Towards the targeted control of gastrointestinal parasitism of grazing calves

by

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Abstract

Gastrointestinal parasitism is a ubiquitous challenge to grazing ruminants with negative impacts on animal production. In recent years control of gastrointestinal parasitism has come under threat due to the emergence of anthelmintic resistance. A dynamic, deterministic simulation model was developed to investigate the consequences of parasitism with *Ostertagia ostertagi* on first season grazing calves. Host-parasite interactions were considered to predict the level of parasitism and performance of an infected calf. Data from published literature were used to parameterise the model, and model sensitivity was tested for uncertain parameters by a Latin hypercube sensitivity design. The model was validated against published literature using graphical and statistical comparisons. Its predictions were quantitatively consistent with the parasitological outputs of published experiments in which calves were subjected to different infection levels.

Subsequently, the model was developed into a stochastic one by considering phenotypic variation amongst the calves and variation in parasite supra-population, i.e. parasite populations at all development stages across all hosts. Model behaviour was assessed against variation in parasite supra-population and stocking rate. The model showed the initial pasture infection level to have little impact on parasitological output traits, such as worm burdens and faecal egg counts, or overall performance of calves, whereas increasing stocking rate had a disproportionately large effect on both parasitological output and performance traits. Stochastic model predictions were validated against published data taken from experiments on common control strategies and showed a reasonable agreement with observations in most cases, reinforcing model accuracy.

Alternative control strategies that aim to slow anthelmintic resistance by maintaining *refugia* on pasture, i.e. ensuring a proportion of the parasite population remains unexposed to anthelmintics, were investigated by using the model. In the first instance, this included targeted selective treatments (TST), whereby only individuals that would benefit most from anthelmintic are treated, according to a phenotypic trait criterion. The simulation model compared: 1) the most appropriate phenotypic trait for treatment selection and 2) the method of selection animals for treatment (i.e. treating a fixed percentage of the population versus treating individuals who exceed a given threshold for treatment). Treatment success was assessed in terms of benefit per R (BPR), the ratio of average benefit in weight gain to change in frequency of resistance alleles R (relative to an untreated population). Overall the most beneficial treatment involved treating calves for which their average daily gain fell

below a threshold level. Subsequently, the effect of different initial pasture contamination levels and stocking rates on the most appropriate phenotypic trait and the most beneficial method of selection for treatment was tested. In general, a greater benefit to treatments was perceived with decreasing initial pasture contamination, with the exception of threshold treatments according to faecal egg counts. Stocking rate had a more variable effect, with the greatest benefit to treatment derived at conventional or high stocking rates, dependent on the determinant criterion and method of selection. It was observed that treating calves when their average daily gain fell below a threshold level was the most beneficial treatment strategy under all investigated scenarios. The work developed here can be used as the basis for the development of TST strategies that minimise the reductions in calf performance whilst simultaneously reducing the rate of development of anthelmintic resistance.

Declaration

I hereby declare this thesis has been composed by myself and has not been submitted as part of any previous application for a degree. All sources of information have been specifically acknowledged by means of referencing.

Zoe Berk

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Publications and Conference abstracts

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Berk, Z., Laurenson, Y.S.C.M., Forbes A. B. and Kyriazakis, I., 2016. A stochastic model to investigate the effects of management treatments on grazing calves exposed to *Ostertagia ostertagi*. *Parasitology*, 143, 1755-1772.

Berk, Z., Laurenson, Y.S.C.M., Forbes A. B. and Kyriazakis, I., 2016. Modelling the consequences of targeted selective treatment strategies on performance and emergence of anthelmintic resistance amongst grazing calves. *International Journal of Parasitology: Drugs and Drug Resistance*, 6, 258–271.

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Berk, Z., Bishop, S.C., Forbes, A.B., Kyriazakis, I., 2015. Evaluation of a model to investigate the host-parasite interactions between first season grazing calves and *O. ostertagi*. Presented at the BSAS annual conference 2015, Chester, UK.

Berk, Z., Bishop, S.C., Forbes, A.B., Kyriazakis, I., 2015. A simulation model to investigate host-parasite interactions between first season grazing calves and *O. ostertagi*. Presented at the 25th WAAVP Conference 2015, Liverpool, UK

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Berk, Z., Laurenson, Y.C.S.M., Forbes, A.B., Kyriazakis, I., 2016. A stochastic, mechanistic model to investigate the effects of management treatments on grazing calves exposed to *Ostertagia ostertagi.* Presented at the 67th annual meeting EAAP Conference 2016, Belfast, UK

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List of common abbreviations

Abbreviation	Definition
AHDB	Agricultural and horticultural development board
BCS	Body condition score
BPR	Benefit per R
CV	Coefficient of variation
COWS	Control of worms sustainably
CP	Crude protein
DM	Dry matter
dpi	Days post-infection
EBLEX	English beef and lamb executive
EBW	Empty bodyweight
IL_0	Initial pasture contamination
L_1	First stage larvae
L_2	Second stage larvae
L_3	Third stage larvae
L_4	Fourth stage larvae
L_5	Fifth stage larvae
LHS	Latin hypercube sensitivity design
ME	Metabolisable energy
ML	Macrocyclic lactones
MP	Metabolisable protein
PC	Pasture contamination
RMSE	Root mean square error
SD	Standard deviation
TST	Targeted selective treatment
TT	Targeted treatment
WB	Worm burden

Chapter 1: Introduction

1.1 Overview

Beef constitutes a major component of human diet, with the number of UK prime cattle slaughtered in 2014 totalling 1.96 million (AHDB, 2015) and demand set to grow (Alexandratos and Bruinsma, 2012). A popular rearing system in the UK is autumn-born suckler beef cattle, generally comprising of cross-bred dairy-beef calves slaughtered at 18-20 months (yard finishing) or 24 months (grass finishing) (Todd et al., 2011). Autumn-born calves are capable of utilising grass from turnout in early spring up to housing in the winter making this preferable to spring calving (Phillips, 2010). However, a ubiquitous threat to grazing livestock is gastrointestinal parasitism with damaging consequences for production, as well as cattle health and welfare, particularly in temperate regions. Generally calves in their first grazing season are most significantly affected, as they have no previous encounters with parasites, resulting in ill health, reduced performance and occasionally mortality in extreme clinical cases. Since the 1960's the number of clinical cases of gastrointestinal parasitism was dramatically reduced due to the development and use of anthelmintic drugs, meaning sub-clinical infections dominate (Sutherland and Scott, 2010), which manifest as reductions in weight gain, inevitably impacting on economic viability (Corwin, 1997). Although anthelmintics are currently successful at controlling the effects of parasitism, an increasing incidence of anthelmintic resistance puts the sustainability of this approach at risk (Rose et al., 2015a). With current control endangered, the likelihood of parasitism arising is expected to increase. For this reason alternative methods of control must be investigated in an attempt to slow the development of anthelmintic resistance, or provide a viable alternative to anthelmintic treatments. In order to justify and explore the use of such control strategies an in-depth knowledge of parasite pathology and epidemiology are essential. The aim of this thesis was to use a model to evaluate alternative control strategies to minimise the impacts of *O. ostertagi* (chapters 4 and 5); in order to do this it was necessary to construct a model that considers important factors, such as host immunity (chapter 2) and parasite epidemiology (chapter 3), to understand the underlying mechanisms of *O. ostertagi* infection.

1.2 Characteristics of *Ostertagia ostertagi*

Gastrointestinal parasitism is a major problem for meat production systems worldwide. Calves in temperate regions, such as the UK, are challenged by two main gastrointestinal parasites: *Ostertagia ostertagi* and *Cooperia oncophora* (Höglund, 2010; Rehbein et al., 2013). The majority of infections in the UK are concurrent, i.e. involve both parasite species, however *O. ostertagi* is the most pathogenic of the two making it a major concern for animal production, health and welfare (Coop et al., 1979; Tisdell et al., 1999). Although proper economic appraisals are challenging, it was estimated that losses associated with *O. ostertagi* are in the region of hundreds of millions of dollars in the US alone (Tisdell et al., 1999, Heizer et al., 2013).

1.2.1 *Free living stages of Ostertagia ostertagi*

O. ostertagi assumes a direct faeco-oral lifecycle in which larvae accumulate on pasture via parasite multiplication within the host calf (figure 1.1). At turnout in early spring an initial underlying level of overwintered larvae subsist on pasture from the previous year. These infective third stage (L_3) larvae are able to survive when exposed on pasture for up to 1 year (Rose, 1961). Pasture contamination (PC) is enhanced by auto-infection, whereby calves consume L_3 larvae and subsequently excrete eggs back to pasture. A proportion of the excreted eggs are expected to hatch and undergo a series of moults within the faecal pat from first stage (L_1) larvae through to the final stage infective L_3 larvae. This process is affected by climatic conditions, affecting the egg hatch rate and the subsequent development up to L_3 larvae in particular. Temperature was found to be the main driver of larval development; higher temperatures tended to result in a more rapid development rate of hatched eggs into L_3 larvae on pasture (Rose, 1961). However, larval mortality was also influenced, with higher temperatures correlating to increased larval mortality rates (Young and Anderson, 1981; Rose et al., 2015b). This occurs up to a point at which extreme temperatures cause the larvae to denature, although this is generally above the normal range of environmental temperature.

Once present and developed on faecal pats, infective L_3 larvae must then migrate onto grass swards to facilitate the intake of larvae by the calf. The crusting of dried faecal pats prevents larvae from escaping the pat to reach grass swards, hence for migration to occur sufficient moisture levels are required (Pandey, 1974). Infective L_3 larvae are the most resilient free-

living stage due to a protective outer sheath (Myers and Taylor, 1989) and migration can occur by a number of mechanisms: translocation through water droplets, splash dispersal through rain drops (Grønvold and Høgh-Schmidt, 1989) and even via transport hosts such as invertebrates (Grønvold, 1979; Holter, 1979) and cattle themselves, via encrusted faeces on limbs and hooves (Hertzberg et al., 1992). As a result, the distribution of larvae across pasture is aggregated, with large numbers of larvae found closer to facecal pats (Boag et al., 1989). As cattle graze on pasture they display faecal avoidance behaviours, hence the greater the distance larvae migrate from the from faecal pats the higher the chance of ingestion (Hutchings et al., 2001; Fox et al., 2013).

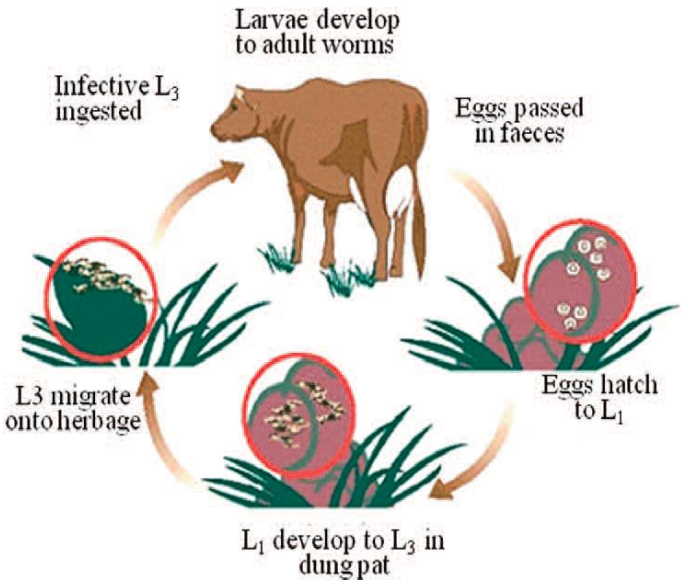

Figure 1.1: The faeco-oral lifecycle of Ostertagia ostertagi worms (source: EBLEX)

1.2.2 *Parasitic phase of Ostertagia ostertagi*

Once ingested, the L_3 larvae migrate to the abomasum where they burrow into the gastric glands and undergo further moulting. At approximately 4 days post ingestion a proportion of the L_3 larvae develop into fourth stage (L_4) larvae and subsequently into fifth stage (L_5) larvae approximately 8 days later (Rose, 1969). L_5 larvae then emerge from the gastric glands into the lumen; this is associated with damage to the parietal cells of the abomasum and in severe clinical cases can result in legions covering the abomasal walls (Murray et al., 1970). Once emerged, the larvae continue to develop into the sexually mature adult stage. The

mature worms then mate and the females produce eggs which are shed in the faeces. The average pre-patent period for the complete lifecycle is ~3 weeks (Anderson, 2000).

1.2.3 *Effects on the host*

Shortly after the emergence of adult worms from the gastric glands a number of biochemical and histopathological changes occur. These changes include hyperplasia and a loss of cellular differentiation, in particular parietal cells which are known to produce hydrochloric acid to maintain a low pH in the abomasum. As a consequence of reductions in parietal cell numbers, a rise in the abomasal pH occurs and can reach as high as pH 7, leading to a series of knock-on effects. Initially increased pH results in the failure to convert pepsinogen into pepsin, resulting in elevated plasma pepsinogen levels; this is further exacerbated by an associated increase in permeability to macromolecules of the abomasal wall (Myers and Taylor, 1989). Additionally, increased pH can cause a reduction in digestive ability, changes in gut bacteria flora or bacterial overgrowth along with hypergastrinemia, the increased secretion of gastrin, which stimulates the production of hydrochloric acid by parietal cells (Myers and Taylor, 1989; Fox et al., 2002).

Clinical disease manifests itself in one of several types: type 1, pre-type 2 and type 2 disease; classification is largely defined by the age of the calf. The most common is type 1 disease which typically affects young susceptible calves in their first grazing season (Myers and Taylor, 1989). Clinical signs include diarrhoea, oedema, anaemia, inappetence, reduced weight gain or weight loss and occasionally even death (Fox, 1993; Jennings et al., 1966). Sub-clinical infections can affect the host through reduced weight gain and competition for nutrient resources, meaning resources previously allocated to calf growth must be used for repairing damage and mounting an immune response (Coop and Kyriazakis, 1999).

There are no visible signs of pre-type 2 disease on the calf; it is characterised by large numbers of larvae becoming arrested in their development upon entering the gastric glands within the calf abomasum. This is triggered by harsh climatic conditions, i.e. cold winters in Northern climates, and larvae can remain arrested in hypobiosis for 4-7 months (Myers and Taylor, 1989). Once climatic conditions improve in the following spring arrested larvae resume development. If large numbers of inhibited larvae accumulate there is an outburst of maturing larvae leading to type 2 disease; typically this only occurs in older calves (yearlings

and above). The clinical and sub-clinical symptoms are the same as for type 1 disease (Myers and Taylor, 1989).

The majority of infections are sub-clinical hence the main problem associated with ostertagiasis is a reduction in bodyweight gain. Poor feed utilisation, changes in gut motility and absorption rates, and loss of blood protein are all proposed contributory factors (Fox, 1997). However it is estimated that reduced voluntary feed intake accounts for ~70% of the reduction in calf bodyweights (Fox et al., 1989b). Larger infections lead to a greater reduction in feed intake, with complete inappetence occurring in the most extreme cases of severely infected calves (Anderson et al., 1967). While this reduction in feed intake may seem paradoxical due to the increased demands on the host (nutritionally and metabolically), explanations for the reduction have been proposed. A simple interpretation would be that worm damage leads to inappetence by some chain of biochemical and physiological reactions. It was proposed that the production of gastrin and appetite related peptide hormones may play a part (Fox et al., 1989a; 2002). However, there is strong evidence that the development of immunity is an integral aspect of the development of parasite-induced anorexia (Parkins and Holmes, 1989; Kyriazakis, 2014). It is widely believed that certain cytokines produced during an immune response to parasitism can cause anorexia; these include interleukins IL6, IL18 and interferon INF-α, which are produced in response to *O. ostertagi* infection (Langhans, 2000; 2007). This is supported by experimental studies in which the calves considered to exhibit the strongest immune response to parasitism were observed to show the most prominent signs of anorexia in comparison to individuals with extremely high worm burdens (WBs), who did not display anorexia to the same extent (Herlich, 1980). Further experiments were conducted on the closely related parasite species *T. columbiformis* within its respective host, lambs. A proportion of lambs were immunosuppressed using corticosteroids and it was observed that these lambs, which also showed high WBs, showed no signs of reduced voluntary feed intake (Greer et al., 2005; 2008). It has also been observed that following anthelmintic treatment there is an immediate recovery from anorectic effect. Hence it can be speculated that anorexia is a consequence of the immune response and is not a side effect of damage to gastrointestinal tract or hypophosphatemia, which would take considerable more time to recover (Kyriazakis et al., 1996a; 1996b).

1.2.4 *Host Immune response*

The development of a host immune response to *O. ostertagi* is generally considered to be slow and incomplete, with cattle generally not considered functionally immune until they have completed at least two grazing seasons (Klesius, 1988; Claerebout and Vercruysse, 2000). The acquisition and expression of immunity is under genetic control and therefore large variations exist between individuals, with the majority of the parasitic burden located in a small proportion of the herd (Barger, 1987; Gasbarre et al., 1990). The emergence of adult worms from the gastric glands can be associated with major changes in immune-related gene expression, possibly as a result of the large increase in antigenic exposure (Mihi et al., 2014). Initially the immune response acts to reduce worm fecundity and stunt worm growth, which may be regulated by the local IgA response (Claerebout and Vercruysse, 2000). Although stunted worm growth contributes significantly to reduction in egg production, morphological changes such as the loss of the vulval flap is also a contributory factor (Michel, 1969a). Subsequently there is increased retardation and arrested development of worms, followed by the expulsion of adult worms and finally reduced establishment of new incoming larvae (Vercruysse and Claerebout, 1997). However, it is important to note that these responses are affected by a number of host and environmental influences, such as nutritional status, hormonal status, age and presence of infection (Claerebout and Vercruysse, 2000).

Different cell types are associated with recognition, processing and presentation of antigens of the gut; however these are not yet well understood. An increase in the production of inflammatory cytokines (IL6, IL17 and IL21) occurs alongside the upregulation of both Th1 and Th2 type cytokines (Mihi et al., 2014). Certain Th2 cytokines (IL4, IL5, IL10, IL13 and IL18) are known to promote protective immunity, whereas Th1 cytokines (INF-α, IL12) promote the survival of the parasite (Else and Finkelman, 1998). Th2 responses will elicit the production of eosinophils and antibodies (immunoglobulins IgA, IgE, IgM, IgG_1, Ig G_2), of which high levels of IgE trigger mucosal mast cells to create an environment inhospitable to the parasite (Claerebout and Vercruysse, 2000). However, there is also upregulation of cytokines associated with immunosuppression which acts to slow the development of a sufficient immune response (Mihi et al., 2014). Current studies into immune-mechanisms have focused on a relatively short time period and therefore further research is required to

identify the important protective immune mechanisms over several months (Mihi et al., 2014).

1.1.1 *Epidemiological Patterns*

Epidemiological patterns of PC are defined by interactions between seasonal factors, host immunity and farm management practices. In cool temperate climates, such as the UK, *O. ostertagi* is well adapted and L_3 larvae are able to survive for long periods of time under winter conditions allowing them to be ingested by cattle the following spring. PC increases as a result of auto-infection. The initial eggs deposited on pasture over the first few months tend to reach the infective stage at a similar time, due to the temperature dependent development from egg to L_3, causing a mid-summer rise in PC. This generally causes *O. ostertagi* to predominate in the latter parts of the grazing season (Nansen et al., 1988). PC subsequently decreases, as a host immune response develops and temperatures begin to fall, meaning eggs deposited late in September are rarely expected to reach the infective stage. These patterns are represented in figure 1.2. PC levels for calf herds experiencing sub-clinical infection levels rarely reach concentrations above 10,000 L_3/kg DM grass (Shaw et al., 1998a). Farm management practices, such as calving systems, can also have significant impacts; autumn-born calves have no prior exposure to parasites at turnout and are consuming large quantities of grass at this stage. Consequently large amounts of L_3 larvae are ingested, making these calves more prone to high-level infections than spring-born calves.

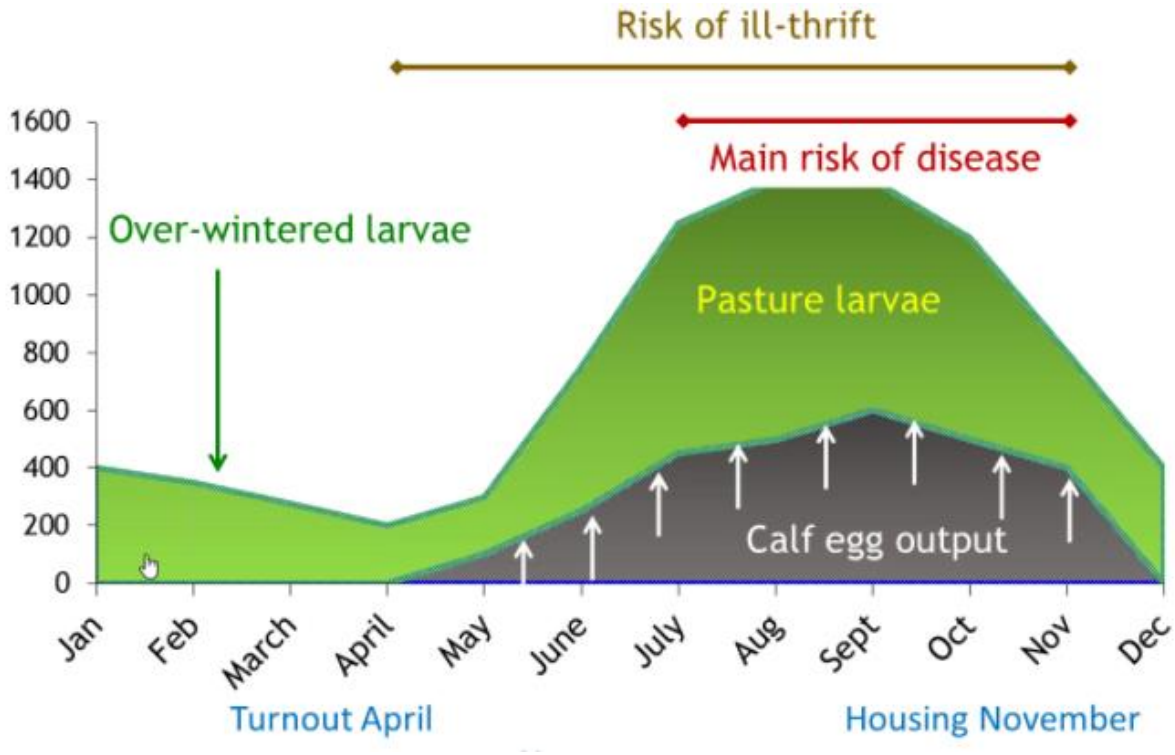

Figure 1.2: Epidemiological patterns of *Ostertagia osteragi* on pasture. Seasonal patterns of the total *O. osteragi* egg output to pasture by a herd of calves, and the resultant effects on contamination of infective L_3 larvae on pasture are shown. (Source: AHDB).

