexia is a consequence of the activation of the immune response by anorexinogenic cytokines (Greer *et al.*, 2008; Kyriazakis, 2010).

Further to the evaluation and modification of the existing individual lamb model, numerous other improvements were made to the model to allow for the investigation of control strategies within commercial livestock systems. The individual lamb model had been extended to a population level by including heritable between-lamb variation in growth attributes, body composition, maintenance requirements and host-parasite interactions (Vagenas *et al.*, 2007c). The between-animal variance of the parameters associated with each of these functions were then parameterised so that output traits matched those reported by Bishop et al. (1996) and Bishop and Stear (1997). A simple description of the epidemiology of *T.*

circumcincta was included, with host ingestion of infective larvae linked to food intake to create a grazing scenario (**Chapter 3**). Further, to correct the under-estimation of the impacts of parasitism on growth rate and food intake, the maximum daily protein loss due to parasitism was re-parameterised to bring the predicted parasite-induced reductions in growth rate in line with the published values (Coop *et al.*, 1982). Finally, a description of anthelmintic resistance genotypes within the nematode population, and the differing phenotypic susceptibility of such genotypes to anthelmintic treatment, was added to the model to assess the impact of differing drenching regimes on lamb performance, parasitological traits and the development of anthemintic resistance (**Chapter 5**).

7.3 Nutritional control

Subclinical host infection with *T. circumcincta* reduces nutrient availability to the host through a combination of parasite-induced anorexia (Coop *et al.*, 1982; Greer *et al.*, 2008) and a loss of endogenous protein into the gastrointestinal tract (Yakoob *et al.*, 1983; Parkins and Holmes, 1989). The reduction in available nutrient resources impacts upon both the growth rate of the host (Coop *et al.*, 1982; 1985) and the ability of the host to mount an effective immune response. Thus, manipulation of host nutrition can increase the ability of the host to cope with the deleterious impacts of parasitism (resilience) (MacRae, 1993; Kyriazakis *et al.*, 1996b; Datta *et al.*, 1998; Knox and Steel, 1999) and improve the ability of the host to contain and overcome parasitism (resistance) by providing sufficient nutrients to allow for a maximal rate of acquisition of immunity (Coop and Kyriazakis, 1999; Greer *et al.*, 2009).

Most research concentrating on the effects of host nutrition on the resilience and resistance to gastrointestinal nematode infection has focussed primarily on protein supplementation (Houdijk *et al.*, 2005; Greer *et al.*, 2009). This is a logical approach due to the loss of endogenous protein as a consequence of parasitism and the proteinaceous nature of components of the immune system (Coop and Holmes, 1996). Indeed, numerous studies have shown that protein supplementation decreases the pathophysiological consequences of infection (Bown *et al.*, 1991; Van Houtert and Sykes, 1996; Fox, 1997) and enhances the expression of immunity to

gastrointestinal parasitism (van Houtert *et al.*, 1995; Coop *et al.*, 1995; Van Houtert and Sykes, 1996; Coop and Holmes, 1996; Datta *et al.*, 1998; Greer *et al.*, 2009). However, significantly less research has been carried out for other components involved in the impact of host nutrition upon the resilience and resistance of livestock to parasitism. Various minerals and trace elements have previously been shown to influence gastrointestinal nematode populations in preliminary studies (Coop and Kyriazakis, 2001); including phosphorus (Coop and Field, 1983), molybdenum (Suttle *et al.*, 1992), copper (Bang *et al.*, 1990), selenium (McDonald *et al.*, 1989) and zinc (Bundy and Golden, 1987; Chandra and Sarchielli, 1993). Whilst further research is still required to improve our understanding of the role of specific nutrients in both the immune response and host resilience, such studies highlight the complexity of the interaction between host nutrition and gastrointestinal parasitism.