Epidemiological patterns may be affected by climate change in the future, resulting in a greater threat of infection and increased reliance on effective control measures (Verschave et al., 2016a). Two major climatic components likely to influence the course of infections, both directly and indirectly, are temperature and rainfall. Increasing global temperatures are expected to lead to a more rapid development of infective L_3 larvae, hence resulting in higher PC earlier in the season before calves have sufficient time to acquire any level of immunity (van Dijk et al., 2010). However, this may be counteracted in part by the higher larval mortality rates observed with increasing temperature. Patterns of rainfall must also be considered, such as future forecasts for periods of drought followed by heavy rainfall. Moisture and rainfall facilitate the migration of L_3 larvae to pasture meaning these patterns could lead to sudden increases in PC (van Dijk et al., 2010). Possible negative effects of global warming on the host must also be considered due to possible implications on animal production efficiency, animal health and welfare (e.g. due to heat stress).

1.3 Control of Infection and Associated Challenges

There are a number of control strategies currently implemented for prevention and reduction of gastrointestinal parasitism in cattle; some of the more common, and seemingly effective, strategies are discussed below.

1.3.1 *Anthelmintic treatments*

Due to a lack of pasture abundance and increasing stocking densities, there is a large dependence on strategic anthelmintic treatments since their introduction in the 1960s. The three broad anthelmintic classes introduced to treat *O. ostertagi* infection include the benzimidazoles, imidothiazoles (levamisole) and macrocyclic lactones (avermectins) (Waller, 2006). Macrocyclic lactones (MLs) are the main class of drugs used to treat *O. ostertagi* infections with ivermectin, doramectin and moxidectin making up 75% of calf treatments to first grazing season calves in the South-West of England (Barton et al., 2006). This is because they are effective against both mature worms and arrested larvae of *O. ostertagi,* and require the lowest dose drug concentration of 0.2mg/kg bodyweight (and 0.5mg/kg for eprinomectin) (Smith, 2014).

Anthelmintic treatment is usually administered in the first grazing season. Methods of application can be therapeutic, whereby calves are treated when signs of infection appear, or preventative whereby calves are treated prior to signs of infection. Strategic dosing is most common, with the key concept to administer drugs early in the grazing season hence preventing the development of large infections, usually through breaking the parasite epidemiology. Although there is no universal practice for dosing frequency, recommendations are for two to three doses administered early in the season (Shaw et al., 1998a). A classic example is dosing with ivermectin for which the frequency of dosing is dependent on the time of turnout. Late turnout generally relates to two doses of ivermectin (3, 8 weeks post-turnout) whereas early turnout generally relates to 3 doses (3, 8, 13 weeks post-turnout). The second dose is generally given in line with the expected mid-summer rise in PC. Although this strategy is relatively successful for treating calves during their first season grazing, host immunity in the second grazing season may be negatively impacted (Claerebout et al., 1999). Excessive use of anthelmintics will reduce the parasitic exposure of a calf and hence may result in a lack of acquired immunity for following grazing seasons (Ploeger et al., 1990). Current anthelmintic practices are fairly successful at preventing type

2 infections and therefore there is no well-structured treatment strategy in place for second grazing season infections. Generally calves experiencing type 2 infection are treated when signs of disease become evident, however this will only prevent clinical disease and not production losses (Myers and Taylor, 1989).

1.3.1.1 Challenges to Current Control

Although modern broad spectrum anthelmintics have provided a convenient and efficient method for controlling parasitic infections in grazing livestock, extensive use has led to the world-wide spread of anthelmintic resistance. Resistance can be defined as 'a greater frequency of individuals within a population able to tolerate doses of compound than in a normal population and is heritable' (Prichard et al., 1980). A parasite is defined as displaying resistance to anthelmintic when treatment reduces either parasite egg counts or adult WBs by less than 95% (Coles et al., 1992). Anthelmintic resistance develops when a parasite has one or more alleles conveying a survival advantage against treatment. Susceptible nematodes are killed by the anthelmintic, whereas nematodes with resistant genotypes survive and reproduce and pass on their alleles to future offspring. Over time a build-up of resistant alleles in the gene pool will lead to high frequency of anthelmintic resistance within the parasite population. Until recently there was little concern for this in cattle, as resistance had not developed as rapidly as in other ruminant parasites. However, anthelmintic resistance of both *Cooperia* and *O. ostertagi* is now documented for all three of the broad-spectrum anthelmintic classes (Rendell, 2010; Sutherland and Leathwick, 2011). *Cooperia* is considered to be the dose-limiting species for MLs, i.e. requires the highest drug concentrations to be effective (Sutherland and Leathwick, 2011). This is likely a result of lower concentrations of ivermectin observed in the intestinal mucosa, where *Cooperia* reside, comparative to the abomasal mucosa, where *O. ostertagi* reside (Lifschitz et al., 2000). Due to the popular use of MLs in the UK, anthelmintic resistance in *Cooperia* has been evident for a number of years (Coles et al., 1998; Stafford and Coles, 1999; Demeler et al., 2009). However, only in recent years have cases of anthelmintic resistance in *O. ostertagi* been documented; some examples of these documented cases are summarised in table 1.1. It is evident that resistance to MLs is becoming more frequent worldwide. Greater concern comes from the more rapid rate of development of resistance to multiple classes of

anthelmintic and for multiple species of parasites than seen for other host species (Sutherland and Leathwick, 2011).

Country	Levamisole	Benzimidazoles	Macrocyclic lactones	
Argentina	x	✓	✓	(Suarez and Cristel, 2007)
	✓	✓	✓	(Fiel, 2009)
Australia	✓	✓	-	(Anderson and Lord, 1979)
	✓	✓	✓	(Rendell, 2010)
	✓	✓	X	(Cotter et al., 2015)
Belgium	✓	-	-	(Geerts et al., 1987)
	-	-	✓	(Demeler et al., 2009)
France	-	-	✓	(Geurden et al., 2015)
Germany	-	-	✓	(Demeler et al., 2009)
	-	-	✓	(Geurden et al., 2015)
Italy	-	-	✓	(Geurden et al., 2015)
Mexico	-	-	✓	(Canul-Ku et al., 2012)
New Zealand	-	✓	-	(McKenna, 1991)
	x	✓	x	(Hosking et al., 1996)
	-	-	✓	(Leathwick and Miller, 2013)
South America	✓	✓	✓	(Ramos et al., 2016)
Spain	-	-	✓	(Martínez-Valladares et al., 2015)
Sweden	-	-	✓	(Demeler et al., 2009)
	-	-	✓	(Areskog et al., 2013a)
United Kingdom	-	-	✓	(Geurden et al., 2015)
United States	-	-	✓	(Gasbarre et al., 2009)
	✓	-	-	(Edmonds et al., 2010)

Table 1.1: A summary of confirmed cases of anthelmintic resistance in *Ostertagia ostertagi;* ✓ signifies confirmed cases of anthelmintic resistance, x signifies cases where no anthelmintic resistance was detected and – signifies cases where the anthelmintic was not tested.

The recent observed increase in *O. ostertagi* resistance to MLs can be attributed to a multitude of factors. Due to the prolonged period of persistence activity associated with MLs, application has been less frequent than other short acting drugs, decreasing the selection pressure for resistance alleles. However, due to recent increases in resistance rates observed for other short acting drug classes and MLs being highly effective at low doses there is an increasing reliance on the use of MLs. The frequent use of MLs in recent years exerted an increased selection pressure on gastrointestinal parasites. There was also a rise in testing for anthelmintic resistance, which may explain the increased awareness of the problem. Methods used to detect resistance have poor sensitivity in cattle, particularly due the low number of parasitic eggs produced by *O. ostertagi* worms. The low egg output combined with longevity of free-living stages on pasture may have helped to slow the anthelmintic resistance in *O. ostertagi*.

The problem of resistance may be exacerbated further by an increasing requirement for anthelmintic control; it is predicted that PC, which ultimately defines the levels of parasitic exposure experienced by calves, will be affected by climate change, as previously detailed, and farm management practices (Morgan et al., 2013). Changes in pasture management, in particular lengthened grazing season, may result in poorer grass quality and may mean higher requirements for anthelmintic treatments, and consequently higher selection for anthelmintic resistance (Skuce et al., 2013). With these challenges in mind, various control strategies have been proposed to maintain effective and sustainable control. Although mechanisms such as nutritional supplementation and biocontrol have been investigated, currently their application is limited and they do not provide a viable alternative (Mansour et al., 1992; Dimander et al., 2003). Other possible alternatives include grazing management, vaccination, selective breeding and targeted (selective) treatment with anthelmintic dosing.

1.3.2 ***Grazing Strategies***

High levels of parasitism can be controlled by implementing lower calf stocking rates. In general high stocking rates lead to greater infection levels from mid-July onwards. This is due to a combination of low grass availability and high egg excretion due to a larger number of hosts. Stocking rates impact on the effectiveness of other management strategies, for example moving calves to fresh pasture mid-season may have a more pronounced effect on the calves kept at a higher stocking density (Nansen et al., 1988).

Further grazing management practices can be implemented to help reduce parasitism; these can be categorised as diluting, preventative or evasive (Michel, 1985). Diluting strategies act to reduce the egg concentration within the total faecal output, hence reducing PC levels. Young calves are liable to reach high levels of infection when grazed together. By co-grazing young calves with a population of older cattle who are immunologically established or an alternative livestock species, in particular sheep, a diluting effect is exerted on total egg output. Grazing with alternate species is beneficial as most worms are host-specific, although co-grazing with sheep may prove dangerous in the presence of high levels of *Haemonchus* (Smith, 2014). Preventative strategies include those whereby cattle are grazed on clean, or safe, pasture; generally clean pasture has not been grazed by cattle for a few years whereas safe pasture has been grazed by an alternate species for a shorter period of time. This includes strategies such as rotational grazing, with the best rotation, although idealistic, described as a three year rotation of calves, sheep and crops (Smith, 2014). Finally, evasive strategies are those whereby no efforts are made to prevent parasitism in the early stages, but calves are moved to safe pasture once infection has developed. Although grazing strategies can prove effective at reducing parasitic burdens they are not intended as an isolated strategy, but in conjunction with other control measures (e.g. anthelmintics).

A classic example of combining anthelmintic treatment with grazing management is the 'dose and move' strategy. Mid-way through the season calves are administered a single dose of anthelmintic and moved immediately (same day) to a new, cleaner pasture. In the UK this is generally in mid-July to coincide with the anticipated peak in PC (Smith, 2014). Most overwinter larvae on the new pasture will have perished by the time of the move, hence preventing large intakes of larvae. However this method is now discouraged by the Control of Worms Sustainably (COWS) programme (Taylor et al., 2010) and care must be taken with subsequent grazing of these pastures, due to concerns over high frequencies of anthelmintic resistant alleles arising in overwintered larval populations. A high concentration of resistant eggs excreted by calves combined with a lack of diluting susceptible genotypes on clean pastures may result in a more rapid development of anthelmintic resistance than would be seen otherwise (Taylor et al., 2010).

1.3.3 *Vaccination*

At present no commercially available vaccinations to counter *O. ostertagi* infections exist, however the EU funded projects PARAVAC and the ongoing PARAGONE are working towards designing prototypes (Claerebout et al., 2005; De Maere et al., 2005; González-Hernández et al., 2016). Potential targets for vaccine development have been identified as the Activation-association Secreted Proteins (ASPs) released by the adult parasite (González-Hernández et al., 2016). Upon immunising cattle with these antigens an increase in effective immune response was observed, manifesting as reduced WBs and worm fecundity. Current research is focused on exploring the protective immune response that may be induced by the vaccine, simplifying antigen production and developing a prototype to take forward to the testing stage by 2020 (www.paragoneh2020.eu). However, this implies it may still be many years before a commercially available vaccine is produced.

1.3.4 *Selective Breeding*

Selective breeding works on the basis of identifying favourable phenotypic traits in individuals and incorporating these into a breeding programme to produce offspring with the advantageous phenotype. The success is dependent on the selected trait having a sufficiently large heritability. A commonly used phenotypic trait is faecal egg counts (FEC), for which heritability for resistance to gastrointestinal parasitism is around 20-30% (Leighton et al., 1989; Gasbarre et al., 1990). Although selection for lower *O. ostertagi* FEC was observed to reduce parasite fecundity, no impact has been observed on the number of worms in the host. This is likely due to the slow acquisition of immunity to *O. ostertagi* and density-dependence effects (Gasbarre et al., 1990).

A second promising trait target for selection is serum antibodies. It was shown that the sire can significantly affect the peak IgG_1 and IgG_2 levels of the calf (Gasbarre et al., 1990), the predominant immunoglobulin associated with the humoral immune response to *O. ostertagi*. Higher IgG titres correlate to fewer and shorter worms with reduced fecundity (Kloosterman et al., 1984). However, the heritability of this trait was only found to be 13% (±11%) (Hayhurst et al., 2010).

Without a complete understanding of the immune mechanisms it is difficult to select for resistance, and the practicalities of selective breeding must also be considered. Selection

may prove more difficult than for small ruminants due to longer generation times, a small number of progeny and the possibility that the parasite will co-evolve to counteract increased host resistance, most likely at a faster rate than selection may occur (Kloosterman et al., 1978). Genetic correlations with performance traits must also be considered: a negative correlation was observed between cow fertility and resistance to worms meaning the most fertile cows displayed large FECs (Mackinnon et al., 1990). Positive correlations were also observed between high IgG levels and reduced performance (Colditz, 2002). This is most likely a result of resource allocation, i.e. through preferential selection of individuals who are pre-dispositioned to allocate a greater proportion of nutrient resources to immune requirements, as oppose to reproduction or growth requirements (Coop and Kyriazakis, 2001). Although an attractive alternative, the application to *O. ostertagi* is complicated and requires further investigation and understanding of underlying immune mechanisms.

1.3.5 ***Targeted (Selective) Treatments***

We can conclude that at present there is no effective alternative to anthelmintic application; therefore methodologies to reduce the development of anthelmintic resistance must be developed. The aim of such strategies is to effectively counteract the effects of parasitism whilst maintaining high drug efficacy. Following the recent success in sheep, strategies such as targeted treatments (TT) and targeted selective treatments (TST) have been considered, whereby animals are treated on the basis of a trigger or a chosen phenotypic trait. TT involves whole-herd treatments at strategic time points based on a measurement of parasitism representative for the entire herd. TST involves the treatment of individuals, dependent on a given measure of parasitism, thus by exploiting individual variation in parasite burden and consequences. Theoretically, by treating only some individuals the others will continue to contribute susceptible parasites to pasture, hence maintaining *refugia*. For such strategies it is necessary to determine phenotypic traits which identify those individuals that would benefit most from treatment. Pathophysiological changes within the host can be indicated by diagnostic markers. For *O. ostertagi* infections such traits include FEC and pepsinogen as measures of parasitism (O'Shaughnessy et al., 2014a; 2015a; 2015b) and average daily gain (ADG) and body condition score (BCS) as measures of host performance and the ability to cope with parasitism (Greer et al., 2010; McAnulty et al., 2011; Höglund et al., 2013a). Discussed below are some of the targeted phenotypic traits that were considered for use in TT and TST strategies, along with the issues they raise.

1.3.5.1 Pasture contamination

Assessments for TT can be made by measuring PC levels as an estimation of the exposure risk experienced by the herd. Theoretically, treatments are administered to the entire population when PC crosses a threshold level. However sampling is laborious and the distribution of larvae on pasture is uneven, both vertically and horizontally (Gruner and Sauve, 1982).

1.3.5.2 Faecal egg counts

FECs have remained popular as they are considered to be a non-invasive measure of parasitism making this an attractive sampling method. FECs are generally considered impractical and are accompanied by large sampling errors, however with the recent development of FECPAK^{G2} (www.fecpak.com) and FLOTAC (Cringoli et al., 2010), remote-location parasite assessment tools, measurements can be obtained with greater accuracy and speed due to on-site application (Bosco et al., 2014; Godber et al., 2015). Currently the COWS recommendation for best practice in anthelmintic usage is to use TT strategies according to the average FEC measured across the total herd. However, correlations between WBs and FECs are usually low, casting doubt on the use of this diagnostic measure as a representation of parasitic burden and the consequences this may have on the maintenance of *refugia* (Michel, 1968; Eysker and Ploeger, 2000). No studies have been conducted using solely FEC as an indicator for TST in cattle, only using combined assessments of FEC and plasma pepsinogen.

1.3.5.3 Plasma pepsinogen

Although more invasive than FEC, plasma pepsinogen levels are also widely accepted as a marker for parasitism. *O. ostertagi* WBs were shown to correlate to plasma pepsinogen levels which also provide a direct marker for abomasal damage (Berghen et al., 1993; Dorny et al., 1999). Plasma pepsinogen concentrations increase as the season progresses to reach a peak in late summer (Jackson, 2013). However, significant differences in plasma pepsinogen levels were observed between different herds of naturally infected individuals. Most of these differences were attributable to variation between calf groups as opposed to variations within calf groups implying that plasma pepsinogen may provide a good indicator for TT (Charlier et al., 2011).This hypothesis was tested using the average herd plasma

pepsinogen levels as a phenotypic trait for TT of calf populations and proved to be successful (Charlier et al., 2010, 2011). Although plasma pepsinogen measurements were found to be repeatable within a given laboratory, huge variations were observed between assessments made in different laboratories, possibly as a result of techniques used (Charlier et al., 2011). The use of plasma pepsinogen as a diagnostic marker is also considered to be poor in older cattle, as clinically healthy individuals are often observed to have elevated pepsinogen levels, likely as a result from a hypersensitivity reaction to previous parasitic exposure (Wiggin and Gibbs, 1989; Berghen et al., 1993; Eysker and Ploeger, 2000). Further to this gastrin was investigated as a diagnostic marker; however it has proved to be both more expensive and less sensitive to low infections than FEC or plasma pepsinogen levels and does not provide any additional information to plasma pepsinogen sampling (Eysker and Ploeger, 2000).

1.3.5.4 Average daily gain

Using ADG as a target for TST, is based on the premise that performance and degree of parasitism are intimately linked (Greer et al., 2010), and partly due to the relative ease of taking measurements. However the correlation between calf ADG and diagnostic measures of *O. ostertagi* parasitism is questionable (Jackson, 2013). To date TST strategies based on ADG were successfully implemented in lamb populations (Leathwick et al., 2006; Greer et al., 2009; Stafford et al., 2009; Gaba et al., 2010; Busin et al., 2013), however limited knowledge of how this may transfer to the cattle industry exists. A retrospective study by Höglund et al. (2009) suggested that ADG was the best trait for TST. Experimental studies have since gone on to support the use of this trait, when comparing the results to routine monthly treatment of the cattle herd (Greer et al., 2010; McAnulty et al., 2011; Höglund et al., 2013a).

1.3.5.5 Body Condition Score

Calves can be allocated a BCS on a scale of 1-5, 1 signifying very thin calves and 5 signifying overweight calves. Although this trait was evaluated for use as a target in TST it has questionable value. TST of lambs presenting a BCS of less than 2 proved to be successful, however it should be noted that in this case FEC was found to be more promising (Gallidis et al., 2009). The successful use of BCS was not found to be repeatable in other populations of growing lambs or calves which deemed BCS to be too insensitive to correctly identify animals

for treatment (Kenyon and Jackson, 2012; Höglund et al., 2013a). A recent experimental study looked at a combination of BCS and FEC to identify calves for treatment and was found to be successful with no impact on ADG in the TST group and a reduction in anthelmintic usage of 98%, comparative to routine monthly treatments (Fahrenkrog, 2013).

Although TT and TST approaches have been developed and are acknowledged to provide practical benefits in multiple species (Charlier et al., 2014), very few experimental studies exist on TST application in cattle. Currently there are no comparisons of the effects of using each of the described phenotypic traits for TST selection basis. Comparisons of such a manner are challenging, partly due to difficulties in evaluating the development of resistance and partly due to confounding variables. For example, although one strategy may seem preferable to another when looking at independent studies this may be heavily influenced by extreme variations in factors such as PC, climatic effects or management practices. Although considerably more support has been drawn for TST in sheep, these findings are not completely transferable due to differences in host genetics, parasite epidemiology and farm management. Mathematical modelling provides an attractive approach to overcome problems of comparing TST strategies in the absence of experimental confounding variables. Such models have been successfully developed to investigate the use of TST within sheep populations (Laurenson et al., 2013a; 2013b; 2016).