Thus, whilst the model developed by Vagenas *et al.* (2007a) aimed to describe the interaction between host nutrition, genotype and gastrointestinal parasitism in growing lambs, focussing solely on the protein and energy content of food may be considered a naive approach. This is particularly evident for the functions describing the impact of parasitism where only a loss of protein is accounted for, and the development of immunity which is driven solely by its protein requirements. However, despite this shortcoming, the simulated outputs for the systematic investigation into the impact of protein and energy content of food used to evaluate potential mechanisms to describe parasite-induced anorexia (**Chapter 2**) were in agreement with reported experimental studies investigating the impact of protein content (Greer *et al.*, 2009; Kyriazakis *et al.*, 1994; Kyriazakis *et al.*, 1996a) and both protein and energy content together (Bown *et al.*, 1991; Coop *et al.*, 1995). Thus, in the absence of sufficient data arising from experimental studies investigating the role of specific nutrients, it may be considered that the main factors (protein and energy) involved in the relationship between nutrition and host resilience and resistance to gastrointestinal parasitism are adequately represented within the model.

In both the simulation studies reported in **Chapter 2** and the experimental studies cited above, increasing protein content of food was shown to result in greater

control of parasitism and reduced impacts on host performance. Hence, protein supplementation may provide an alternative control strategy capable of reducing the requirement for anthelmintic treatment. However, these studies were carried out for trickle challenged animals and thus further research may be required to investigate the impact of protein supplementation in grazing animals. Further, whilst it may be easy to manipulate the nutrition of penned animals, supplying additional protein may be considerably more difficult in grazing sheep and needs to be considered when assessing its potential implementation. This may be achieved by giving grazing animals access to a feed of high protein content, thus parasitized sheep may then choose to select a diet of higher nutrient content which allows them to improve their resilience (Kyriazakis *et al.*, 1994).

7.4 Selective breeding for host resistance

Breeding for host resistance to nematodes provides an alternative control strategy which may reduce the need for anthelmintic treatment by improving the host control of parasitism within a sheep population (Kemper *et al.*, 2010). Commercial sheep breeding programmes require genetic parameter estimates for host resistance and performance traits, including the heritability of individual traits and the genetic correlation between traits, in order to design appropriate breeding goals. Whilst reported heritabilities for faecal egg count (FEC) and body weight (BW) are relatively consistent (Safari and Fogarty, 2003; Safari *et al.*, 2005; Bishop and Morris, 2007), estimates of genetic correlations between these traits are variable, ranging from -0.8 (Bishop *et al.*, 1996) to +0.4 (McEwan *et al.*, 1992; 1995). Variation observed in such correlations may be due to interactions between host resistance genotype, the intensity of infection and anthelmintic drenching practices (Coop and Kyriazakis, 1999). Robust estimates for genetic correlations are required before incorporating them into multi-trait breeding objectives and thus such effects must be accounted for in selection strategies.

The intensity of infection has previously been implicated in the variations reported for the genetic correlation between FEC and BW (Bishop and Stear, 1999). In both these studies, the genetic correlation between FEC and productivity became

increasingly negative (i.e. favourable) as pasture contamination increased. Such results are consistent with the predictions reported in **Chapter 3**. Therefore it is also unsurprising that an absence of any relationship was predicted when anthelmintic treatments are administered, as the pathogenicity of parasite infection may be expected to reduce with reduced worm burdens. However, whilst the intensity of infection was identified as a potential cause of much of the variation in genetic parameter estimates, it does not fully account for the variability observed in published values. Further, no scenarios were predicted where the correlation was positive and unfavourable such as those reported by McEwan *et al.* (1992; 1995).

Extreme values reported within field studies may arise from small population sizes which are associated with large standard errors. Increasing the population size would therefore be expected to lead to smaller standard errors and hence more robust genetic estimates. Further, other factors may be involved in the reported variation of genetic parameter estimates such as sheep breed, nutritional status and nematode species (Bishop and Stear, 1999). Sheep breeds differ in their growth and resistance attributes (Friggens *et al.*, 1997; Bishop and Morris, 2007), and may therefore lead to variation in reported genetic parameter estimates. The model presented here also assumed that traits underlying resistance and growth were uncorrelated, however, altering this assumption can lead to more extreme relationships between FEC and sheep performance (Doeschl-Wilson *et al.*, 2008). The nutritional status of sheep may also affect this relationship as poor protein nutrition may suppress expression of the immune response to gastrointestinal parasitism (Coop and Holmes, 1996). Finally, there are many differences between nematode species, including the differing pathogenicity of parasite species and the induction of differing immune responses, which may be considered as a cause of variation in genetic parameter estimates (Bishop and Stear, 2003).

Whilst this study focussed on the Scottish Blackface as the most numerous sheep breed in the UK, and *T. circumcincta* as the most abundant nematode species in temperate climates (Stear *et al.*, 2007), it may be possible to further characterise the expected impact of both sheep breed and nematode species on genetic parameter estimates using this model in the future. Both sheep breed and nematode species may

be investigated by re-parameterising the host and parasite input trait values. Further, the mathematical model may also be further extended to enable the exploration of a more realistic scenario involving mixed species infections and thus further clarify potential sources of variation in genetic parameter estimates.

Variation in the genetic correlations between FEC and BW poses as a particular problem to animal breeders as breeding goals aimed at improving both sheep performance and resistance will have to be formulated specifically according to environment, disease status and the genotype of the animals to be improved. The predictions reported in **Chapter 3** for the impact of intensity of infection on genetic parameter estimates may provide sufficient information to aid the formulation of breeding goals. In general, the genetic correlations between FEC and productivity strengthen as pasture contamination increases, with the favourable (i.e. negative) genetic correlations predicted being beneficial to selective breeding for both performance and resistance. However, other factors may also require further investigation to provide a complete understanding of the variation arising in genetic parameter estimates. These include environmental variations, breed differences and a re-evaluation of the effect of variation in resource allocation. It may be advantageous to achieve this using a modelling approach as this method allows for the investigation of genetic correlations calculated under differing assumptions which would not be feasible under field conditions.

7.5 Grazing management

Additional benefits may be derived from breeding for host resistance via a reduction in FEC. Reduced FEC may be expected to lead to reduced larval contamination of pasture and consequently reduced host exposure to infective larvae. However, predicting the benefits of selection for host resistance in grazing ruminants can be difficult due to complex interactions between parasite epidemiology and host resistance to nematode infections (Bishop and Stear, 1997). The combination of selection for resistance and other control methods, such as grazing management, may provide further complementary benefits and lead to reduced anthelmintic use (Coop and Kyriazakis, 2001).

The impact of host resistance genotype on parasite epidemiology has previously been the focus of experimental studies where the difference between average FEC of resistant and susceptible lambs grazed separately were reported to be around 2 to 3-fold greater than when grazed together (Gruner *et al.*, 2002; Bisset *et al.*, 1997; Leathwick *et al.*, 1998). This is consistent with the predictions of the mathematical model reported in **Chapter 4** when resistant and susceptible lambs were grazed separately for at least two grazing seasons. Such differences in the impact of host resistance on parasite epidemiology may therefore be exploited using grazing management strategies to provide further control of gastrointestinal parasitism (Bishop and Stear, 1999).

Whilst the experiments cited above focussed solely on the impact of host resistance genotype on parasite epidemiology, the mathematical model included a description of the impact of parasitism on host performance and thus can provide further information on the potential to exploit differences in host resistance genotype to control gastrointestinal parasitism. In the simulation study, anthelmintic treatment was predicted to have little impact on BW predictions for resistant lambs, whilst causing improvements in BW gain for susceptible lambs. This suggests that the administration of anthelmintic treatments may be reduced by targeting only susceptible lambs without a reduction in the BW gain across a population. Further, susceptible lambs were also predicted to benefit from grazing with more resistant lambs which reduce the population's exposure to infective larvae. Thus, sheep breeds known to be susceptible to nematode infections may benefit from grazing the same pasture as breeds known to be more resistant. Finally, grazing strategies aimed at reducing host exposure to infective larvae using pasture relocation were predicted to have little impact on FEC and BW predictions. Such findings suggest that control strategies aimed at impacting the propagation of nematode infections are of much greater benefit than those that aim to control free-living stages. Although, it should be noted that the epidemiological model used in these simulation studies was simplistic and did not include the effects of temperature and humidity on the rate of development and mortality of free-living stages, and the migration of infective larvae onto herbage as described in the model of Leathwick *et al.* (1992). Inclusion of such factors in the model may alter the predicted potential of pasture relocation as a