1.4 The development of gastrointestinal nematode transmission simulation models

Simulation models have been around for many years with the intention of understanding, analysing and forecasting disease (Taylor et al., 2003). Developing a simulation model provides a non-invasive way to investigate the expected outcomes of various scenarios that a host species can be subject to whilst simultaneously reducing time and resource demands. Models can be classified as empirical, whereby observational data is used to define relationships. However many aspects of the parasitic lifecycle are unclear and difficult to extrapolate to new conditions (Verschave et al., 2016a). Mechanistic models provide an attractive alternative; such models are based on knowledge and understanding of the system in question and can provide an in-depth understanding of underlying processes and hence are better suited to predicting responses to novel situations.

Deterministic models are those in which outcomes are pre-determined by defined relationships, and models with the same inputs will (generally) lead to the same output, i.e.

point estimate. They lack an element of random sampling which leads to the variable outcomes characteristic of stochastic models. Stochastic models may have randomly sampled variation at various levels, for example spatially, across time or between animals. Elements of stochasticity can be described as demographic, which relates to random differences between individuals, or environmental, which relates to effects due to environmental fluctuations (e.g. temperature). For any given input, outputs may vary between different simulations using the same input parameters and typically they result in a statistic distribution of outcomes. Demographic stochasticity within the host population, specifically immune responses and growth characteristics, is necessary to simulate TST treatment strategies based on treating individuals dependent on a given phenotype. Logically, if all calves are considered to show the characteristics of an 'average' calf there will be no variation between traits and therefore each individual will exhibit similar outputs meaning exploitation of various phenotypic traits for treatment cannot be investigated. At present no stochastic models exist to describe gastrointestinal parasitism in individuals for cattle, although such models have successfully been developed for sheep (Laurenson et al., 2012b).

Creating a complex model is constrained by the availability of data and thus model development and parameterisation is a continuous, cyclic process. In order to place confidence in a model it is important to validate it, often via comparisons to existing data. The possibility of validation is dependent on the purpose and type of the model and can of course prove difficult for models designed to extrapolate data. Models can be validated qualitatively, but also quantitatively by which statistical testing on the likeness between observed and predicted results can be conducted (Mayer and Butler, 1993; Rykiel, 1996). However model agreement does not always necessarily indicate model validity, there may be alternate hypothesise that also fit to reality (Taylor, 2003). Although model validation is important, a major drawback is in a consistent lack of real life data available for comparison (Taylor, 2003).

1.4.1 *Previous simulation approaches*

Previous attempts to model gastrointestinal parasitism of cattle have focused on *O. ostertagi* for reasons detailed above, i.e. pathogenicity and economic significance. Models have either simulated the entire lifecycle, or more recently focused on specific aspects such as the free-living or parasitic stages. A summary of the current simulation models used to investigate *O. ostertagi* infections are summarised in table 1.2. Most early models have attempted to look at simplified representations of the entire lifecycle, from eggs on pasture through to mature adult worms; this is advantageous in that they can be run on a continuous time frame without the requirement of inputs over time. The first model for gastrointestinal parasitism of calves largely focused on free-living stages with the aim of producing model predictions for PC and clinical disease alone (Gettinby et al., 1979; Gettinby and Paton, 1981). Within-host dynamics were kept very simple with WBs considered as a function of both larval intake and the WB on previous days. Fecundity was considered to be unaffected by immunity and consistent across time. The model also relies on empirical data in order to simulate the outputs.

Subsequently the PARABAN model was developed to explore and evaluate control strategies (Smith and Grenfell, 1985; Grenfell et al., 1987a; 1987b, Smith et al., 1987a; 1987b). The model was deterministic in that it assumed all calves to exhibit the same behaviours, thereby only considering the 'average' calf. The model was comprised of a series of differential equations representing changes in state of the nematode from eggs to free-living stages to mature adult. Upon considering the free-living stages, key environmental influencers, such as temperature and moisture, were incorporated into larval development and loss. It was then assumed that calves ingest a set number of larvae as defined by a transmission constant; this made no consideration for the interactions between infection levels, calf feed intake, and calf nutrition which will all affect grass intake and subsequently larval intake levels. Once L_3 larvae were ingested an immune response was assumed to occur, the effect of host immunity was considered for parasite establishment, mortality and fecundity. Immune exclusion (establishment rate) was assumed to be a sigmoidal function of time from turnout, with no consideration for host experience of the parasite which was shown to affect immune development. Worm mortality was assumed to be a linear function of the total number of parasitic larvae ingested. Worm fecundity was assumed to be density dependent and a function of the time from turnout and mature WB, again making no

consideration for host exposure to parasites. Additionally the overcrowding effect was calculated purely on the number of worms without taking into consideration the effects of immunity on worm length.

Model	Complete lifecycle?	Environmental stochasticity?	Demographic stochasticity?	Control strategies modelled?	Model validated?
Gettinby et al. (1979) Gettinby and Paton (1981)	✓	✓	x	x	✓
Smith and Grenfell (1985) Grenfell et al. (1987a,b) Smith et al. (1987a,b)	✓	✓	x	✓	X
Ward (2006a,b)	✓	✓	x	✓	✓
Chaparro and Canziani (2010) Chaparro et al. (2011)	Free-living	-	-	x	✓
Chaparro et al. (2013)	Parasitic	-	-	x	x
Rose et al. (2015b)	Free-living	✓	-	x	✓
Verschave et al. (2014a, 2015)	Parasitic	✓	x	x	✓

Table 1.2: A summary of mechanistic models for *O. ostertagi* infections in cattle. ✓ signifies the presence of a feature within the model and x signifies the absence of a feature within the model

Expansions of the PARABAN model enabled users to account for a number of control strategies involving anthelmintic treatments. Although multiple drug regimes were explored using the model, this fails to consider the interactions between the rate of immune acquisition and anthelmintic usage. The authors also stressed that although anthelmintics were based on those used in the field, simulations were based on hypothetical parameters to illustrate the model potential (Smith et al., 1987b). The model also fails to consider or predict animal performance, making it difficult to make any economic evaluations on the various control strategies tested (Smith and Galligan, 1988).

To address the issue of interactions between calf nutrition, larval intake and calf performance the model was further extended by Ward (2006a; 2006b). The aim was to model animal growth and interactions with the parasitic lifecycle in order to make economic evaluations. Bodyweight was considered as a descriptor of calf state. New components such as grass availability, metabolisable energy (ME) and feed intake were also incorporated into the model. The assumption was made that ME was the first limiting resource; metabolisable protein (MP) was not considered in the model. Interactions between MP and the effects of parasitism are important to consider as often this is the limiting factor, as was demonstrated experimentally whereby MP supplementation resulted in improved resistance to parasitism (Bown et al., 1991; Kahn, 2003). ME requirements were considered for maintenance, bodyweight growth and energy 'wasted' by parasites. In the case of insufficient ME no consideration was made for the impacts this may have on immunity. To account for parasite-induced anorexia the adult WB was taken as a measure of the reduction in feed intake. This impacted on the dry matter intake, and therefore intake of ME and larvae. As the mechanism of parasite-induced anorexia is not well understood and does not always correlate well to adult WBs (Herlich, 1980; Kyriazakis, 2014), further investigation of this trait in its relation to immunity may be of interest. Again, this model accounted for a population comprised of calves all of the same description representing 'average' calf characteristics and did not account for variations in growth rates of body composition.

More recently models based on *O. ostertagi* were focused on a specific phase of the lifecycle, as oppose to previous models where the entire lifecycle was modelled (Chaparro and Canziani, 2010; Chaparro et al., 2011, 2013; Verschave et al., 2014; Rose et al., 2015b). Although this may allow for a more detailed analysis of certain factors, it requires a constant input of data, implying that the model cannot be used without data or predictions for the

counterpart of the lifecycle (e.g. egg output or PC levels). The GloWORM project aimed to model the lifecycle of *O. ostertagi* and *Cooperia* and was split into two sections, GloWORM-FL (Rose et al., 2015b), focusing on the free-living stages, and GloWORM-PARA (Verschave, 2015), focusing on the parasitic stages. The aim was to append the two models, however by considering each stage separately important interactions in the acquisition of immunity and subsequent effects on PC may be overlooked, impacting on the overarching messages taken from the model. Additionally developing separate model components can be considered inflexible and can increase levels of uncertainty. Rose et al. (2015b) focused on environmental fluctuation and the effect of these on the free-living stages, producing an output for PC levels. GloWORM-PARA focused on the parasitic stages to produce outputs of FEC by attempting to classify acquired immunity. Akin to PARABAN the model does not account for demographic stochasticity within the herd or the effects of parasitism on the calf bodyweights. The model was targeted towards dairy cattle and therefore much of the validation of the model focused on older cattle, meaning the descriptive of younger calves which experience the greatest impacts of infection may not be modelled to the same degree of accuracy (Verschave, 2015).

Alternative methods for modelling include the fuzzy logic rule for which scale parameters are derived from fits of predicted outputs. This technique was used to describe free-living and parasitic phases of *O. ostertagi* (Chaparro and Canziani, 2010; Chaparro et al., 2011; 2013). However, in this approach many of the parameters are localised to the specific environment of the experimental data to which they were fitted making them vulnerable to changing conditions. Additionally, the model for the parasitic phase produced by Chaparro et al. (2013) does not account for interactions between changes in calf bodyweight and nutrition and parasitism with no predictions of calf performance.

Although these models provide a thorough, well-balanced representation of *O. ostertagi* infections there are still a number of issues to address. To summarise, the main focus of the early models was on free-living parasitism with only a very basic consideration made for the parasitic phase within the host (Gettinby et al., 1979; Gettinby and Paton, 1981). PARABAN (Smith and Grenfell, 1985; Grenfell et al., 1987a; 1987b, Smith et al., 1987a; 1987b) constituted a much more thorough description of the parasitic life stages and did so with relatively few parameters. However, the classification of acquired immunity may be considered over-simplistic. Although the model succeeded in considering a wide range of

current management and control practices, it was deterministic and therefore unable to consider strategies such as TST whereby calves must be modelled on an individual basis. The model made no consideration for calf performance and therefore Ward (2006b) attempted to extend the model to account for this. Although this was successful in making the first inference in modelling performance, the only descriptor of calf state was bodyweight and the only consideration for resource requirements was ME, ignoring MP requirements all together. Further models have focused on one aspect of the life-cycle (Chaparro and Canziani, 2010; Chaparro et al., 2011; 2013; Verschave et al., 2014; Rose et al., 2015b); however these models may fail to consider important interactions between different phases during the model parameterisation. At present no demographically stochastic models exist for gastrointestinal parasitism of calves.

1.5 Current gaps in our knowledge of *O.ostertagi* infection

From reviewing the literature it is clear that there are still a number of issues to address with regards to *O. ostertagi* infections of cattle. Host-parasite interactions are difficult to investigate experimentally due to the necessity for calf necropsies and associated issues with animal welfare and large financial costs incurred. Consequently a number of unanswered questions exist; in particular the acquisition of calf immunity is not well-understood and has not been characterised. A further understanding of how calf immunity interacts with nutrition could help in developing an understanding of the benefits and limitations of supplementary feeding. It has been observed that the relationship between infection and performance is complex, although there is suggestion that stimulation of immune activity can have a direct negative effect on performance intake (Parkins and Holmes, 1989; Kyriazakis, 2014), this has not been directly investigated in cattle. These interactions must be better understood to assess and implement effective treatment of parasitism by enabling an understanding of the distribution of parasitism and performance across individuals within a population.

With the increased incidence of anthelmintic resistance (Sutherland and Leathwick, 2011; Rose et al., 2015a) alternative control strategies are becoming of increasing importance, in particular TST. Currently there are limited studies on the application of TST strategies in cattle and no direct comparison of TST strategies in terms of method of selection or phenotypic target trait for selection. This is largely a result of experimental designs constrained by difficulties in quantifying resistance and confounding variables, e.g.

management practices. To exploit the phenotypic traits of a set of individuals it is important to know the range of individual characteristics and how these may vary within populations. Currently there are no such demographically stochastic models investigating the effects of gastrointestinal parasitism on cattle to enable the investigation of TST strategies. Various management and environmental factors can be expected to influence the development of resistance, however due to difficulties in quantifying resistance it is challenging to understand the extent. This may be expected to have subsequent implications for the most beneficial treatment strategy.

1.6 Thesis Aims

From reviewing the literature it is clear that *O.ostertagi* is a problem for cattle production in the UK and worldwide, exacerbated by increased occurrence of parasite resistance to anthelmintic drugs. Modelling approaches can help to provide an in-depth understanding of parasite infections dynamics by breaking down various aspects that would otherwise be impractical to explore experimentally, for example comparison of different phenotypic traits for TST strategies. By identifying important factors in the development of infection, models can help to make predictions. Various management scenarios can also be projected by simulations, as demonstrated by previous models of livestock gastrointestinal parasitism that corresponded well to experimental observations (Smith et al., 1987b; Laurenson et al., 2012b). Therefore the overall aims of the project were: 1) to construct a model that considers important factors, such as host-parasite interactions and parasite epidemiology, in order to understand the underlying mechanism of *O. ostertagi* infection, and 2) to use the model to evaluate alternative control strategies that could be used to minimise the impact of *O.ostertagi* in beef cattle systems, while preventing the build-up of resistance. In order to achieve this, the thesis took the following steps (specific objectives):

1. Developed a model to account for calf- *O. ostertagi* interactions, including the parameterisation of key features such as host acquired immunity, parasite-induced anorexia and calf body composition, and ultimately produce output predictions for important parasitological and performance characteristics; this initial model was deterministic. The sensitivity of key model outputs to important, but uncertain, parameters was ascertained to quantify the uncertainty associated with the model. To place any levels of confidence in the model relevant outputs were validated against published literature to ensure consistency with experimental trials and to

identify any potential causes of variation between the model predictions and observed patterns (**Chapter 2**).

2. The deterministic model was expanded to account for both demographic and environmental stochasticity. This would allow for underlying mechanisms to be investigated and a range of different individual responses to be predicted, enabling future scope for investigating individual-based treatments (e.g. TST). In order to place confidence in the model a validation was performed against published experimental trials involving common methods of parasite control. The developed model was then used to test the hypothesis that 1) initial pasture contamination (IL_0) affects the dynamics of infection experienced by a population of calves and 2) high stocking rates negatively impact on the level of infection experienced by a population of calves (**Chapter 3**).
3. Due to the aforementioned uncertainties with comparing the effectiveness of TST strategies, the model was developed to investigate different methods of selection for TST application and to make a comparison on the possible phenotypic trait criteria for treatment selection and investigate the effects of each on calf performance and the emergence of anthelmintic resistance. Ultimately, the aim was to conclude the best method and best phenotypic trait for selection for maintaining effective control against parasitism whilst protecting against anthelmintic resistance, under standard conditions of medium IL_0 and at conventional stocking rates (**Chapter 4**).
4. The rate at which anthelmintic resistance in parasites develops is believed to be strongly influenced by seasonal PC, patterns of which are affected by IL_0 levels and calf stocking rates. The model was used to test the hypothesis that 1) IL_0 has an effect on the outcomes of different TST strategies and 2) stocking rate has an effect on the outcomes of different TST strategies. Ultimately the aim of this chapter was to assess whether there is an overarching best practice for application of TST under a wide-range of scenarios (**Chapter 5**).

The general discussion (**Chapter 6**) combines findings of the simulations with published literature to assess our understanding of parasitic infections and relevant interactions, and thus identify further gaps in our knowledge. The application of alternative control strategies and combinations of control methods are discussed along with potential future model

insights and developments. Recommendations on the use of TST are provided, along with the practical issues associated with implementing such a strategy.

Chapter 2: A simulation model to investigate interactions between first season grazing calves and *Ostertagia ostertagi*

2.1 Abstract

A dynamic, deterministic model was developed to investigate the consequences of parasitism with *Ostertagia ostertagi*, the most prevalent and economically important gastrointestinal parasite of cattle in temperate regions. Interactions between host and parasite were considered to predict the level of parasitism and performance of an infected calf. Key model inputs included calf intrinsic growth rate, feed quality and mode and level of infection. The effects of these varied inputs were simulated on a daily basis for key parasitological (worm burden, total egg output and faecal egg count) and performance outputs (feed intake and bodyweight) over a 6 month grazing period. Data from published literature were used to parameterise the model and its sensitivity was tested for uncertain parameters by a Latin hypercube sensitivity design. For the latter each parameter tested was subject to a 20% coefficient of variation. The model parasitological outputs were most sensitive to the immune rate parameters that affected overall worm burdens. The model predicted the expected larger worm burdens along with disproportionately greater bodyweight reductions with increasing daily infection levels. The model was validated against published literature using graphical and statistical comparisons. Its predictions were quantitatively consistent with the parasitological outputs of published experiments in which calves were subjected to different infection levels. The consequences of model weaknesses are discussed and point towards model improvements. Future work should focus on developing a stochastic model to account for calf variation in performance and immune response; this will ultimately be used to test the effectiveness of different parasite control strategies in naturally infected calf populations.

2.2 Introduction

There are increased concerns about prospects for sustainable control of gastrointestinal parasites in grazing ruminants. These stem from a variety of risks, including the loss of infection resistance as hosts are selected for production intensity (Mackinnon et al., 1991), the effects of climate change on parasite dynamics (Skuce et al., 2013), and the increased incidence of parasite resistance to anthelmintics (Rose et al., 2015a). Although the latter has been more commonly identified for small ruminants, there is increasing evidence that it is also happening for cattle (Edmonds et al., 2010; O'Shaughnessy et al., 2014b). Amongst others, Sutherland and Leathwick (2011) have reported parasite resistance to the three broad-spectrum anthelmintic classes (benzimidazoles, levamisole and macrocyclic lactones (MLs)) used on cattle.

For this reason there is a need to develop strategies that would enable sustainable control of gastrointestinal parasites and maintain the effectiveness of chemoprophylaxis (Charlier et al., 2014). Several strategies that may achieve this have been proposed, including targeted selective treatment (TST), breeding cattle resistant to parasites and grazing management. Testing for the effectiveness and interactions of such strategies is very difficult both experimentally and in practice. This is due to cost and difficulties in making fair comparisons, in the absence of confounding variables; for example although traits have been independently evaluated for TST in cattle, a direct comparison with other applied control strategies has not yet been conducted (Höglund et al., 2009; 2013a).

Recently, simulation models have been used to make such direct comparisons for control strategies on parasitised sheep (Laurenson et al., 2012a; 2013a; 2013b). Investigating the consequences of such strategies in silico for cattle may be one cost effective and time efficient way of overcoming the above limitations. Currently there are only two simulation models which investigate host-parasite interactions for cattle (Smith, 1987b; Ward, 2006a). Both models have their limitations; for example, the former model cannot make predictions about the consequences of parasitism on performance, whereas the latter uses bodyweight as the only descriptor of the animal. The objective of this paper was to develop a novel simulation model to account for the interactions between *Ostertagia ostertagi*, the most prevalent parasite of cattle worldwide, particularly in temperate regions (Tisdell et al., 1999), and immunologically naïve calves, which are most at risk from parasitism. Emphasis in model development was given to accounting for within host parasite dynamics and their effects on

host performance. The model was developed with the view of introducing between-animal variation in later steps.

2.3 Materials and Methods

2.3.1 *Model development*

The model stems from the ideas presented by Laurenson et al. (2011) to simulate the effects of *Teladorsagia circumcincta* challenge on growing lambs; however, it was developed to account for the interactions between the host and parasite in question. The developed model is dynamic and deterministic, as it predicts the responses of a single calf to infection, but contains elements of stochasticity to account for some of the variation within parasite worm populations. As a first step the growth and performance of a healthy calf were simulated, taking into account the calf genotype and management conditions. Subsequently the effects of *O. ostertagi* infection on the calf in question were simulated. Previously published generic equations and relationships are provided in Appendix A, along with more detailed justifications.

2.3.1.1 Parasite-free animal

2.3.1.1.1 Basic intrinsic growth model

The calf considered was a weaned, castrated male (steer) Limousin X Holstein Friesian born in autumn; this common cross currently represents the majority of beef cattle in the UK (Todd et al., 2011). Autumn born calves are capable of utilising grass in spring and hence are turned out at approximately 6 months of age and left at pasture until late autumn (Phillips, 2010). The growing calf is described in terms of its empty body weight (EBW) (body weight minus gut-fill), as dictated by the expected protein and lipid body content (Emmans and Kyriazakis, 2001).

The EBW composition of a calf comprises of its components protein, lipid, ash, water and a negligible amount of carbohydrates; each of these have an expected growth rate (Appendix A) defined by animal genotype (Emmans and Kyriazakis, 2001). According to Wellock et al. (2004) intrinsic growth of mammals can be modelled using a sigmoidal growth function, where the calves grow at a rate relative to their current and mature mass. Thus in order to predict intrinsic, henceforth called 'desired', growth, only three parameters were required: the current body mass of the animal, its growth rate parameter B (day^{-1}) and its mature body

mass (Emmans and Kyriazakis, 1997). It was further assumed that the animal has an intrinsic body fatness, which was defined by the lipid to protein ratio at maturity (Emmans, 1997). The mature EBW (EBW_M) was estimated at 680 kg and the *B* rate parameter as 0.0071 day^{-1} for steers from the data of English Beef and Lamb Executive (EBLEX) Better Returns Programme (2005) (Appendix A). The total bodyweight (*BW*) of the calf at any given time point was the sum of the EBW and the gutfill (*GF*) of the calf. The gutfill largely depends on the quantity (and therefore quality) of the feed (grass).