control strategy particularly in the case where such a strategy is employed to take advantage of seasonal variation. However, whilst pasture relocation strategies may reduce the need for anthelmintic treatment by reducing host exposure to infective larvae, they may also be a high-risk practice for the selection for anthelmintic resistance (Barnes *et al.*, 1995; Waghorn *et al.*, 2009). Thus inclusion of such practices in nematode control strategies should only be undertaken with a full understanding of parasite epidemiology and the potential of such practices to select for anthelmintic resistance.

7.6 Targeted selective treatment

Maintaining a proportion of the nematode population *in refugia* (unexposed to anthelmintic) preserves susceptible parasite genotypes, thus slowing the development of anthelmintic resistance (van Wyk, 2001; Nielsen *et al.*, 2007; Soulsby, 2007; Torres-Acosta and Hoste, 2008). Targeted selective treatment (TST) reduces the number of anthelmintic treatments administered, and thus increases the nematode population *in refugia* (unexposed to anthelmintic), by selective treatment of only those animals that will most benefit from treatment whilst leaving the rest of the flock untreated (van Wyk *et al.*, 2006; Kenyon *et al.*, 2009). This approach requires determinant criteria for the identification of animals susceptible to parasitism, and consequently various phenotypic traits have been proposed for this purpose. These include parasitological traits such as FEC as an indicator of host resistance (Cringoli *et al.*, 2009; Gallidis *et al.*, 2009), and performance traits such as live weight (Leathwick *et al.*, 2006a; b) and weight gain (Waghorn *et al.*, 2008; Stafford *et al.*, 2009; Gaba *et al.*, 2010) as indicators of host resilience. In principle, three factors may be considered to influence the effectiveness of TST regimes in maintaining effective parasite control and slowing the emergence of anthelmintic resistance. These are the proportion of animals drenched at each treatment occasion, the number and timing of treatments (Jackson and Coop, 2000; Coles, 2005; van Wyk *et al.*, 2006), and the determinant criterion used to rank animals for treatment (Kenyon *et al.*, 2009).

Chapter 7 – General Discussion

Field studies investigating refugia-based strategies, including TST, have focussed on the short-term impacts upon flock performance and parasitic burdens, whilst simulation studies have previously been used to provide insights into the long-term relationship between such control strategies and the emergence of anthelmintic resistance (Barnes *et al.*, 1995; Leathwick *et al.*, 1995). However, comparison between epidemiological simulation studies and performance based field studies is difficult. Thus, the mathematical model was used to make a comparison of traits previously proposed for use as determinant criteria for TST regimes and investigate the short- and long-term impacts of TST regimes and drenching frequency on both sheep performance and the emergence of anthelmintic resistance (**Chapter 5**). TST is a relatively recent approach to the control of gastrointestinal parasitism and thus many experiments evaluating their use are still on-going (Kenyon and Jackson, 2012). Therefore, greater emphasis on the predictions of our *in silico* exploration into TST strategies is discussed here.

The best determinant criteria for a TST may be considered to be the trait that allows for the greatest reduction in the number of anthelmintics administered whilst maintaining the highest level of flock performance within a grazing season. Under this premise, using FEC as the determinant criterion was predicted to be the best trait for use in a TST regime out of those previously proposed. This finding was proposed to be due to various factors that may cause variation (noise) in the phenotypic measurements for each of the traits investigated as determinant criteria. Whilst FEC measurements are affected by variation arising from sampling errors (Bishop *et al.*, 1996; Stear *et al.*, 2009) and faecal output (Niezen *et al.*, 1998; Athanasiadou *et al.*, 2005) that may obscure the identification of susceptible lambs, performance traits such as BW and weight gain may be considered to be subject to many more sources of variation in growth attributes (Bishop *et al.*, 1996; Bishop and Stear, 1997) and environmental factors affecting the link between food intake and growth rate (Cammack *et al.*, 2005) that may obscure the identification of lambs with a low resilience to parasitism.