2.3.1.1.2 Resource requirements and feed intake

As with previous models (Vagenas et al., 2007a; Laurenson et al., 2011) only protein and energy requirements were considered, as all other nutrient requirements were assumed to be fulfilled by the feed and were not limiting to the calf (Wellock et al., 2004). It is generally accepted that healthy ruminants allocate feed resources to three functions: maintenance, growth and reproduction (Coop and Kyriazakis, 1999). Because the model considers steers, the reproduction-associated requirements were ignored. Equations for the protein and energy requirements for the processes of maintenance and growth are given in Appendix A.

It was assumed that the calf attempts to eat to fulfil its requirements for the first limiting feed resource (Emmans and Kyriazakis, 2001). As feed quality declines, feed intake initially increases, to a maximum defined by gut capacity (Kyriazakis and Emmans, 1995). Hence feed bulk is the only constraint that may prevent a healthy calf from satisfying its requirement. Equations to describe the feed intake needed to fulfil protein and energy requirements are given in Appendix A. In order to reflect the day to day variation in calf feed intake, a random effect was assumed (Doeschl-Wilson et al., 2008). This was done by generating random numbers to create the day to day variation in feed intake of up to, proportionately, 0.01.

2.3.1.1.3 Allocation of constrained resources

There are numerous circumstances under which intake of resources may be insufficient to meet the needs of all primary functions (requirements). For the purposes of the model, it has been assumed that as feed quality declines feed intake would initially increase; this would be up to a maximum defined by gut capacity (Kyriazakis and Emmans, 1995). When this happens, the animal has the problem of how to allocate its limiting feed resources (Coop and Kyriazakis, 1999). Here, it was assumed that the requirements for maintenance were met first, and any excess was allocated to growth. The efficiency of protein deposition and lipid deposition were considered to be 0.50 and 0.59, respectively (AFRC, 1993). If there are

insufficient resources to fulfil maintenance requirements then the host will undergo catabolism of protein and lipid body reserves and ensure calf survival in the short-run. If either of these deficiencies is maintained over a significant time period the calf will continue to catabolise stores until death occurs.

2.3.1.2 Parasitised calf

The model describes the host-parasite interactions presented in figure 2.1. The process starts with the ingestion of larvae, a proportion of which will establish in the gastrointestinal tract and develop into adult worms resulting in a cost to the host in terms of protein loss (Fox, 1993). Of these adult worms a proportion will die on each given day and any surviving adult female will produce eggs. These three processes (establishment, mortality and fecundity) are affected by the host through its immune responses.

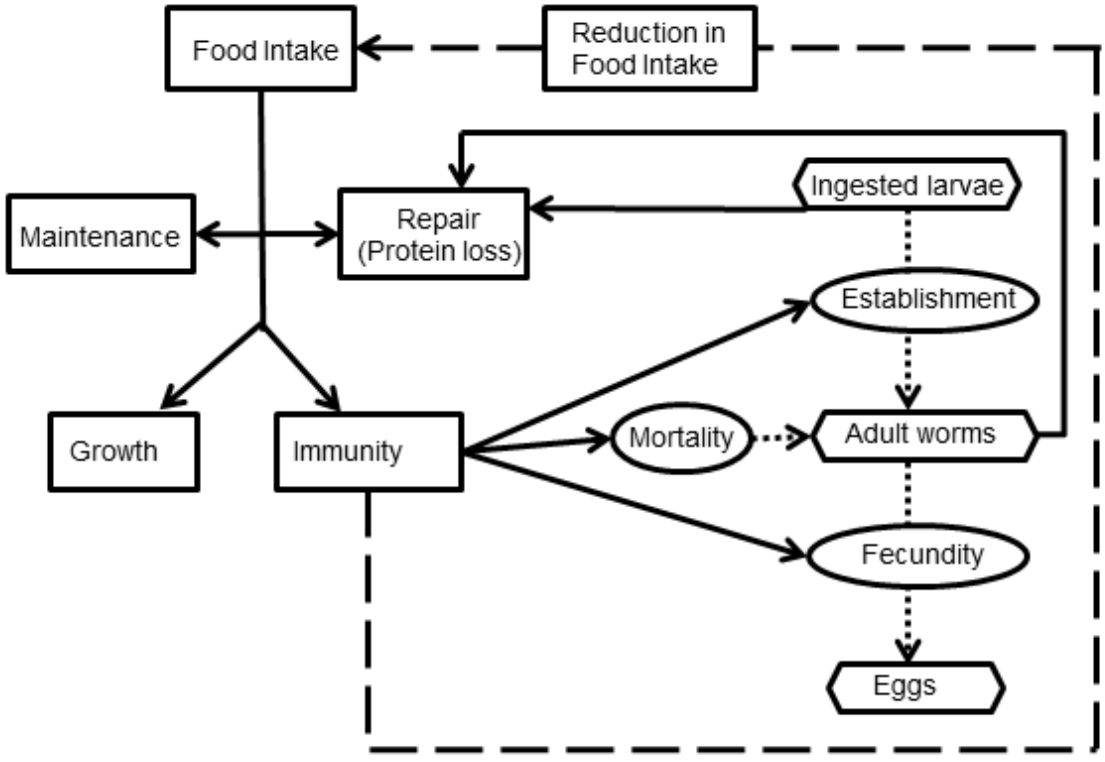

Figure 2.1: A schematic description of the parasite-host interactions. The rectangular boxes and solid lines indicate the flow of ingested feed resources; the oval boxes indicate the host-parasite interactions and the hexagonal boxes represent the the key measurabe stages of the parasite life-cycle. Host immune response is assumed to lead to parasite-induced anorexia (broken line)**.**

2.3.1.2.1 Immune response

Calves were assumed to have had no prior parasitic exposure at turnout to pasture. Although the immune response to *O. ostertagi* is currently not well understood (Li et al., 2010), worm burden (WB) has been found to show significant negative correlation to level of parasitic exposure over time (Vercruysse and Claerebout, 1997). Immune development following exposure was reflected in three parasite within-host relationships: establishment (ε), mortality (μ) and fecundity (F) (Bishop and Stear, 1997). To quantify the degree of parasite exposure, and hence the acquisition of an immune response, the measure of *larvaldays* was devised. *Larvaldays* is a measure of the cumulative exposure to parasites, a function of the larval dose administered and the length of time the host experiences each individual larva, and was chosen to represent immune development due to its ability to account for the larval intake of one day to have effects on exposure in subsequent days, in addition to further incoming larvae (equation 2.1). *Larvaldays* does not take into account larvae that have died or failed to establish, because the effect was found to be inconsequential, due to the relationship between *larvaldays* and the immune response (see below). All three affected responses (establishment, mortality and fecundity) were expressed as functions of *larvaldays*.

$$Larvaldays = Larvaldays_{t-1} + \sum LI \quad (2.1)$$

where $\sum LI$ is the cumulative larval intake and t is time in days

2.3.1.2.2 Defining and parameterising parasite burdens

In the absence of an immune response a maximum proportion of ingested larvae will establish; as the animal develops immunity, the proportion of the larvae that establish will decline until a plateau is reached (Smith and Grenfell, 1985). The immune response to *O. ostertagi* is incomplete; therefore the plateau occurs at a non-zero minimum establishment rate (Klesius, 1988). A proportion of the established adult worms will die on any given day: in the absence of immunity a minimum mortality rate applies and as immunity develops this increases towards a maximum (Kao et al., 2000). Available data that measures the WB of parasitised calves for given larval challenges reflects the combination of the above two processes. These data alone cannot be used to show the separate effects of establishment and mortality.

Initially, the combined effect of establishment and mortality was plotted against *larvaldays* from the experiment A of Michel (1969b), one of the very few experiments with such data. The data suggested an exponential relationship between *larvaldays* and the combined effect of establishment and mortality (*EM*), taking the form:

$$EM = (EM_{max} - EM_{min}) \cdot \exp\ (-k_{EM} \cdot larvaldays) + EM_{min}$$

(change in adult worm numbers/day) (2.2)

where EM_{max} is the maximum of combined establishment and mortality, EM_{min} is the minimum of combined establishment and mortality and k_{EM} is the constant relationship between *larvaldays* and the combined establishment and mortality level. The parameter values obtained from fitting the equation (2.2) to data were 0.82 (EM_{max}), 0.08 (EM_{min}) and 2.6E-08 (k_{EM}) (R=0.738, RMSE=0.119). However, it was necessary to separate the effects of establishment and mortality in order to capture WB dynamics. It was, therefore, assumed that worm mortality rate followed the same sigmoidal pattern as described by Louie et al. (2005):

$$\mu = \frac{(\mu_{max} - \mu_{min})\ \cdot\ (Larvaldays)^2}{{k_\mu}^2 +\ (Larvaldays)^2} + \mu_{min}$$

(proportion adult worms/day) (2.3)

where μ_{max} is the maximum mortality, μ_{min} is the minimum mortality and k_μ is a constant of the relationship between *larvaldays* and the mortality. The parameters were estimated using the values of Vagenas et al. (2007a) as a baseline, and adjusted to produce similar patterns of WB to those observed by Michel (1969b). Values were estimated at 0.12 (μ_{max}), 0.01 (μ_{min}) and 4E+06 (k_μ). The remaining effect on the adult worm numbers after accounting for mortality was assumed to be attributable to the establishment rate (ε):

$$\varepsilon = \frac{EM}{1 -\ \mu}$$

(Proportion larvae establishing/day) (2.4)

The modelled WBs were fitted to experimental data from experiment A of Michel (1969b) to estimate establishment and mortality rate parameters within a dynamic system.

The pre-patent period of *O. ostertagi* can vary from 17 to 25 days (Parkins and Holmes, 1989; Williams et al., 1974). To take into account the likely stochastic nature of the pre-patent period was assumed to be normally distributed across this time period (mean=21 days, SD= 1.64 days), and was estimated at whole day increments. This allowed for the gradual appearance of a WB rather than the otherwise sudden maturation of all larvae on a single day and can be represented as follows:

$$MatureL_x = Larvae_{t-16} \cdot \varepsilon_{t-16} \cdot P_x \quad (2.5)$$

where $MatureL_x$ is the number of larvae maturing on day x from a given larval cohort, $Larvae_{t-16}$ is the total number of larvae that will mature into adult worms from each larval cohort (administered 16 days previously) and P_x is the normal probability density function integrated over 1 day (and assumed to be negligible for t<17 and t>25).

The WB could then be defined at time *t* as a function of the previous day's WB and the newly matured adult worms (summed across all larval cohorts):

$$WB_t = (1-\mu) \cdot WB_{t-1} + \sum_t MatureL_x \quad (2.6)$$

where WB_t is the new worm burden, WB_{t-1} is the previous days worm burden, μ is the parasite mortality and $\sum MatureL_x$ is the sum of newly matured adult worms across all larval cohorts.

2.3.1.2.3 Defining and parameterising worm fecundity and worm mass

As with parasite establishment, the fecundity (eggs/female) was assumed to decline towards a plateau as immunity was acquired (Michel, 1969b). The immune response effect on fecundity was assumed to develop at a different rate to the establishment and mortality due to different underlying immune mechanisms (Stear et al., 1995; Prada Jiménez de Cisneros et al., 2014). As with *EM* the eggs per female was plotted against *larvaldays* from the experiment A of Michel et al. (1969b); the data suggest an exponential relationship between *larvaldays* and fecundity (*F*), taking the form:

$$F = (F_{max} - F_{min}) \cdot \exp(-k_F \cdot larvaldays\ ) + F_{min}$$

(Eggs/female/day) (2.7)

where F_{max} is the maximum number of eggs per female worm, $F_{min}$is the minimum number of eggs per female worm and k_F is the constant of the relationship between *larvaldays* and fecundity. After fitting the equation to the data of Michel (1969b) parameter values of 39 (F_{max}), 6 (F_{min}) and 2.9E-07 (k_F) were obtained (R=0.673, RMSE=4.781). Key assumptions made were that the proportion of female worms was 0.55 (Verschave et al., 2014) and eggs develop at the same rate, irrespective of the age and length of the worm.

The WB does not provide a full description of the parasitic infection, as both the total mass of the adult worms and the density dependence effects have not been considered thus far. Worm mass was calculated to provide a more complete measure of parasite infection (Michel et al., 1978; Bishop and Stear, 1997); this accounted for worm length as affected by the density dependence effect, whereby worm size (and fecundity) decrease with increasing worm numbers (Michel et al., 1978). Worm length has been found to display strong positive correlation to adult worm fecundity (Stear and Bishop, 1999). The density dependence effect on worm mass was described according to Vagenas et al. (2007a) (equations 2.8 and 2.9):

$$F_{Scaled} = F \cdot \left(\frac{WB}{WB_{Av}}\right)^{DD}$$

(Eggs/female/day) (2.8)

where WB_{Av} is the WB at which F_{Scaled} is equal to *F* and provides an estimate at which intraspecific competition between worms occurs for limited resources, this was taken to be 15,000 adult worms per calf (Michel, 1969b); and DD is a constant density dependence factor (-0.5).

Given the strong positive correlation between worm length and fecundity (Stear and Bishop, 1999), worm mass (*WM*) was calculated as:

$$WM = WB \cdot F_{Scaled} \quad (2.9)$$

FECs (eggs/g faeces) were calculated as the total daily egg output divided by the daily faecal output as estimated from the passage of undigested dry matter (DM). The random nature of sampling FEC was modelled as a Poisson distribution (Torgerson et al., 2012), after taking into account the limit of detection of the modified McMaster technique to measure 25 eggs/g of faeces (Borgsteede and Hendriks, 1979; Geldhof et al., 2002). Grazing beef calves

average a faecal DM content of 140-350 g DM/ kg faeces (Allen et al., 1970; Bellosa et al., 2011; Jalali et al., 2015; Young and Anderson, 1981), hence it was assumed that faecal DM comprised 0.25 of the faecal matter.

2.3.1.2.4 Parasite-induced anorexia

A reduction in voluntary feed intake accompanies parasitic infections (Kyriazakis et al. 1998: Kyriazakis 2014) and may be linked to cytokines associated with the development of the immune response (Greer et al., 2008; Herlich et al., 1980; Kyriazakis, 2011, 2014). In *O. ostertagi* infection anorexia does not appear on average before 21 days post-infection (Szyszka and Kyriazakis, 2013), which coincides with the first appearance of adult worms. Anorexia was modelled as a direct function of the rate of acquisition of immunity as per Laurenson (2011). The anorexia was then applied to actual feed intake, as described below, through a reduction parameter (RED). This was calculated as a direct function of the rates of firstly the combined effect of establishment and mortality and secondly of fecundity. Due to the differing physical units of the two immune measurements it was necessary to include a scaling factor; the rate of change in each response was scaled by the maximum possible change in the immune rate as follows:

$$RED = C_1\left(\frac{dEM/dt}{EM_{max} - EM_{min}} + \frac{dF/dt}{F_{max} - F_{min}}\right) \quad (2.10)$$

where C_1 is the scaling parameter, dEM/dt is the rate of change in combined establishment and mortality and dF/dt is the rate of change in fecundity.

A maximum RED for subclinical infections was considered (0.20 (Sandberg et al., 2006)). During the course of an infection RED will start at zero, rise to a maximum and then decline towards zero as immunity is acquired, however due to the slow development of immunity complete recovery may not occur over the time period considered. The reduction is considered a function of the desired feed intake to fulfil all requirements:

$$FI_{anorexic} = (1 - RED) \cdot FI_{desired} \quad \text{(kg/day)} \quad (2.11)$$

where $FI_{anorexic}$ is the feed intake of an anorexic calf and $FI_{desired}$ is the desired feed intake of the calf to fulfil all resource requirements.

2.3.1.2.5 Protein loss

One of the consequences of *O. ostertagi* infection is damage to the abomasal tissue of the host, resulting in protein loss (Fox, 1993; Holmes, 1993). Incoming larvae penetrate the gastric glands where they moult and turn into adult worms, which subsequently emerge; the damage to the gastric glands is proportional to the size of the parasite as it grows and is extended through metaplastic changes in the surrounding mucosal cells. The protein loss is a function of both larval burden and worm mass (Parkins and Holmes, 1989; Scott et al., 2011); the general trend observed for both is a sigmoidal increase up to an asymptote as the mass increases (Vagenas et al., 2007a). The simplest equation to describe this was proposed to be a logistic equation with the rate values that have been determined heuristically to fit bodyweight losses in literature (Szyszka and Kyriazakis, 2013). Equations for the potential protein losses were represented as:

$$PLM_{Pot} = \frac{Ploss_{max}.Ploss_{target} \cdot \exp(rLB \cdot LB)}{Ploss_{max} + Ploss_{target} \cdot (\exp(rLB \cdot LB) - 1)} \quad \text{(kg/day)} \tag{2.12}$$

$$PWM_{Pot} = \frac{Ploss_{max}.Ploss_{target} \cdot \exp(rWM \cdot WM)}{Ploss_{max} + Ploss_{target} \cdot (\exp(rWM \cdot WM) - 1)} \quad \text{(kg/day)} \tag{2.13}$$

where $Ploss_{target}$ is the target protein loss (0.0001 (Vagenas et al., 2007a; Laurenson et al., 2011)), $Ploss_{max}$ is the maximum protein loss (0.5kg/d, see equation 2.14), rLB (8.5E-5) and rWM (8.0E-6) are the rates of protein loss associated with larval burden (*LB*) and worm mass (*WM*) respectively.

The total protein loss is considered as the sum of the protein loss caused by both larval burden and by worm mass (see Appendix A, equations A.20 and A.21), up to a capped maximum protein loss. The maximum protein loss caused by parasitic burden is the maximum protein loss the host can withstand; if this is sustained across time calf mortality may eventually occur. As far as we are aware measurements of maximum protein loss for infected calves do not appear in the literature but have been reported for sheep, estimated as 0.01 kg/day (Laurenson et al., 2011). An allometric scaling parameter linking mature weight of sheep and cattle was used to scale the maximum protein loss for lambs to give a maximum value of 0.5 kg/d in calves.

$$Ploss_{Max(Steer)} = \left(\frac{BW_{M\ (Steer)}}{BW_{M\ (Sheep)}}\right)^{0.73} * Ploss_{Max(Sheep)}$$

(kg/day) (2.14)

where $BW_{M\ (Steer)}$ is the mature weight of a steer, BW_M is the mature body weight of a sheep, $Ploss_{Max(Steer)}$ is the maximum calf protein loss and $Ploss_{Max(Sheep)}$is the maximum protein loss in lambs.

2.3.1.2.6 Partitioning limited protein resources

Parasitised calves were assumed to have two additional functions to which they must allocate resources; damage repair and an immune response. As with healthy calves the maintenance requirements, along with damage repair were satisfied first (Coop and Kyriazakis, 1999). If these needs are not met then protein stores would be catabolised and eventually the calf would succumb to the consequences of the infection. Conversely, if nutrients remain after allocation to maintenance, they would be allocated between the two remaining functions of immunity and growth in proportion to their requirements (Coop and Kyriazakis, 1999). This allocation strategy is consistent with evidence of both reduced growth and immune development in nutritionally limited calves (Mansour et al., 1991; 1992). Proportional allocation may allow the host to tolerate a small number of parasites providing opportunity for parasite recognition to develop over time, and hence prevent a large infection arising (Viney et al., 2005). The resource requirements for maintenance and growth are given in section 8.1.3 of Appendix A, whereas the requirements for damage repair and the immune response were calculated as per Laurenson et al. (2011).

Due to protein allocation to the immune response there will be a reduction in protein loss caused by the parasites per se. The protein loss is then re-estimated following the reduction in worm mass and the spared protein added back to the available protein. The allocation to growth was estimated as:

$$PAC_{Growth} = P_{Avail} - (PAC_{Imm} + PLoss)$$

(kg/day) (2.15)

where PAC_{Growth} is the actual protein allocated to growth, PAC_{Imm} is the protein allocated to immunity, $PLoss$ is the protein loss after taking into account immunity and P_{Avail} is the protein available to allocate to these processes.

The remaining protein spared by the immune response was allocated to the growth function to prevent the model from enter a continuous loop whereby allocation to immunity will continually reduce protein loss and require re-allocation to growth and immunity.

2.3.1.3 Investigating model behaviour

The model was used to investigate predictions for a range of parasite infection intensities. The default values for the model were Limousin x Holstein-Friesian steers allowed ad-libitum access to high quality grass (AFRC,1993) for one grazing season (6-7 months from turnout). The default calf genotype was characterised according to EBLEX (2005) (Appendix A) with 106kg of protein at maturity (P_M), 207kg of lipid at maturity (L_M) and 0.0071 per day growth rate (B).

Model outputs were simulated for two challenge situations: the first tested the effect of different trickle doses of infective larvae administered daily. These were 3,500, 7,000 and 14,000 L_3/d representing a range of larval intakes that might lead to subclinical infections (Szyszka and Kyriazakis, 2013). The second investigated the effect of weekly as opposed to daily trickle infections, to match the common experimental protocol for parasite administration (Wiggin and Gibbs, 1989; Xiao and Gibbs, 1992; Szyszka and Kyriazakis, 2013). The number of infective larvae administered for this purpose was a total of 210,000 L_3 administered within a three week period. This was given either as a single dose, 3 doses of 70,000 L_3 per week or as 21 doses of 10,000 L_3/d. The daily outputs predicted by the model were WB, calf total egg output, FEC, feed intake and bodyweight.

2.3.2 *Model sensitivity*

In order to determine which parameters have the most significant effect on the model outputs a sensitivity analysis was conducted. An ANOVA was performed to determine the contribution of selected model parameters to variance of each output measure (Saltelli et al., 2010; Campolongo et al., 2011). The parameters selected were those for which the least confidence in actual values existed, but which appeared mechanistically important for model behaviour; this included 5 categories with a total of 12 parameters between them.

The following five categories were targeted for investigation:

1. Larval establishment and adult worm mortality as defined by 3 parameters: $\boldsymbol{EM_{max}}$ – maximum proportion of larvae establishing and surviving as adult worms; $\boldsymbol{EM_{min}}$ **–** minimum proportion of larvae establishing and surviving as adult worms; $\boldsymbol{k_{EM}}$ **–** the constant relationship between *larvaldays* and surviving adult worms as affected by establishment and mortality.
2. Adult worm mortality as defined by 3 parameters: $\boldsymbol{\mu_{max}}$ – maximum effect of mortality on adult worms; $\boldsymbol{\mu_{min}}$ **–** minimum effect of mortality on adult worms; $\boldsymbol{k_{\mu}}$ **–** the constant relationship between *larvaldays* and adult worm mortality.
3. The fecundity of female adult worms defined by 3 parameters: $\boldsymbol{F_{max}}$, – maximum number of eggs per female worm; $\boldsymbol{F_{min}}$ **–** minimum number of eggs per female worm; $\boldsymbol{k_F}$ **–** the constant relationship between *larvaldays* and number of female worms.
4. The rate of reduction in feed intake dependent on rate of immune acquisition: $\mathbf{C_1}$
5. The rate of protein loss, as defined by two rate parameters: $\boldsymbol{rWM}$ **–** the rate of protein loss associated with adult worm mass and $\boldsymbol{rLB}$ **–** the rate of protein loss associated with larval burden.

It was assumed that each parameter was normally distributed (Vagenas et al., 2007c), using the best-estimate value as the parameter mean and assuming a coefficient of variation of 20%. The possible values for the constant relationships with *larvaldays* levels (k) of establishment, mortality and fecundity were considered to follow a log-normal distribution in order to take into account the possible variation of a rate parameter over orders of magnitude. For the same reason, the likely rates of protein loss were also assumed to follow a log-normal distribution. The distributions of parameter values were divided into 5 sections,

each section assumed to be of equal probability, and the mid-point value selected. This allowed for a simpler and more consistent comparison in the analysis by selecting 5 possible values for each of the 12 parameters and then generating random combinations of these values. Using Latin hypercube sampling (LHS), parameters were sampled without replacement for each section to give 5 sets of parameter combinations. This was repeated 50 times to give a total of 250 parameter combinations; this was considered a sufficient number of combinations to allow a 12-way ANOVA due to the large number of parameters that may affect each output. Each of the 250 combinations was then modelled over a 200 day period for the three separate challenge levels of 3,500, 7,000 and 14,000 L_3/d and a record was taken of relevant outputs simulated. Each output set was then compared to the "best-estimate" output values (produced by the initial "best-estimate" parameters).

An ANOVA of constrained (Type III) sum of squares was conducted to analyse five defined outputs, viz. peak WB, time of peak WB, the peak total egg count, the peak reduction in feed intake and finally the final bodyweight. Significance was tested at the 99% level ($p<0.01$) in all cases. A multiple linear regression was then conducted to determine the percentage change in outputs with respect to changes in parameter values. All model simulations and statistical analyses (ANOVA) were programmed in Matlab (2012).

2.3.3 *Model validation*

The model was parameterised using data from experiment A of Michel (1969b) due to its utility. To validate the model, graphical comparisons and statistical analyses were made on independent data from sets of published experiments. Model performance was assessed in terms of goodness-of-fit of the observed against predicted values for three selected outputs on a daily basis: adult WBs, total egg counts and FECs (Symeou et al., 2014). The literature studies selected for evaluation were based on the following criteria: (1) Infections were only with *O. ostertagi* and no other species were involved; (2) calves were infected during the growth phase; (3) calves were allowed access to ad-libitum, high quality feed; (4) calves were parasite naïve, i.e. had no prior experience of parasites before the experiment; (5) larval doses were administered either weekly or more frequently.

Only eleven studies met the above criteria and were used to test for the effects of different trickle doses on (1) WBs (Michel and Sinclair, 1969; Michel, 1969b experiment B; Michel, 1970); (2) total egg counts (Michel and Sinclair, 1969; Michel, 1969b experiment B); (3) FECs (Wiggin and Gibbs, 1989; Claerebout et al., 1996; Mansour et al., 1992; Xiao and Gibbs, 1992; Hilderson et al., 1993; 1995; Satrija and Nansen, 1993; Forbes et al., 2009). The experimental larval challenges were used as inputs to the model. It was assumed that there has been little to no selection for resistance to *O. ostertagi* and hence the parasitological parameters that can be seen as host specific, have remained unchanged over the time period considered by all experimental studies (Prakash, 2009). In order to compare the model outputs to observed FECs the former must be considered as eggs per gram of wet faecal matter, however the DM content will vary dependent on the feed. For all studies where feed type was specified, calves were fed corn silage, hay or concentrates which lead to a higher faecal DM content than when fed on grass (Young and Anderson, 1981; Van Bruchem et al., 1991); in these case the faecal DM content was assumed to be 350g DM/kg.

The statistical analyses conducted to assess the goodness of fit for the purpose of model evaluation were as follows: (1) the correlation coefficients (R) were used to assess whether the simulated outputs followed the same pattern as observed values, with a value of unity signifying a perfect fit. (2) The coefficient of variation for the root mean square error (CV-RMSE) measured the closeness of observed and predicted values; a lower value signifies a closer match. (3) The relative error (E) determined the bias of predicted results, which is the total difference between predictions and observations. This revealed whether the results

have been consistently over or under estimated in relation to the observed data; a positive E value indicates over estimation and a negative E value under estimation (Symeou et al., 2014).

$$E = \frac{\sum \frac{(O_i - P_i)}{O_i}}{n - 1}$$

where *E* is the relative error, $O_i$is the observed value, $P_i$is the predicted value and n is the total number of observations made.

The statistical significance of CV-RMSE was assessed by CV-RMSE$_{95\%}$, a value greater than this suggests that the predicted values are not within the 95% confidence intervals of the observed data (Symeou et al., 2014). The statistical significance of E was also tested with $E_{95\%}$, again an E value below this signifies predicted values fell within the 95% confidence intervals for the observed measurements (Symeou et al., 2014). Due to the nature of experimental infections conducted on cattle it was difficult to find an appreciable number of studies giving values taken from multiple calves at repeated time points. Thus for a subset of studies, it was possible to estimate the 95% confidence intervals on the experimental data (to compare with model deviation as measured by CV_RMSE and E).

2.4 Results

2.4.1 *Model exploration*

The model predictions on the effects of different trickle infectious doses are detailed below; the same predictions for the effects of different modes of administration of the same infectious doses are shown in Appendix B.

2.4.1.1 The consequences of different levels of infection

The WBs of a single calf infected with different trickle doses of *O. ostertagi* are shown in figure 2.2A. The rate of increase in WBs increased with increasing number of larvae administered, reaching a peak at 53, 48 and 44 days post infection (dpi) for the 3,500, 7,000 and 14,000 L_3/d respectively. WBs and their negative gradient of reduction started to decline faster at higher tickle doses. WBs never reached zero even when immunity was developed in full. This is due to the assumption that a small number of larvae (8%) will continue to establish and from those a number will survive as adult worms (88%).

The FEC (eggs/g faeces) are a representation of the number of parasitic eggs found in a random sample of faeces (figure 2.2B). The distribution of eggs throughout the faeces is random and therefore the FEC had the potential to be largely under or overestimated, which is represented by the large day to day variation. A clear pattern in total egg numbers produced by all female worms per day in a calf is in figure 2.2C. The total egg counts show a similar pattern to WBs as this is reflective of the female worm populations, however the peak is slightly earlier at 33, 38 and 29 dpi for 3500, 7000 and 14,000 L_3/d respectively. When comparing the relative maximum values of WBs and total egg outputs for different trickle doses, there was a greater difference across WBs. When compared to the low infection level of 3,500 the peak WBs for 7,000 and 14,000 L_3/d were 1.65 and 2.72 times greater, whereas for the peak total egg counts the differences were not as pronounced, being 1.17 and 1.34 times greater respectively.

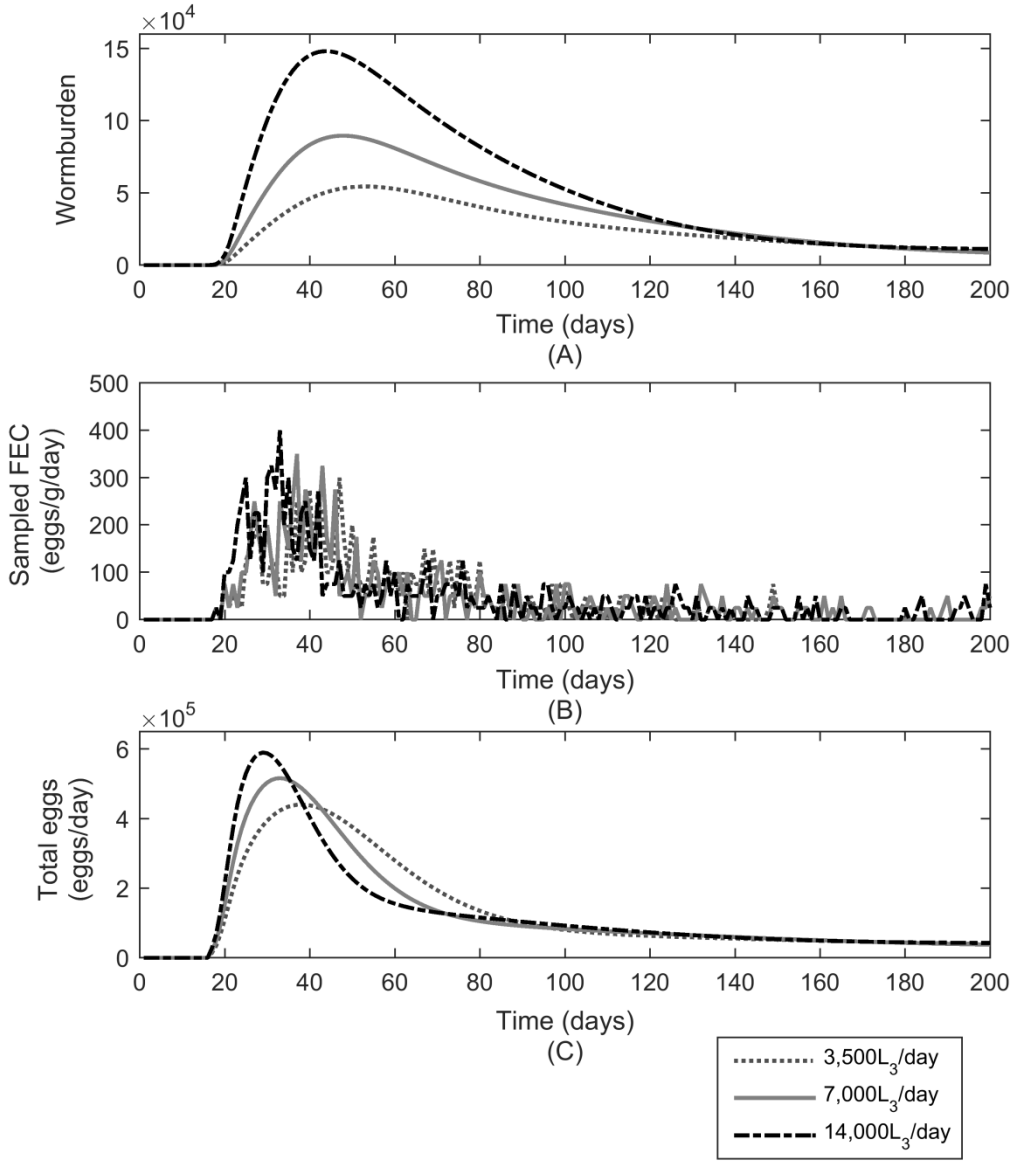

Figure 2.2: Predicted worm burdens (A), sampled daily faecal egg counts (FEC) (B) and daily total egg outputs (C) produced over time in calves administered one of 3 different infection doses of *Ostertagia ostertagi* L_3 larvae: 3,500, 7,000 and 14,000 L_3/day over a 200 day period. The FEC were subject to a random sampling error.

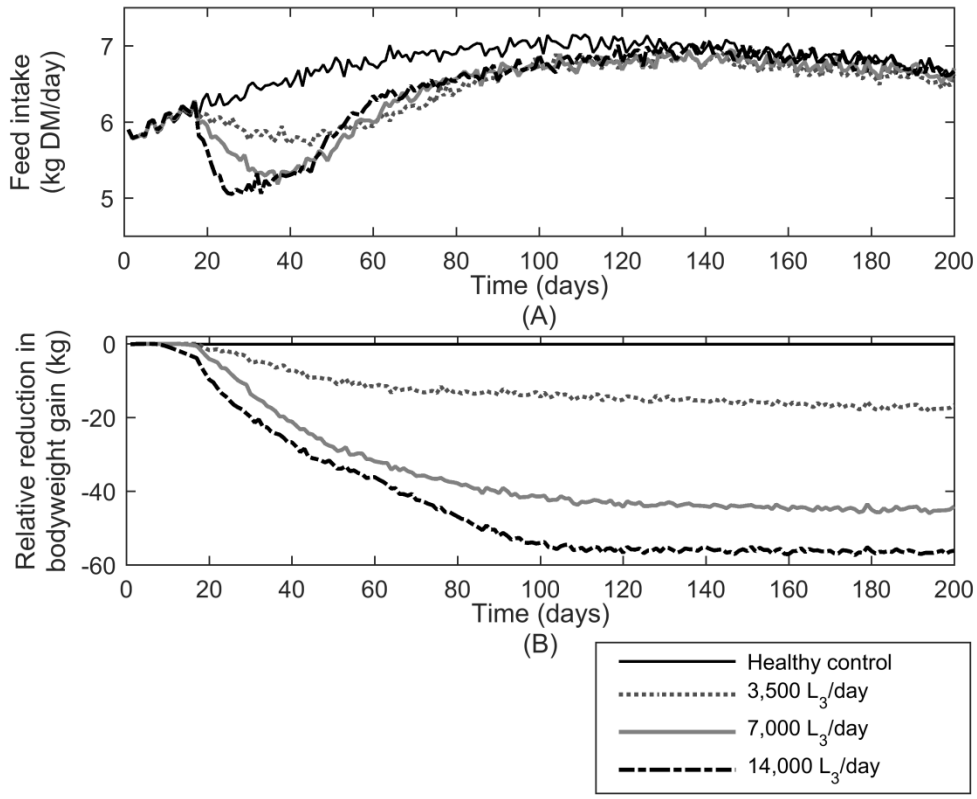

Figure 2.3: The predicted daily feed intake (A) and total relative bodyweight losses (in comparison to uninfected controls) (B) over time in calves administered 3 different infection levels of *Ostertagia ostertagi* L_3 larvae: 3,500, 7,000 and 14,000 L_3/day.

The feed intakes of calves given different trickle doses are shown in figure 2.3A, together with the intake of a healthy calf for comparison. A reduction in feed intake was observed for all infection levels; the extent of the reduction was greater for larger challenges. The point at which the maximum reduction in intake was observed was earlier for larger infection levels with recovery for 3,500, 7,000 and 14,000 L_3/d starting at d 42, 37 and 25 pi respectively in reflection of the immune development. Feed intake returned to levels similar to those displayed by the uninfected calf for the larger infection level by day 130; this was not the case for the lower levels of infection, where intake was slightly below that of the uninfected calf.

The reductions in bodyweight gain of infected calves when compared to a healthy calf for different trickle doses are in figure 2.3B. The effect on bodyweight was greater with larger infection levels; this was predominantly due to reduced feed intake and the damage caused by worms. As the challenge level increased, disproportionate losses in bodyweight gain were observed: a 152% increase in reduction was observed from 3,500 to 7,000 L_3/d compared to a 25% increase from 7000 to 14,000 L_3/d. The maximum effects on the bodyweight appeared

in the early stages of infection, where maximum reductions in bodyweight gain of 3%, 9% and 12% were observed, for the three trickle doses respectively.

2.4.2 *Model sensitivity*

Table 2.1 shows the range of values for simulated outputs of the three traits: peak WB, time of peak WB (days) and final bodyweight (kg), when the selected model parameters were simultaneously varied. The numerical ranges of the outcomes of maximum WB were largest for higher challenge levels. The range for final bodyweights, however, was the same for all challenge levels. Parameters that had a significant effect are reported in order of magnitude of effect on the given output (i.e. the output is most sensitive to the first noted parameter). P values are given in Appendix C.

Larval Challenge (L_3/day)	**Peak worm burden**	**Time of peak worm burden (days)**	**Final Bodyweight (kg)**
3,500	0.146-2.06 $x10^5$	31-132	465-564
7,000	0.241-4.15 $x10^5$	29-112	463-563
14,000	0.389-5.06 $x10^5$	27-96	463-563

Table 2.1: The range of model outcomes for the three parasitological outputs of peak worm burden, timing of peak worm burden, and final bodyweight are shown for simulations of the model run at three challenge levels of 3,500, 7,000 and 14,000 L_3/d. The simulations for each challenge level were run using parameter combinations generated using the Latin hypercube sampling method whereby combinations were randomly selected to best cover the area of possible outcomes. Each parameter was tested at a coefficient of variation of 20%.

2.4.2.1 Parasitism outputs

WB was significantly affected by 3 parameters: $\boldsymbol{k_{EM}}$ **(**the constant relationship between *larvaldays* and its effect on establishment and mortality); $\boldsymbol{EM_{max}}$ (maximum effect of establishment and mortality) and $\boldsymbol{k_{\mu}}$ (the constant relationship between *larvaldays* and mortality) when significance was fixed at the 99% significance level ($p<0.01$). Time of peak WB was significantly affected by $\boldsymbol{k_{EM}}$, $\boldsymbol{EM_{max}}$ and $\boldsymbol{\mu_{max}}$ (maximum mortality) for all infection levels. The total egg counts were found to be sensitive to a large number of parameters with 4 having significant effect for all infection levels. Affecting parameters were $\boldsymbol{k_{EM}}$; $\boldsymbol{F_{max}}$ (maximum fecundity) and $\boldsymbol{EM_{max}}$. Additionally, total egg counts were significantly affected by $\boldsymbol{\mu_{max}}$ at 14,000 L_3/d, whereas the effect was not significant for other infection levels.

The relative effect of changing each parameter can be seen in the linear regression plots, as demonstrated for the infection level of 14,000 L_3/d (figure 2.4). The sensitivity ratio plotted indicates the relative change in the output for a given relative change in the parameter; for example, a coefficient of 1 indicates that a 10% increase in the parameter produces a 10% increase in the particular model output. The largest infection level of 14,000 L_3/d was chosen as this appeared to be the most sensitive to parameter changes. From these plots it was clearly seen that measures of parasitism were most sensitive to $\boldsymbol{k_{EM}}$, the constant relationship between *larvaldays* and the combined effect of establishment and mortality. Conversely, changes in the parasite-related parameters of $\boldsymbol{EM_{min}}$ (minimum effect of establishment and mortality), $\boldsymbol{\mu_{min}}$ (minimum mortality), $\boldsymbol{F_{min}}$ (minimum fecundity), $\boldsymbol{k_F}$ (the constant relationship between *larvaldays* and mortality) and performance-related parameters $\mathbf{C_1}$ (the rate of reduction in feed intake dependent on rate of immune acquisition), $\boldsymbol{rWM}$ (rate of protein loss associated with worm mass and $\boldsymbol{rLB}$ (rate of protein loss associated with larval burden) barely affected the outcomes.

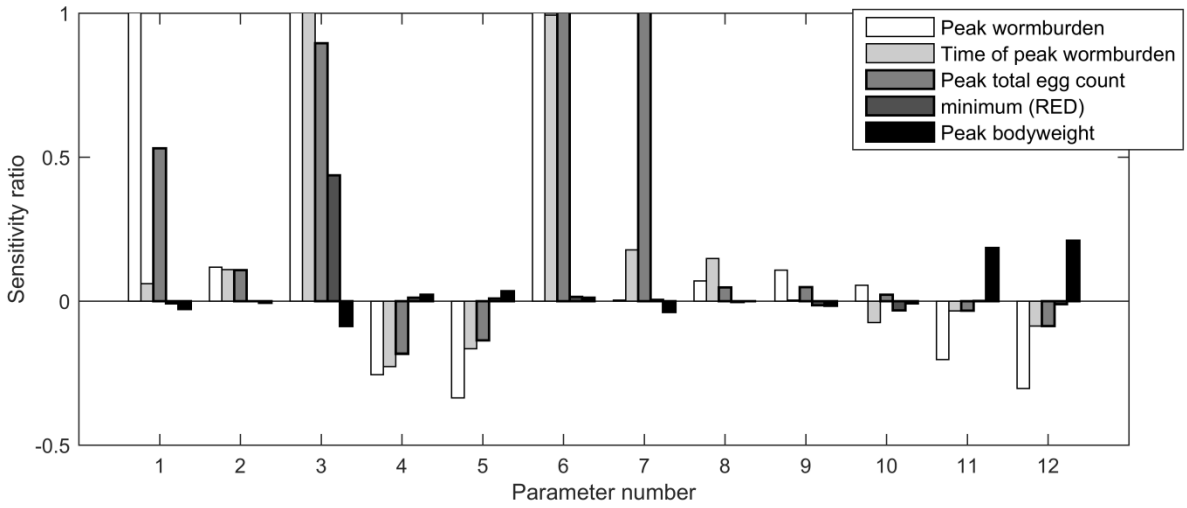

Figure 2.4: The sensitivity ratio of each of the 5 outputs considered (value and time of peak worm burden, peak faecal egg count, peak of reduction in feed intake and final bodyweight) in relation to each of the model parameters considered (1-12) when a calf was infected with 14,000 L_3/d. The parameters were firstly the immune parameters (1-9): the combined effect of establishment and mortality on adult worm burdens (maximum, minimum and rate):$EM_{max}(1)$, $EM_{min}(2)$, k_{EM} (3); the effect of mortality of adult worms (maximum, minimum and rate): $\mu_{max}(4)$, $\mu_{min}(5)$, $k_{\mu}(6)$; the fecundity (eggs) of female adult worms(maximum, minimum and rate): F_{max} (7), F_{min} (8), $\mathrm{k_F}$ (9). The performance parameters (9-12) considered were; the rate of reduction in feed intake dependent on rate of immune acquisition: C_1 (10); the rate of protein loss caused by adult worms rWM (11) and by larvae rLB (12). The sensitivity analysis was conducted by the Latin hypercube sampling technique.