In the short-term (within a single grazing season) decreasing the proportion of the lamb population drenched was predicted to reduce the benefits of anthelmintic

treatment on average empty body weight (EBW), however this was also predicted to lead to a decreasing rate at which anthelmintic resistance develops. Such predictions reflect the trade-off between resistance management and the effective control of parasitism (Besier, 2012). This trade-off therefore identifies a conflict in the short- and long-term goals of TST strategies, and thus requires the evaluation of the long-term impact of TST regimes on both sheep performance and the emergence of anthelmintic resistance. Further, increasing the frequency of anthelmintic drenching was predicted to lead to an increased rate at which anthelmintic resistance develops, however, the beneficial impacts of each consecutive drench on sheep performance were predicted to decrease. This suggests that increasing the treatment frequency impacts upon the balance between these short and long-term goals, with suppressive drenching protocols impacting upon the emergence of anthelmintic resistance to a greater extent than maintaining effective parasite control.

In the long-term, across grazing seasons and for the effective life-time of an anthelmintic, no major differences were predicted in total weight gain benefits when treating differing proportions of the lamb population, however, the duration of anthelmintic efficacy was predicted to increase with reducing proportions of the lamb population drenched. This suggests that the benefits derived from using TST strategies may be observed as an extension of the duration of anthelmintic efficacy whilst having no impact upon sheep production. Further, it should be noted that whilst decreasing the proportion of the lamb population drenched extended the duration of anthelmintic efficacy, the number of anthelmintics administered before resistance was reported reduced due to increased selection pressure being exerted when effectively targeting lambs with a high parasitic burden. This may be a beneficial outcome for farmers as it may reflect a reduction in the number of unnecessary drenches, and hence less money wasted. Finally, increasing the frequency of treatment was predicted to decrease the net beneficial impacts upon EBW and the duration of anthelmintic efficacy. This may be considered to be a consequence of stronger selection for anthelmintic resistance than maintaining effective parasite control described for the short-term impacts described above. Thus, suppressive drenching protocols which may be used to maximise short-term

production in commercial farms should be avoided in favour of drenching only on occasions of absolute requirements for maintaining animal health and welfare.

Determinant criteria for a TST strategy can be optimised by reducing the sources of variation that may obscure the identification of susceptible lambs (as described above). Estimated breeding values (EBV) for individual animals based upon FEC data (Morris *et al.*, 1998; Bisset *et al.*, 2001; Woolaston and Windon, 2001), such as may be used in a selective breeding program, along with genetic markers for host resistance (Kemper *et al.*, 2011) may thereby provide a better means of identifying susceptible animals for treatment by reducing environmental variation and sampling errors. A study into the potential of using determinant criteria that may derive from such methods (**Chapter 6**) concluded that whilst EBVs provided a better indication of the animal's resistance genotype, phenotypic traits provided a better indication of the state of parasitism and thus the phenotypic experience of the lamb at a given time point. However, this study did identify that the key factor involved in the optimisation of determinant criteria was the reduction of sampling errors during the measurement of FEC. Such sampling errors may be easily reduced through taking duplicate samples for each lamb (Stear *et al.*, 1995) and thereby provide better identification of susceptible animals.