2.4.2.2 Performance outputs

The maximum reduction in feed intake was significantly impacted by $\boldsymbol{C_1}$ (the rate of reduction in feed intake dependent on rate of immune acquisition) and $\boldsymbol{k_{EM}}$ **(**the constant relationship between *larvaldays* and its effect on establishment and mortality). Bodyweights were significantly impacted by $\boldsymbol{k_{EM}}$, $\boldsymbol{rWM}$(rate of protein loss associated with worm mass), and $\boldsymbol{rLB}$ (rate of protein loss associated with larval burden) for all infection levels.

2.4.3 ***Model validation***

The model was tested using published experimental studies, the statistical comparsions are displayed in table 2.2. Graphical comparison were made to WBs from Michel et al. (1970) (figure2.5), Michel and Sinclair (1969) (figure 2.6A) and Michel (1969b; Experiment B) (figure 2.7). Comparisons for total egg outputs were made for Michel and Sinclair (1969) (figure 2.6B), and Michel (1969b; Experiment B) (figure2.7). Finally, comparisons on FEC were made for against the published experiments of Claerebout et al. (1996) (figure 2.8), Forbes et al. (2009) (figure 2.9), Hilderson et al. (1993) (figure 2.10), Hilderson et al. (1995) (figure 2.11), Mansour et al. (1992) (figure 2.12), Satrija and Nansen (1993) (figure 2.13), Wiggin and Gibbs (1989) (figure 2.14) and Xiao and Gibb (1992) (figure 2.15). Comparsions for the best and worst fits are described.

In the majority of cases the comparsion between experimental and model observations showed a similar pattern for WBs with increasing WBs up to a peak followed by a decline; this was reflected in the high positive R correlation coefficients between 0.581 and 0.834. A graphical comparison of model predictions and observations for Michel (1970) is presented in figure 2.5. Although the CV-RMSE did not fall within the 95% level, suggesting a large amount of dispersal from the observed results, the E value fell well within the $E_{95\%}$ suggesting there was no bias and predictions were not consistently over or under estimated compared to observed values. The exception to this pattern was Michel and Sinclair (1969) in which a faster decline in WBs was observed (figure 2.6A). This was reflected in the lower R correlation coefficient and larger negative E value, showing a consistent overestimation by the model.

Measurement output	Source	R	CV RMSE (%)	CV $RMSE_{95\% CI}$	E (%)	$E_{95\%}$
Worm burdens	Michel (1970)	0.834	39.2	36.1	3.58	24.3
Worm burdens	Michel (1969b) Experiment B	0.728	43.0	N/A	-4.30	N/A
Total eggs		0.684	61.4	N/A	-16.7	N/A
Worm burdens	Michel and Sinclair (1969)	0.581	27.6	N/A	-28.9	N/A
Total eggs		0.926	28.4	N/A	-45.4	N/A
Faecal egg counts	Claerebout et al. (1996)	0.728	71.3	N/A	67.2	N/A
Faecal egg counts	Forbes et al. (2009)	0.671	56.6	N/A	48.7	N/A
Faecal egg counts	Hilderson et al. (1993)	0.368	80.5	N/A	-8.28	N/A
Faecal egg counts	Hilderson et al. (1995)	0.798	62.1	N/A	66.2	N/A
Faecal egg counts	Mansour et al. (1992)	0.654	35.9	N/A	-13.2	N/A
Faecal egg counts	Satrija & Nansen (1993)	0.699	29.1	N/A	-17.7	N/A
Faecal egg counts	Wiggins & Gibbs (1989)	-0.0590	97.1	N/A	65.0	N/A
Faecal egg counts	Xiao & Gibbs (1992)	0.813	64.8	N/A	65.2	N/A

N/A- not applicable

Table 2.2: The outcomes of statistical analyses used to assess goodness-of-fit between predictions and observed and experimental results of worm burdens, total egg outputs and faecal egg counts. Values for the R correlation coefficient, the coefficient of variation of the root mean square error (CV RMSE) and the relative error (E) are all given to 3 significant figures. The 95% confidence interval of experimental data is estimated where possible; in some cases standard deviations were not provided as only one calf was used for each measurement.

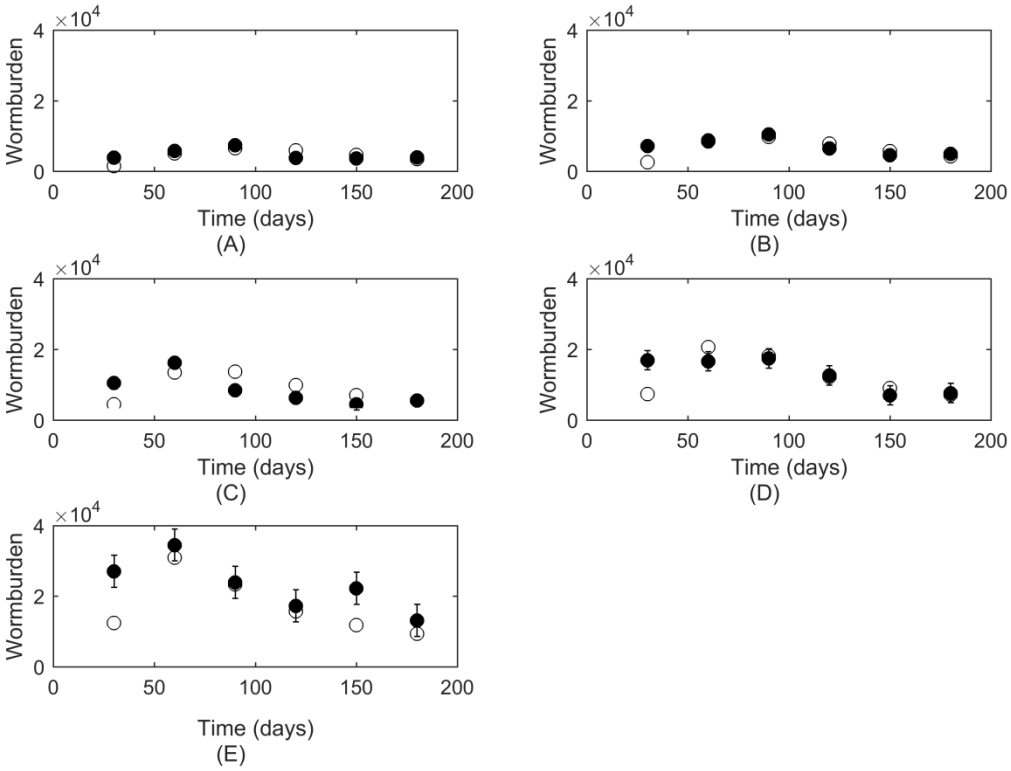

Figure 2.5: A comparison of the observations (●) by Michel (1970) to simulated predictions (o) for worm burdens produced by *Ostertagia ostertagi* infections of A) 200 L_3/d; B) 340 L_3/d; C) 570 L_3/d; D)950 L_3/d; E) 1600 L_3/d. Each measurement was taken from 5 calves for each point.

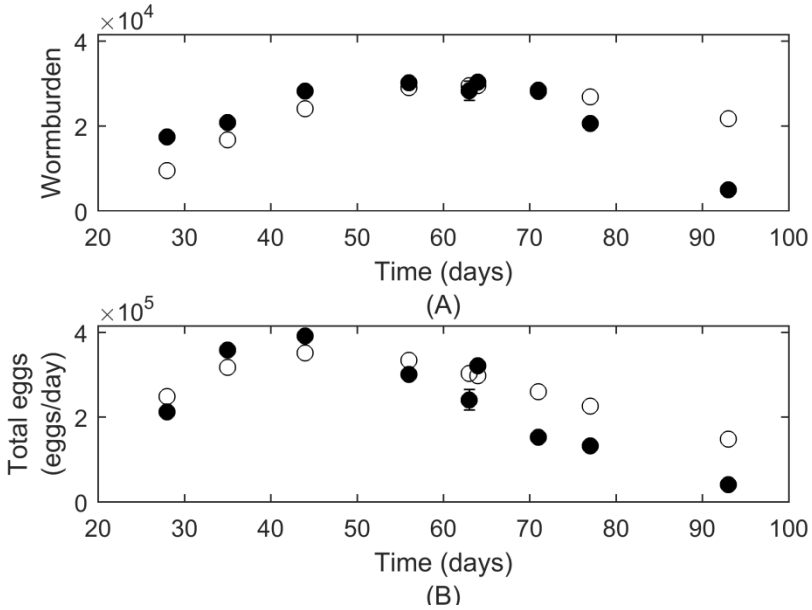

Figure 2.6: A comparison of experimental observations (●) by Michel and Sinclair (1969) to simulated predictions (o) for A) worm burdens and B) total eggs counts produced by an infection level of 1500 L_3/d. Each point is based on measurements from one calf, with the exception of day 63 which is based on measurements from 2 calves.

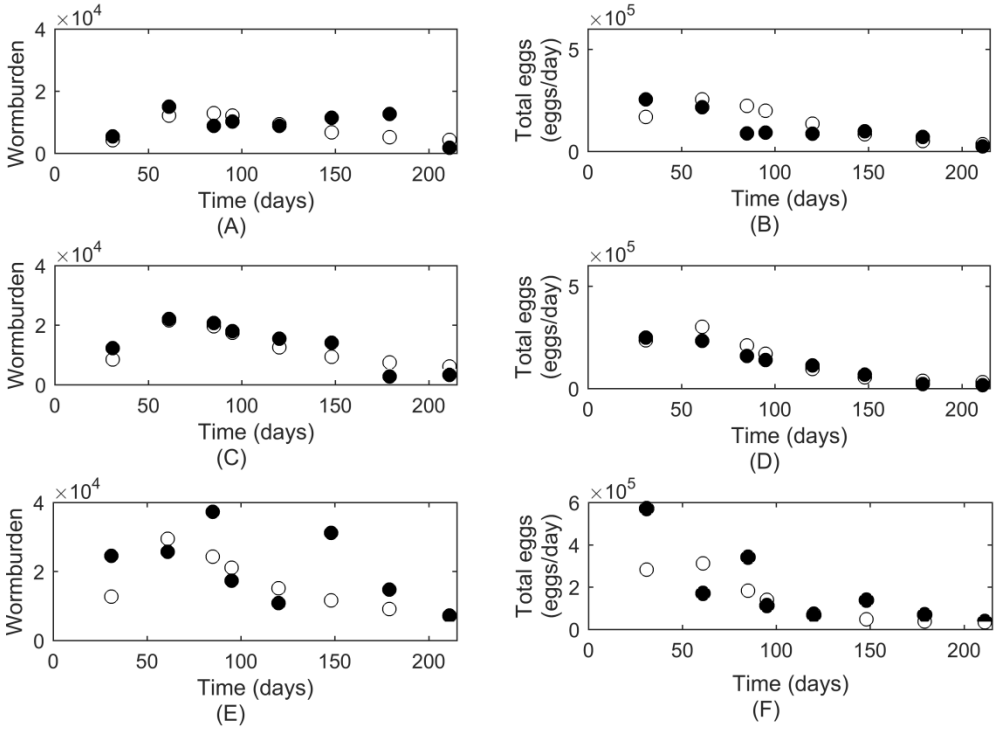

Figure 2.7: A comparison of experimental observations (●) by Michel (1969b) experiment B to simulated predictions (o) for worm burdens resulting from infection doses of A) 500 larvae per day; B) 1000 larvae per day; C) 1500 larvae per day and total eggs per day resulting from infection levels of D) 500 larvae per day; E) 1000 larvae per day; F) 1500 larvae per day. Each experimental data point is based on measurements from a single calf.

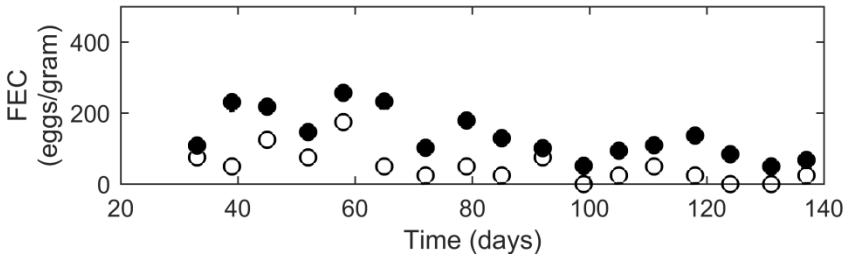

Figure 2.8: A comparison of experimental observations (●) by Claerebout et al. (1996) to simulated predictions (o) for faecal egg outputs per gram of fresh faeces produced by an infection level of 20,000 larvae per week, administered in 3 doses, for 21 weeks. Each measurement was taken for 6 calves.

Of the aforementioned studies meeting the validation criteria only two provided total egg outputs; similarly to the WBs the observations revealed total eggs reached a maximum early on in the infection and decreased from this point onwards. Model predictions were in reasonable agreement with the observed values for both experiment B of Michel (1969b) (figure 2.7) and Michel and Sinclair (1969) (figure 2.6B). The latter showed a close correspondance with a high R correlation coefficient of 0.926; however as a consequence of the pattern of WB the E value showed again a consistent overestimation of results by the model.

In general the observed pattern of FECs was similar to that of total egg outputs: increasing to a peak early on in the infection and then consistently decreasing. The pattern was not as clear due to the sampling error incorporated for FEC counting; this was reflected in the R values given in table 2.2. An example of a good fit was Satrija and Nansen (1993) in which a relatively low CV-RMSE and E value indicate a close fit between results and minimal bias, this is represented graphically in figure 2.13. However not all experiments provided such strong support to the model, in particular Wiggin and Gibbs (1989) for which FEC offered an extremely weak R coefficient of -0.059 suggesting the observed pattern was not well replicated by model predictions (figure 2.14). This was accompanied by an extremely large CV-RMSE value of 97.1 and a largely positive E value suggesting a gross underestimation by the model, which can clearly be seen in figure 2.14. However, it can be observed that the FEC values reported in Wiggin and Gibbs (1989) are noticeably larger than typical published values.

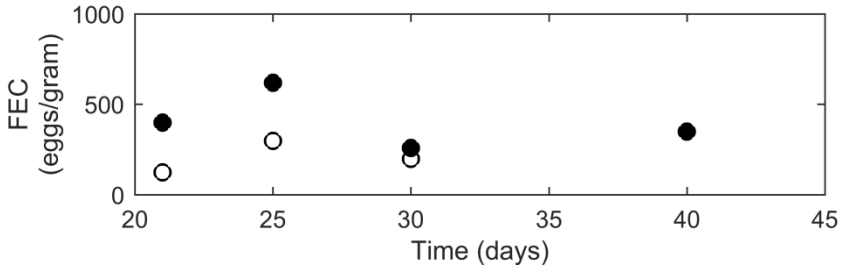

Figure 2.9: A comparison of experimental observations (●) by Forbes et al. (2009) to simulated predictions (o) for faecal egg outputs per gram of fresh faeces produced by an infection level of 70,000 larvae per week, administered in 3 doses, for 8 weeks. Each measurement was taken for 5 calves.

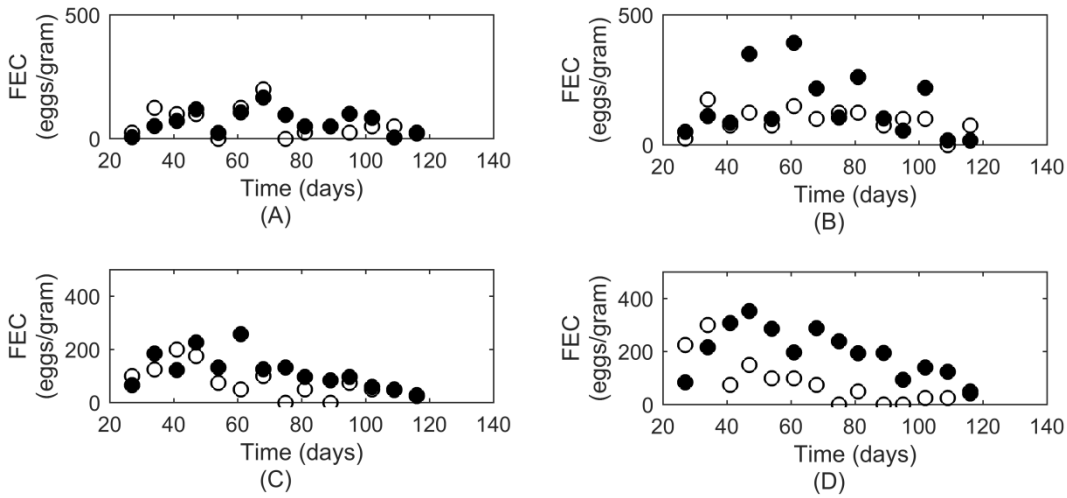

Figure 2.10: A comparison of experimental observations (●) by Hilderson et al. (1993) to simulated predictions (o) for faecal egg outputs per gram of fresh faeces produced by infection levels of (A) 5,000 larvae per week; (B) 10,000 larvae per week; (C) 20,000 larvae per week; (D) 40,000 larvae per week, all administered in 3 doses a week for 17 weeks. Each measurement was taken for 4 calves.

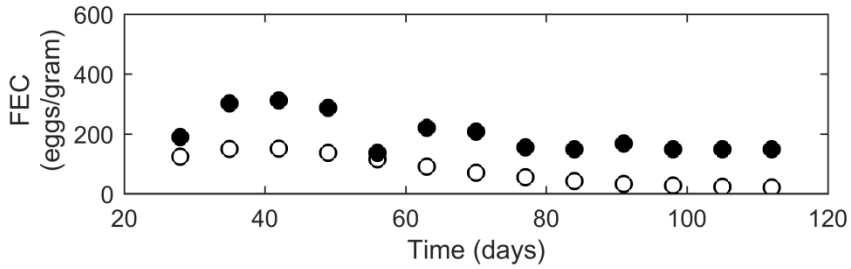

Figure 2.11: A comparison of experimental observations (●) by Hilderson et al. (1995) to simulated predictions (o) for faecal egg outputs per gram of fresh faeces produced an infection level of 20,000 larvae per week, administered in 3 doses, for 17 weeks. Each measurement was taken for 5 calves.

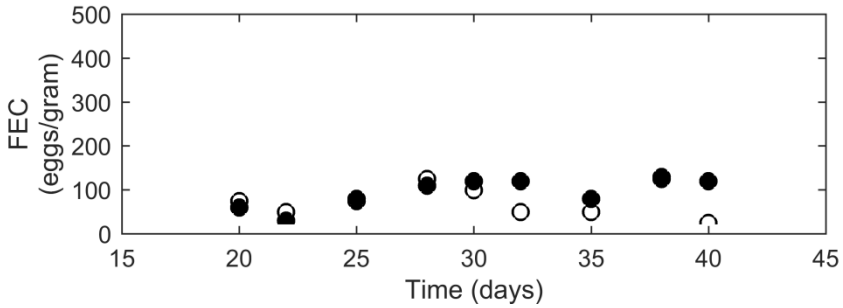

Figure 2.12: A comparison of experimental observations (●) by Mansour et al. (1992) to simulated predictions (o) for faecal egg outputs per gram of fresh faeces produced by an infection level of 3,000 larvae administered every other day for 6 weeks. Each measurement was taken for 6 calves.

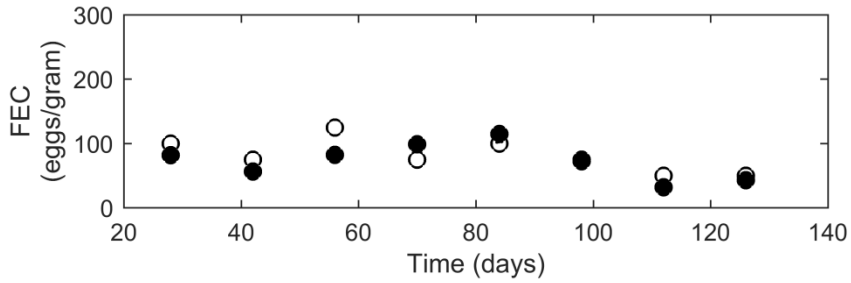

Figure 2.13: A comparison of experimental observations (●) by Satrija and Nansen (1993) to simulated predictions (o) for faecal egg outputs per gram of fresh faeces resulting from a weekly infection of 1,250 larvae. Each measurement was taken for 6 calves.

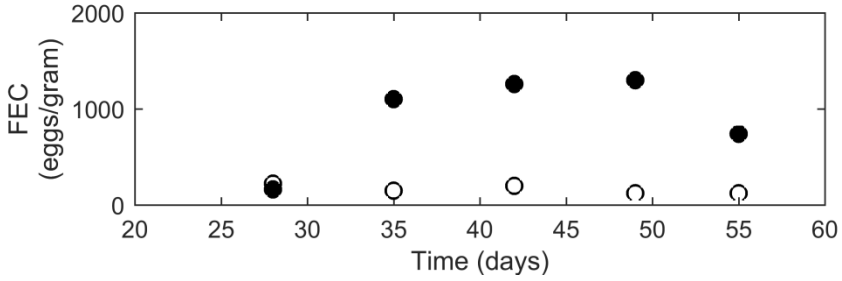

Figure 2.14: A comparison of experimental observations (●) by Wiggin and Gibb (1989) to simulated predictions (o) for faecal egg outputs per gram of fresh faeces produced by a weekly infection of 30,000 larvae. Each measurement was taken for 12 calves.

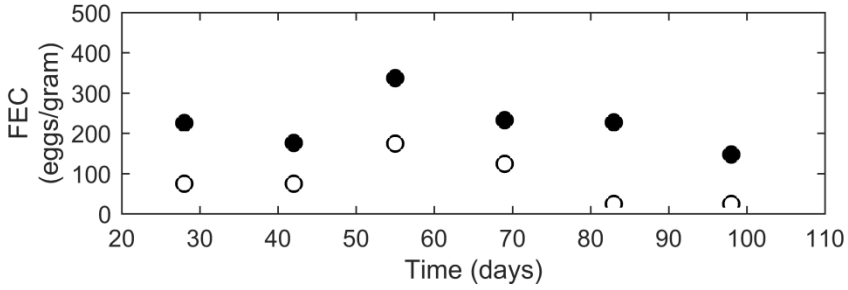

Figure 2.15: A comparison of experimental observations (●) by Xiao and Gibb (1992) to simulated predictions (o) for faecal egg outputs per gram of fresh faeces produced by a weekly infection of 10,000 larvae for 14 weeks. Each measurement was taken for 5 calves.

2.5 Discussion

The overall aim of this paper was to develop a model that accounted for the interactions between *O. ostertagi* parasitism and first season grazing calves, under UK conditions. Although the model was deterministic, it was constructed with the view of developing it into a stochastic one, to allow for the investigation of different methods of control of the parasite, including selection for host resistance (Laurenson et al., 2012a; 2012b). Larval intake was considered an input to the model, but there are plans to account for parasite populations in the environment in the manner similar to Laurenson et al (2012b).

Although there are a number of models focusing on predicting the epidemiology of *O. ostertagi* (Chaparro and Canziani, 2010; Gettinby and Paton, 1981; Gettinby et al., 1979), currently there are only two models that specifically aim to investigate within-host interactions between calf host and *O. ostertagi*. The PARABAN model (Grenfell et al., 1987a; 1987b; Smith and Grenfell, 1985; Smith et al., 1987a; 1987b) was specifically developed to account for the rate of change in parasite populations within hosts and the environment, and has been used to investigate the effectiveness of anthelmintic treatment on parasite dynamics. This model, however, does not account for the consequences of parasitism on host performance and its creators recognised its limitations in this respect (Smith, 1997). This was ascribed mainly to the fact that there is not a linear relationship between parasitism and performance, and the lack of clarity for which indicators of parasitism could be used to define a relationship with production.

Ward (2006a) attempted to account for the consequences of parasitism on calf performance by developing an animal growth model and by considering the effects of parasitism on host feed intake and metabolism. Parasite dynamics were expressed by the same equations that formed the basis of the above model (Smith et al., 1987b). This implies that parasite establishment and fecundity were considered a function of time, as opposed to being a function of the development of the immune response (Smith and Grenfell, 1994); the only description of calf state used in the model was its bodyweight. A consequence of these assumptions would be an under- or over-estimation of calf performance during parasitism, as was indeed the case in the validation of the model by Ward (2006b). This could arise, for example, by over or under expression of the immune function to parasites as a consequence of nutrition (Ploeger et al., 1995; Coop and Kyriazakis, 1999).

The previously developed models identify the challenges associated with the development of a model that predicts the interactions between *O. ostertagi* and calves. In our model the animal state was characterised by calf degree of maturity (current protein mass divided by mature protein mass) and level of fatness, consistent with other animal growth models (Emmans and Kyriazakis, 2001), and by the cumulative exposure to parasitic challenge (*larvaldays*). The former feature enables simulation of different genotypes. A further attraction of describing the calf through these traits is that it is possible to introduce variation and co-variation in them and as a consequence to convert a deterministic model into a stochastic one (Vagenas et al, 2007c; Laurenson et al, 2012b). The consideration of *larvaldays* enabled the immune response of the animal to be linked to the duration of parasite exposure, which is hypothesised to have greater effect on immune acquisition than the level of infection per se (Hilderson et al., 1993). Hence this model was able to portray differences in rate of immune development at different levels of infection.

Protein loss, which is the main consequence of gastrointestinal parasite challenge (Taylor et al., 1989), was related to current worm mass and larval burden, as opposed to WB and larval intake (Ward, 2006a). It was not possible to treat the impact of larvae mass similarly to worm mass, due to the difficulties in estimating the impact of immunity on larval mortality. On entering the host the model immediately discarded any larvae that failed to establish hence potentially resulting in an underestimation of the larval burden. Although there is currently little quantitative information about parasite-induced protein loss in calves, some assumptions were made, consistent with the quantitative estimates of protein loss during abomasal parasitism in sheep (Laurenson et al., 2011) and our current estimates of the effects of *O. ostertagi* on calf productivity (Szyszka and Kyriazakis, 2013). Better estimates of these relationships will enhance model accuracy.

The basis of the causal reduction in feed intake during parasitism has been the subject of considerable debate (Fox et al., 1989a; Kyriazakis et al., 1998; Laurenson et al., 2011). Feed intake reduction during parasitism was related to the rate of change in each of the immune parameters: this was in order to relate parasite-induced anorexia to the development of the immune response, as has been suggested by Sandberg et al. (2006) and Kyriazakis (2011; 2014). The rapid recovery in feed intake post administration of anthelmintics in cattle (Bell et al., 1990) and other ruminants (Kyriazakis et al., 1996b), suggests that anorexia is not a consequence of pathology, but is inextribaly linked to the stimulation of the immune

response caused by the exposure to the parasites. Feed intake recovers when the immune reponse is fully developed (Kyriazakis et al., 1996b, Sandberg et al., 2006); however it was assumed that there would be no compensatory increase in feed intake and perfomance (Kyriazakis and Houdijk, 2007). The existence of such compensatory response would affect the predictions of the model in terms of calf performance, but not its parasitological outputs.

The assumptions made about within host parasite populations and the interactions between host and parasite lead to a number of model behaviours. The rate of reduction in WB was more rapid for higher infection pressures; this was a reflection of the model assumption that the development of immunity was dependent on the cumulative exposure to larvae. WBs never reached zero even when immunity had developed in full, consistently with the idea of incomplete and slow development of immunity to *O. ostertagi* in relation to other parasite species (Klesius, 1988; Hilderson et al., 1993). This is consistent with the suggestion that complete parasite clearance is resource expensive and hence a low level of parasite challenge may be comparatively resource cheap (Medley, 2002; Viney et al., 2005). We did not observe a relationship between infection pressure and the plateau of within host WB, as suggested by Cattadori et al (2005); this was a reflection of the absence of an epidemiological component in our model.

Anorexia became evident around the same time for all infection levels; this was a result of a threshold level of immune acquisition achieved at a similar time for each challenge dose, consistent with Szyszka and Kyriazakis (2013). In addition it has been shown that feed intake is not affected during the stage of larval development (Michel, 1969b; Fox et al, 1989a; Szyszka and Kyriazakis, 2013). Feed intake also began to recover earlier for higher infection levels. This was a reflection of the assumption for faster immune acquisition and a higher desired intake to meet increased nutrient demands; more heavily parasitised calves must have larger requirements for repair and immunity (Sandberg et al, 2006). The total duration of anorexia was shortest for larger infection levels with no clear recovery in feed intake occurring for the lower levels. This is consistent with Herlich (1980) who found that the duration of anorexia seemed to be unrelated to the size of WB across cattle age groups infected with *O. ostertagi,* with cattle of 24 months showing large WBs but without signs of anorexia. In contrast Herlich (1980) concluded that of the age groups considered (2, 4/5, 12 and 24 months) only the 2 month old calves appeared to show ‘resistance’ to parasitic

infection, implying the highest development of immunity, and coincidentally the highest incidence of anorexia.

A sensitivity analysis was conducted to identify parameters of key influence; the LHS was chosen as this method attempts to cover the widest space of possible parameter combinations. As far as we are aware, this has been the first attempt to apply the methodology in the validation of parasitological models. The approach requires fewer simulations than the Monte Carlo method as it is guaranteed to cover more uniformly the complete range of possibilities. Conversely a Monte Carlo simulation, which selects values at random, may generate clusters of similar parameter combinations while failing to probe other important regions of the parameter space.

In order to place any confidence in the model it was necessary to validate it against published literature. Identifying suitable data sets to perform such validation was by no means an easy task. Parasitological traits were validated by comparing observed and simulated outputs for WBs, total egg outputs and FEC. As far as parasitological measurements are concerned, most experiments do not report calf WBs at a particular time point of infection. This is due to the large costs and animal welfare concerns associated calf necropsies. The experiments by Michel (1969b; 1970) and Michel and Sinclair (1969) are the few exceptions that report such data, but their data were mostly based on single calf measurements. As a consequence they were subject to individual variation in calf responses, as demonstrated by the relatively large CM-RMSE values. For similar reasons few experiments also report total egg outputs, however due the ease of faecal collections the usual parasitological traits reported is FEC. In most cases FECs also showed a good fit, although these were subject to enormous variation, even for the same host, due to the pattern of feed intake, as well as the volume and consistency of faeces produced (Vagenas et al., 2007b). Many of the experiments are likely to have involved smaller size calves, resulting in an overestimation of feed intake and hence in faecal matter. Experiments will always be restricted by the number of animals involved; simulations studies are not limited by this, but can take into account between animal variation.

Of the relevant studies many were performed a number of years ago; since then calves have been selected for performance traits, but little to no selection for resistance appears to have taken place (Prakash, 2009). Owing to a lack of experimental studies investigating the effect

of sub-clinical challenge levels on calf DM intake it was not possible to validate performance; inference was made from bodyweight reductions comparatively to control animals. A general review of the literature on feed intake during *O. ostertagi* infection showed varied patterns of feed intake between studies (Fox et al, 1989a; Szyszka and Kyriazakis, 2013). It has been observed that duration and magnitude of parasite-induced anorexia are both strain dependent with a variance of up to 8 days between strains (Herlich et al., 1984).

The limitations of the model predictions point towards the need to develop a population model, as opposed to a deterministic model to account for calf – *O. ostertagi* interactions. To account for discrepancies between studies and for variation within them resulting from calf genetic variation, a stochastic herd-based model needs to be developed. Vagenas et al. (2007c) and Laurenson et al. (2012b) have described the challenges associated with this task for the development of a simulation model that accounted for the interactions between sheep and *T. circumcincta*. Nevertheless, such a development is a necessary step to address the consequences of management on the parasitism of a population of calves, especially given the move towards the development of TST in order to reduce the rate of selection for anthelmintic resistance (Charlier et al., 2014) whereby only individuals who would benefit most from treatment receive it.

2.6 Conclusions

A dynamic, deterministic model to account for the interactions between calves and *O. ostertagi* has been developed. Although the model was developed for a specific calf genotype given *ad libitum* access to high quality grass, the model is able to apply to other genotypes and be extended for different nutritional scenarios. Comparisons of model outputs to experimental observations highlighted both model strengths and weaknesses. Reliance of the model on expressing the development of the immune responses affecting parasite populations within the host, points towards the need to collect further data to define such relationships. In this respect the model has a heuristic value. A major strength of the model is its ability to be converted into a population model and hence be used as a tool to investigate the consequences of parasitism in a group of calves subjected to different management treatments.

Chapter 3: A stochastic model to investigate the effects of control strategies on calves exposed to *Ostertagia ostertagi*

3.1 Abstract

Predicting the effectiveness of parasite control strategies requires accounting for the responses of individual hosts and the epidemiology of parasite supra- and infra-populations. The first objective was to develop a stochastic model that predicted the parasitological interaction within a group of first season grazing calves challenged by *Ostertagia ostertagi*, by considering phenotypic variation amongst the calves and variation in parasite infra-population. Model behaviour was assessed using variations in parasite supra-population and calf stocking rate. The model showed the initial pasture infection level to have little impact on parasitological output traits, such as worm burdens and faecal egg counts, or overall performance of calves, whereas increasing stocking rate had a disproportionately large effect on both parasitological and performance traits. Model predictions were compared to published data taken from experiments on common control strategies, such as reducing stocking rates, the 'dose and move' strategy and strategic treatment with anthelmintic at specific times. Model predictions showed in most cases reasonable agreement with observations, supporting model robustness. The stochastic model developed is flexible, with the potential to predict the consequences of other nematode control strategies, such as targeted selective treatments on groups of grazing calves.

3.2 Introduction

Gastrointestinal parasitism of calves, in particular with *Ostertagia ostertagi*, is a significant challenge to their health, welfare and productivity. As such, a variety of control strategies have been proposed to reduce the negative effects of parasitism (Cockroft, 2015). These include the Weybridge 'dose and move' strategy, a reduction in stocking rate and dosing at strategic time points of the grazing season (Michel and Lancaster, 1970; Hansen et al. 1989; Cockroft, 2015). More recently, targeted selective treatment (TST), where specific individuals of a population as opposed to the whole population are treated, has been suggested as an alternative control strategy (Höglund et al. 2013a; O'Shaughnessy et al. 2014a; 2015a; 2015b).

Quantifying the effectiveness of such strategies is both time consuming and expensive, and in many respects it is difficult, if not impossible to make comparisons between them due to confounding variables (Höglund, 2010). Simulation modelling is a potential alternative to experimentation and, provided that a model is based on sound principles and data, it has the potential to evaluate different approaches to control. In order to be able to assess the effectiveness of such control strategies, a stochastic (i.e. probabilistic, population-based) model allowing for individual-response differences is required. This is because individuals will affect parasite epidemiology and subsequently influence the effectiveness of control. Stochastic models (Renshaw, 1991) can help to evaluate such strategies, by simulating identical scenarios allowing a direct comparison of treatment effectiveness, and to identify potential interactions, thereby aiding in the assessment of the feasibility of novel control strategies. Currently, we are not aware of published simulation models that allow us to account for variation between individual calves within a group and variation in parasite supra population, i.e. parasite populations at all development stages across all hosts.

The aim of this paper was to develop a stochastic simulation model that was capable of accounting for such variation and can be utilised in future studies of parasite control strategies. The stochastic model was based on the deterministic approach previously developed in chapter 2. The deterministic model is able to account for the interactions between gastrointestinal parasites and an individual calf to predict parasite infra-populations, i.e. populations within individual hosts. By introducing variation in growth and resistance traits amongst calves, along with an epidemiological-transmission layer, we aimed to develop a model which considers *Ostertagia ostertagi*-calf interactions along with their

epidemiological consequences. Following model development, its behaviour was evaluated under simple manipulations such as variations in stocking rate and initial larval pasture contamination (IL_0). Finally, the model was validated against the prevailing management control strategies, such as reduced stocking rate, the 'dose and move' strategy and strategic anthelmintic drenching.

3.3 Materials and Methods

A previously developed dynamic, deterministic model (Chapter 2) to describe the interactions between gastrointestinal parasites and an individual calf, was extended to a stochastic one for a grazing population/herd of calves. A brief description of the individual calf model is given below, followed by a more detailed description of the additional features incorporated towards the development of a grazing population model.

3.3.1 *Individual calf model*

A schematic diagram representing the model interactions for an individual calf infected by *O. ostertagi* is provided in figure 3.1. Briefly, it was assumed that a healthy calf attempts to ingest sufficient nutrients to meet demands for growth and maintenance (Coop and Kyriazakis, 1999). In the presence of parasitic infection, a parasitised calf experiences an endogenous protein loss (Fox, 1993). Consequently, the calf is assumed to invest in an immune response to reduce the impact of infection (Claerebout and Vercruysse, 2000). However, despite the endogenous protein loss and the increased resource requirement for the development of immunity, a reduction in feed intake occurs as a result of immune components, e.g. cytokines, and related pathological and inflammatory responses (Fox et al. 1989b; Kyriazakis, 2014). This reduction was modelled as a function of the rate of acquisition of immunity (Laurenson et al. 2011). Consequently, the calf consumes insufficient feed resources to fulfil its requirements. Ingested protein, after the loss due to parasitism, was assumed to be first allocated to maintenance and repair requirements (Coop and Kyriazakis, 1999). Remaining feed resources were then allocated between growth and immunity, proportional to their requirements (Kahn et al. 2000; Doeschl-Wilson et al. 2008; Laurenson et al. 2011). Such requirements were defined in accordance to Vagenas et al. (2007b).

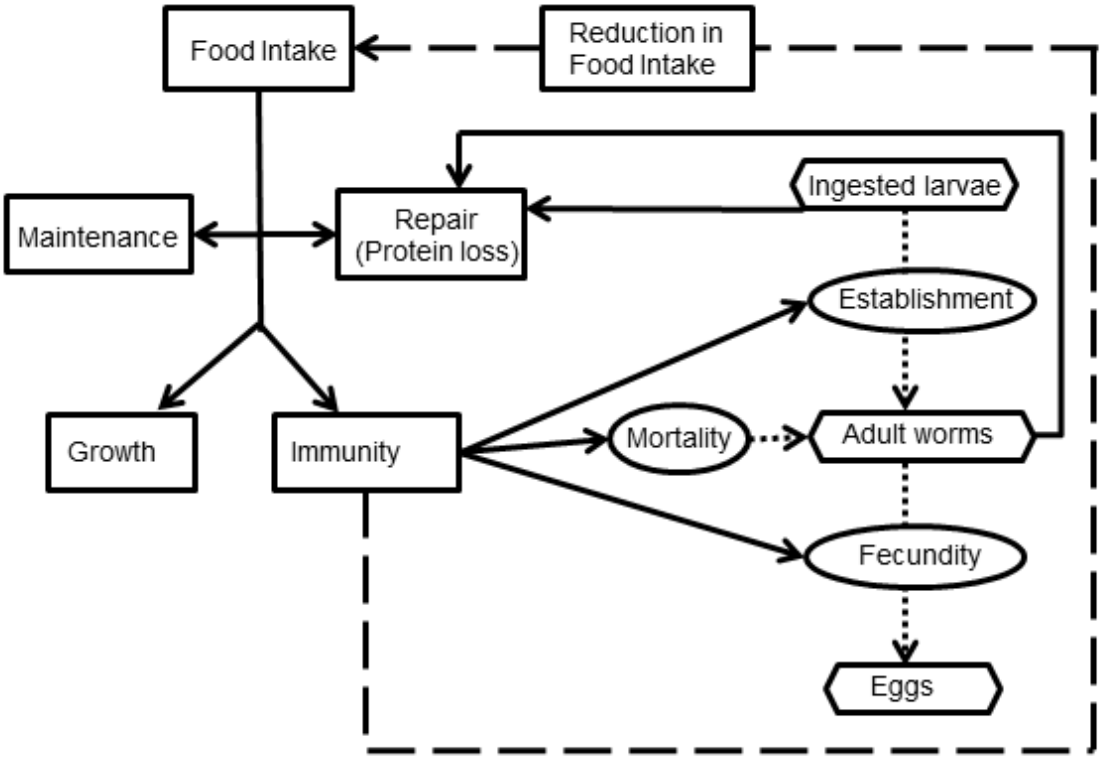

Figure 3.1: A schematic description of the parasite-host interactions. The rectangular boxes and solid lines indicate the flow of ingested feed resources; the oval boxes indicate the host-parasite interactions and the hexagonal boxes represent the key measurable stages of the parasite life-cycle. Host immune response and related pathological and inflammatory responses were assumed to lead to parasite-induced anorexia (broken line).

3.3.2 ***Herd Population Model***

In contrast to previously published models (Vagenas et al. 2007c; Doeschl-Wilson et al. 2008; Laurenson et al., 2012b), between-animal variation was only modelled at the phenotypic level, for the sake of simplicity. Phenotypic variation was assumed to occur in animal growth characteristics, maintenance requirements and host immunity to gastrointestinal parasitism.

3.3.2.1 Variation in growth characteristics.

A growing calf was described by its initial empty bodyweight at weaning (EBW_i), protein mass at maturity (P_M), a growth rate parameter (B^*), and the lipid to protein ratio at maturity (LPR_M). These parameters were selected to minimise correlation to one another, hence preventing problems that would arise from correlated parameters for stochastic simulations (Symeou et al. 2016). Growth was assumed to be driven by protein and lipid

retention, with expected growth rates described by adaptations of existing functions (Emmans, 1997; Emmans and Kyriazakis, 1997), such that:

$$\Delta PGrowth_{max} = P \cdot \left(\frac{B^*}{{P_M}^{0.27}}\right) \cdot \ln\left(\frac{P_M}{P}\right) \quad (3.1)$$

$$\Delta Lipid_{des} = \Delta PGrowth_{max} \cdot LPR_M \cdot d \cdot \left(\frac{P}{P_M}\right)^{(d-1)} \quad (3.2)$$

where $\Delta PGrowth_{max}$ is the expected rate of protein retention (kg/day), $\Delta Lipid_{des}$ is the expected rate of lipid retention (kg/day), P is the current body protein mass (kg), and d =$1.46 \cdot {LPR_M}^{0.23}$.

Thus, differences in EBW_i, P_M, B^*, and LPR_M can result in between-animal variation in initial body weight, growth rate, mature body composition and mature body weight. As such, these input parameters were assumed to vary phenotypically and are given in table 3.1.