Finally, it should be noted that the description of anthelmintic resistance used in this model was simplistic, such that resistance was under monogenic control (Elard and Humbert, 1999), conferred by a recessive allele (Silvestre and Cabaret, 2002), with all genotypes being assumed to be equally fit (Barrett *et al.*, 1998; Elard *et al.*, 1998). However, this may only be the case for benzimidazole, as resistance to anthelmintics such as avermectins and levamisole appear to be multigenic (Gilleard, 2006). As such, alterations to the assumptions made here may be expected to alter the rate at which anthelmintic resistance develops (Barnes *et al.*, 1995). Further experimental studies may provide more details on the mechanisms involved in the development of anthelmintic resistance in the future, and modification of the current model may allow for the exploration of assumptions described above and provide further information on the expected impacts of such mechanisms on the emergence of anthelmintic resistance. Further, if a detailed understanding of the genetic

mechanisms underlying resistance to differing classes on anthelmintics is attained, then it may be possible to extend the duration of efficacy of anthelmintics by using them in combination with anthelmintic compounds from another class (Dobson *et al.*, 2011; Leathwick, 2012).

7.7 Integration of parasite control strategies

In the future, it is unlikely that nematode control in small ruminants will rely on one single approach. Whilst the simulation studies detailed here, along with the cited experimental studies, have primarily focussed on the ability of alternative control strategies to manage the effects of gastro-intestinal parasitism independently to each other, sustainable parasite control may be expected to be largely dependent on the ability to achieve an integrated parasite management strategy combining the various options available (Waller, 2006b). Currently there is a wealth of knowledge on the impact of alternative control strategies on host-parasite interactions, however, there are still gaps in our present knowledge on the full implications of each strategy. In this thesis we used a mathematical modelling approach to describe the potential impacts and implications of control strategies on host performance, parasite epidemiology and the emergence of anthelmintic resistance, which may be considered to clarify our understanding of such interactions. However, further points of interest were identified that still require exploration, whether through further simulation studies or experimental research. These include whether parasite-induced anorexia is present for foods of sufficiently low energy content as to impose the maximum gut capacity, the effects of environmental fluctuations and breed differences on genetic correlations between BW and FEC, and an experimental evaluation and comparison of the determinant criteria proposed for a TST strategy.

When assessing the merit of different strategies for nematode control, it is important that comparable information is obtained for each strategy. Whilst, the final benefit of such strategies may be observed in their ability to maintain sustainable levels of productivity in livestock systems, alternative control strategies are predominantly aimed at achieving effective parasite control and thus should be evaluated on their impact upon parasitological traits such as FEC. This parameter

may also provide an appropriate measure for comparison of the impact of differing control strategies as FEC determines the level of on-going host exposure to infection.

However, whilst exploration of the impact of these control strategies on FEC may provide information on the potential benefit of each strategy separately, there is little understanding of how these control strategies may interact. Initial experimental studies into the interaction of control strategies (Eady *et al.*, 2003) suggests that there are no major antagonisms and therefore differing control strategies may be used effectively together, each adding value in a sustainable nematode control program. Further research is still required to identify the interactions between control strategies, and to assess the benefits upon productivity, although the indication is that using integrated control strategies may markedly reduce the requirements for anthelmintic treatment (Barger, 1999).

The optimal combination of control strategies may be expected to be dependent on production objectives and factors governing the management of livestock systems. Production objectives may be affected by market needs which could increase intensive farming practices and thereby make strategies such as grazing management incompatible with requirements to maximise production systems. When livestock systems are put into a financial/economic context it becomes apparent that a cost-benefit analysis of each control strategy, as well as in combination, is also required. Whilst alternative control strategies are expected to reduce the requirement for pharmaceutical intervention and thus reduce the cost of parasite control, there are also financial costs involved in the alternative strategies proposed. These may include the cost of nutrient supplementation, and the labour intensive practice of monitoring parasitism within flocks using FEC measurements required for implementation of strategies such as TST. Thus, integrated control strategies would need to be tailored for different farming systems (Krecek and Waller, 2006) taking into account production objectives, available resources and potential constraints, as well as factors such as sheep breed and regional environmental differences that may impact upon the prevalence of differing nematode species, disease epidemiology and host-parasite interactions. Whilst this may appear to be a daunting task, integrated parasite control schemes that are

practically and financially feasible may provide a way of reducing the need for anthelmintic treatment thereby delaying the development of anthelmintic resistance and ensuring the long-term sustainability of livestock systems.

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