3.3.2.2 Variation in maintenance requirements.

The body maintenance requirements for protein (PR_{maint}, kg/day) and metabolisable energy (ER_{maint}, MJ/day) were modelled in accordance with Emmans and Kyriazakis (2001):

$$PR_{maint} = p_{maint} \frac{P}{{P_M}^{0.27}} \quad (3.3)$$

$$ER_{maint} = e_{maint} \frac{P}{{P_M}^{0.27}} \quad (3.4)$$

where p_{maint} is the constant associated with protein maintenance requirements and e_{maint} is the constant associated with energy maintenance requirements. Phenotypic variation in the parameters p_{maint} and e_{maint} was assumed, as it signifies differences in maintenance requirements for protein and energy (Knap and Schrama, 1996; Laurenson et al. 2012b).

3.3.2.3 Variation in host immunity.

The immune response was represented by the host-controlled traits of parasite establishment, mortality (μ, proportion of adult worms/day) and fecundity (*F*, eggs/female/day). Establishment was determined by subtracting the effect of mortality from the combined effect of establishment and mortality (*EM*, change in adult worm numbers/day). The functions used to describe these traits were characterised in Chapter 2 as:

$$EM = (EM_{max} - EM_{min}) \cdot \exp\ (-k_{EM} \cdot larvaldays) + EM_{min} \quad (3.5)$$

$$\mu = \frac{(\mu_{max} - \mu_{min}) \cdot (larvaldays)^2}{{k_\mu}^2 + (larvaldays)^2} + \mu_{min} \quad (3.6)$$

$$F = (F_{max} - F_{min}) \cdot \exp(-k_F \cdot larvaldays\ ) + F_{min} \quad (3.7)$$

where *larvaldays* is a measure of parasite exposure; EM_{max}, μ_{max} and F_{max} are the maxima of the combined effect of establishment and mortality, mortality and fecundity, respectively; EM_{min}, μ_{min} and F_{min} are the minima of the combined effect of establishment and mortality, mortality and fecundity, respectively; k_{EM}, k_μ and k_F are the rate constants of the relationships between *larvaldays* and the combined effect of establishment and mortality, mortality and fecundity, respectively.

The calves were assumed to be initially naïve to gastrointestinal parasites and gradually acquired immunity as calf exposure to infective larvae increased. The rate of immune acquisition was therefore determined by the length of temporal exposure to infective larvae and the rate parameters k_{EM}, k_μ, k_F, for each of the host-controlled immunity traits. All parameters describing the maxima, minima and rate of acquisition for each of the host-controlled immunity traits were assumed to exhibit between animal variation.

3.3.2.4 Variation in feed intake.

In addition to variation in the specified traits, a degree of random variation was assumed to reflect the influence of external factors controlling variation in day to day feed intake that were not explicitly accounted for by the model. Due to the correlation between growth and feed intake tending towards unity in this model, daily random deviation in feed intake was

adjusted to give a more realistic phenotypic correlation between feed intake and growth rate of approximately 0.8 (Cammack et al. 2005; Laurenson et al. 2012b).

3.3.2.5 Parameter values and distributions.

The model was parameterised such that the calf and its growth represented a weaned, castrated male (steer) Limousin x Holstein Friesian born in autumn; this common cross currently represents the majority of beef cattle reared in the UK (Todd et al. 2011). Autumn born calves are capable of utilising grass in spring and hence are turned out at 6 months of age and left at pasture until late autumn (Phillips, 2010). Parasitological parameters were based on those gathered from published literature (Chapter 2). Each trait selected to be phenotypically variable was assigned a population mean and coefficient of variation (CV) as provided in table 3.1 based on several sources. The immune development traits were assumed to follow a log-normal distribution, whereas all other traits were assumed to be normally distributed (Vagenas et al. 2007c; Laurenson et al. 2012b). Over recent years calves have been selectively bred to show favourable traits, such as growth rate (Prakash, 2009). However, immune traits are rather more difficult to select for (Frisch, 1981; Prakash, 2009). Log-normal distributions were assigned to the immune rate parameters to allow for higher levels of variation (several-fold increase or decrease) without the negative values that could arise from utilising a normal distribution for these parameters. For the growth attributes the mean values were taken as presented in Chapter 2 and CVs based on estimates for other ruminants (Vagenas et al. 2007c; Laurenson et al. 2012b). Similarly, the mean value of immune traits were taken as presented in Chapter 2 and, owing to a lack of data to provide confident estimates, CVs were based on values for lambs infected with the closely related parasite *Teladorsagia circumcincta* (Laurenson et al. 2012b).

All traits, other than those representing the host immune response, 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 (components of the Th2 immune response; Mihi et al. 2014). Thus, the rate-determining parameters (k_{EM}, k_{μ}, k_F) were assumed to be strongly correlated (coefficient of correlation r = +0.5) (Laurenson et al. 2012b). Establishment was calculated as the combined effect of establishment and mortality minus the effect of mortality alone, as such predictions for establishment and mortality were correlated. In order to counteract this, a negative correlation (r = -0.2) was applied to the

parameters describing the maximum effect of combined establishment and mortality and the minimum mortality. For correlated traits a Cholesky decomposition of the variance-covariance matrix was used to generate the co-variances between the phenotypic input parameters of the individual animals.

Category	Parameter (units)	Description	Mean	CV
Growth	P_M (kg)	Mature protein content	106	0.125
	LRR_M (kg)	Mature lipid to protein ratio	1.95	0.15
	B* (day^{-1})	Protein growth rate constant	0.025	0.15
	EBW_i (kg)	Initial empty body weight	255	0.15
Maintenance	PR_{maint} (kg/day)	Coefficient for protein maintenance requirements	0.004	0.15
	ER_{maint} (kg/day)	Coefficient for lipid maintenance requirements	1.63	0.15
Immunity	EM_{max} (day^{-1})	Max. combined establishment/mortality	0.82	0.1
	EM_{min} (day^{-1})	Min. combined establishment/mortality	0.08	0.1
	μ_{max} (day^{-1})	Max. mortality	0.12	0.2
	μ_{min} (day^{-1})	Min. mortality	0.01	0.1
	F_{max} (egg/female/day)	Max. fecundity	39	0.3
	F_{min} (egg/female/day)	Min. fecundity	6	0.1
	k_{EM}	Rate change parameter for combined establishment/mortality	-2.7x10^{-8}	0.01
	k_{μ}	Rate change parameter for mortality	4x10^{6}	0.01
	k_F	Rate change parameter for fecundity	-2.9x10^{-7}	0.01

Table 3.1: Calf traits for which phenotypic variation between individuals was assumed to occur within the model, with corresponding parameter values for their mean and coefficient of variation (CV). See text for sources of parameter values.

3.3.3 ***Epidemiological module***

To simulate natural infection of calves in the herd, it was necessary to consider external environmental conditions, including the epidemiology of free-living parasite stages. Many aspects of parasite epidemiology are affected by environmental conditions, in particular temperature and moisture (Stromberg 1997). Temperature was considered to have the most prominent effect as described below, and moisture was assumed non-limiting under UK conditions. The potential effects of other environmental factors, such as moisture or UV light, were not considered (Stromberg 1997).

3.3.3.1 Grass quantity and quality.

The total grazing pasture available to the calf herd was defined in hectares (*H,* ha). The initial quantity of grass per hectare (GPH_0) was defined as 2500 kg DM/ha in accordance with AHDB Grazing Planning (2016a) and an even grass coverage was assumed. As such, the initial quantity of grass available for grazing (G_0, kg DM) was calculated as:

$$G_0 = GPH_0 \cdot H \tag{3.8}$$

Each day (t), the total grass available for grazing (G, kg DM) was updated to take into account the grass consumed by the calf population and new grass growth. Thus, G_t was estimated in accordance with Laurenson et al. (2012b):

$$G_t = G_{t-1} - \sum FI_{t-1} + (GG \cdot H) \tag{3.9}$$

where $\sum FI$ is the total feed intake for all simulated calves, and GG is daily grass growth (kg DM/ha) which was estimated for the relevant grazing period using the average grass growth per day for each month reported by AHDB (2016a). *GG* ranged from 30 to 60 kg DM/ha over the 180 day simulated grazing season.

A reasonably consistent relationship between calendar month and quality of grass has been reported (Trouw Nutrition, 2010; AHDB, 2013). Consequently, the crude protein (*CP*, g/kg DM) and metabolisable energy (*ME*, MJ/kg DM) content of grass were time-dependent according to data obtained from fields grazed by cattle in the UK (Woodward et al. 1938; Dale et al. 2012). As such, over the simulated grazing period of 180 days, *CP* ranged from 165 to 199 g/kg DM, and *ME* ranged from 11.2 to 12.0 MJ/kg DM.

3.3.3.2 Pasture contamination.

A given number of overwintered infective L_3 larvae were assumed to be resident on pasture and comprise the initial L_3 larval contamination (IL_0, L_3/kg DM). As such, the initial total infective L_3 larval population on pasture (LP_0) was calculated as:

$$LP_0 = IL_0 \cdot G_0 \tag{3.10}$$

On subsequent days a small number of additional larvae were assumed to become resident on pasture as a result of the maturation and migration of a low level of overwintering eggs, L_1 and L_2 (Bairden et al. 1995; Urquhart et al. 1996). This was modelled as an exponential decay function (Pandey, 1972; Myers and Taylor 1989), such that the infective L_3 larvae arising daily from an initial underlying contamination of eggs, L_1 and L_2 (IL, L_3/kg DM) was estimated on day t as:

$$IL_t = 0.05\exp(-0.05t) \cdot IL_0 \tag{3.11}$$

For simplicity, the assumption was that there is a constant relationship between the initial L_3 contamination and subsequent development of L_3 from overwinter eggs, L_1 and L_2 larvae. However, this consideration was only made prior to the appearance of infective L_3 larvae arising from eggs deposited by the calf population. The time to earliest appearance of egg-producing adult female worms within the host population, and hence eggs deposited onto pasture, was assumed to be 17 days (Williams et al. 1974). The proportion of eggs that develop into infective L_3 larvae was assumed to be 0.15 (Young and Anderson, 1981). The number of days taken for the eggs to reach the infective L_3 stage, and the mortality rate of infective L_3 larvae, were assumed to be temperature dependent (Pandey 1972; Smith et al. 1986).

To model temperature-dependent effects over the simulated grazing season, the mean of the average monthly temperatures observed by the UK Meteorological Office over a 3-year period (2010-2012) were used. A 4th-order interpolating polynomial was fitted to the average monthly temperatures to produce a six-months temperature curve (Emmanouil et al. 2006), such that the maximum temperature (*Temp*, °C) on day t was given by:

$$Temp_t = 0.000000013t^4 - 0.0000077t^3 + 0.00067t^2 + 0.084t + 6.3 \tag{3.12}$$

As such, over the simulated grazing period of 180 days, *Temp* ranged from 7.8 to 15.4 °C.

An exponential relationship was fitted between paired data describing temperature and development time, i.e. number of days taken to develop from egg to an infective L_3 larva on pasture (Rose, 1961). As a result, the mean development time of eggs deposited on day t, *DT* (days, rounded to the nearest integer), was assumed to be dependent on *Temp*:

$$DT_t = 146\, e^{-0.189 \cdot Temp_t} + 2.92 \qquad (3.13)$$

The stochastic nature of development time was represented as a uniform distribution (mean=DT_t days, range= ±4 days), over whole day increments (Rose, 1961). As such, *DT* ranged from 7 to 40 days over the simulated grazing period. Thus, the number of new infective L_3 larvae (*newIL*) arising from eggs previously deposited by the calf population was calculated from a convolution of egg deposition and egg maturation time distributions:

$$newIL_t = \left(\sum_{i=0}^{i=t} U[(t-i) - DT_i]\; PEI \cdot E_i \right) \qquad (3.14)$$

where *U[~]* is a uniform probability distribution centred at zero with a range of -4 to +4 days, and *t* is the current day, *i* any previous day (from 0 to current day), E_i the total egg output of the calf population on day *i*, DT_i the mean development time for eggs deposited on day *i*, and *PEI* the proportion of eggs that develop into infective L_3 larvae. *U* has a value of ~11% probability of maturing on day *DT* after deposition on pasture, and on the 4 days previous and following day *DT*.

The relationship between *Temp* and the larval mortality rate (L_3M, proportion of infective L_3 larvae dead/day) was defined using data from Young and Anderson (1981) for the temperature ranges observed in the UK. A linear relationship was assumed (Grenfell et al. 1986), such that L_3M on day *t* was given as:

$$L_3M = 0.0014 \cdot Temp_t + 0.018 \qquad (3.15)$$

Over the simulated grazing period, L_3M ranged from 0.029 to 0.040 (Young and Anderson, 1981).

Consequently, the total infective L_3 larval population on pasture (*LP*) at the start of day *t* was given as:

$$LP_t = (LP_{t-1} - \sum LI_{t-1}) \cdot (1 - L_3M_t) + (IL_t \cdot H) \quad \text{when } newIL_t = 0 \quad (3.16)$$

$$LP_t = (LP_{t-1} - \sum LI_{t-1}) \cdot (1 - L_3M_t) + newIL_t \quad \text{when } newIL_t > 0 \quad (3.17)$$

where $\sum LI$ is the total larval intake of the calf population.

3.3.3.3 Larval intake.

Calves were assumed to graze randomly across pasture. However, the spatial distribution of the larvae across the pasture was assumed to be aggregated (Boag et al. 1989; Grenfell et al. 1995; Verschave et al. 2015). A negative binomial probability distribution was used with the mean being mean larval contamination of pasture (L_3/kg DM) and the exponent describing the degree of aggregation *k* = 1.41 (Verschave et al. 2015). Hence, the larval intake (*LI*, infective L_3 larvae) of an individual calf was determined by its feed intake (*FI*, kg DM) and by sampling the pasture according to the negative binomial distribution, such that:

$$LI_t = FI_t \cdot NB\left(\frac{LP_t}{G_t}, k\right) \quad (3.18)$$

where LP_t/G_t (L_3 larvae/day) is the mean number of L_3 larvae per ha grazed on day t.

3.3.4 ***Simulations***

The modelled herd comprised of 500 calves generated using a stochastic Monte-Carlo simulation, created in MATLAB (2015*b*). For the model inputs defined in table 3.1, this population size resulted in a maximum relative standard error of 1.34% (estimated for F_{max}), which was considered sufficiently large given that further increases in population size showed no further reduction in standard error.

3.3.4.1 Model behaviour.

Model behaviour was evaluated by simulating a selection of IL_0 levels and stocking rates. To investigate model behaviour under differing IL_0 levels (0, 100, 200 or 500 *O. ostertagi* L_3/kg DM), the grazing area was set to 100ha to represent a conventional stocking rate of 5 calves/ha (AHDB, 2016a). To investigate model behaviour under differing stocking rates, IL_0 was set to 200 *O. ostertagi* L_3/kg DM, and the grazing area adjusted for low (3 calves/ha), conventional (5 calves/ha) and high (7 calves/ha) stocking rates, as defined by AHDB (2016a). In all cases, calves were assumed to be parasitologically naïve when turned out in early April for 180 days. Model outputs were calculated on a daily basis and presented as the population mean for: (1) parasite worm burden (WB, worms); (2) faecal egg count (FEC, eggs/g faeces); (3) feed intake (kg DM); (4) relative reduction in calf bodyweight gain (kg) (comparative to a non-parasitised healthy calf); and (5) pasture larval contamination (PC, L_3 larvae/kg DM).

3.3.4.2 Model validation (controls strategies).

To validate model outputs, predictions were compared to observations made in experimental studies investigating the impact of a variety of nematode control strategies (stocking rates, 'dose and move' and strategic anthelmintic treatment). Where possible, experimental observations were compared to the population mean for the following model outputs: (1) FEC (eggs/g faeces); and (2) PC (L_3/kg DM). Where observed percentages of *O. ostertagi* present in relation to other parasites were recorded, direct quantitative comparisons were made. In cases where parasite species differentiation was not made the total numbers of strongyle eggs or pasture larval counts were used to provide a qualitative validation.

Experimental studies from available literature were selected for comparison on criteria stated in table 3.2. A thorough literature review identified the following 8 studies that met the specified criteria and were therefore used to validate model predictions for: (1) stocking rate (Nansen et al. 1988); (2) strategic dosing (Jacobs et al. 1989; Fisher and Jacobs, 1995; Taylor et al. 1995; Vercruysse et al. 1995; Satrija et al. 1996; Sarkũnas et al. 1999); (3) 'dose and move' (Michel and Lancaster 1970). Initial model input values were taken from each study and included (1) IL_0 (L_3/ kg DM); (2) calf stocking rate; (3) day of turnout and; (4) experimental treatment strategy. For cases where calves received unplanned supplementary feed or emergency anthelmintic treatments part way during the experimental period, measurements taken beyond these points were not included. The actions taken to ensure that the simulations were comparable to experimental observations are below.

	Criteria
1	The only available feed was grass
2	The experiment was conducted on calves grazing in spring months and maintained in a temperate environment
3	All calves were infected during the growing phase
4	No calves had exposure to parasites prior to the experiment (i.e. first grazing season calves)
5	Infections were either single *O. ostertagi* or mixed with *Cooperia* spp. (due to the lack of single species *O. ostertagi* infections in literature it was necessary to consider mixed infections; the consequences of *Cooperia* infections were accounted for as described in section 3.3.4.2.3)
6	Any dosing with ivermectin was administered at the recommended dose of 200µg/kg by subcutaneous injection
7	Any dosing with thiabendazole was administered orally at the recommended dose of 200mg/kg

Table 3.2: A list of the required criteria that were achieved by experimental studies in order for them to be appropriate for use in validating the model.

3.3.4.2.1 Growth rates.

The model required P_M and B* as inputs. All studies meeting the criteria described above were performed a number of years ago and hence it was necessary to account for changes that may have occurred in these traits as a result of selective breeding. This was done according to the method detailed in Chapter 2. It was assumed that calf body composition has remained the same with no direct selection for lean cattle, but rather for heavier mature weights (Emmans and Kyriazakis, 2001; Hays and Preston, 2012).

Following this, the mean of parameter *B** (table 3.1) was adjusted such that model outputs reflected the growth rates observed for un-infected calves in each study. In the absence of un-infected experimental control groups, calves under a strategic ivermectin treatment were assumed to reflect the growth rate of un-infected calves. For example, in Michel and Lancaster (1970) calves receiving repeated anthelmintic treatments were assumed to reflect growth rates of un-infected calves.

3.3.4.2.2 Epidemiological components.

To account for the variations in turnout date, the date of turnout was used as an input for each experiment. This allowed for seasonal factors such as grass growth, grass quality and temperature-dependent effects to be adjusted accordingly.

3.3.4.2.3 Mixed Cooperia infections

It was necessary to consider mixed infections of *O. ostertagi* and *Cooperia* due to limitations in the published literature for model validation. Such infections have been observed to cause a greater depression in growth than mono-specific infections (Kloosterman et al. 1984; Satrija and Nansen, 1993). It is widely recognised that although in a single *O. ostertagi* infection any protein loss can be reabsorbed in the small intestine, in a mixed infection the presence of *Cooperia* in the small intestine hinders the reabsorption process (Fox, 1993; Holmes, 1993). Thus, parameters describing the protein loss associated with both larval and worm mass were increased by 10% (Kloosterman et al.1984).

3.3.4.2.4 Control via stocking rate.

The constant population size of 500 calves was used for all simulations. As such, the total grazing area (*H*, ha) was adjusted to match the differing stocking rates of each experimental study. In the experimental study of Nansen et al. (1988), which investigated two stocking rates, a mid-season rotation was incorporated whereby half of the calves were moved to clean pastures, thus halving the stocking rate on current pasture. To account for this, *H* was

doubled at the appropriate time-point. Further, to simulate calves that moved to a clean pasture the same parameters were defined; however, at the time of the mid-season rotation when *H* was increased, the PC was also reset to 10 L_3/kg DM as representative of a 'clean' pasture.

3.3.4.2.5 Control via 'dose and move'.

During the period for which Michel and Lancaster (1970) conducted their study, ivermectin was not available and thiabendazole was the drug of choice; the efficacy of this drug is likely to have been high at the time of this experiment and hence an efficacy of 0.99 and no persistent activity (Prichard et al. 1981) was assumed. Following anthelmintic drenching, calves were immediately moved to a 'cleaner' pasture by resetting the grass available for grazing (G_t) to 2500 kg DM/ha (ABDH, 2016a) and PC to 50 L_3/kg DM (with no resident egg, L_1 or L_2 population).

3.3.4.2.6 Control via strategic anthelmintic treatment.

Although there are no universal guidelines for strategic anthelmintic dosing, the recommended timings for administration of ivermectin are 3, 8 and 13 weeks post-turnout in order to minimise worm egg output until mid-July, when most over-wintered larvae have died (Cockroft, 2015). Ivermectin, the most widely used anthelmintic, was assumed to have an efficacy of 0.99 against *O. ostertagi* with persistent activity for three weeks (NOAH, accessed 2015). Following this period of persistent efficacy against *O. ostertagi*, ivermectin efficacy was assumed to decrease by 0.15 per day. This was parameterised such that model predictions for FEC and PC exhibited similar patterns to those observed in ivermectin treated calves (Vercruysse et al. 1988). Ivermectin was assumed to be equally effective against all worm and larval stages residing within the host.

3.4 Results

3.4.1 *Model behaviour*

3.4.1.1 Frequency distribution of output traits

Output performance traits were normally distributed at all times. For example, the means (and SD) for bodyweight were 363 (32.7), 429 (41.5), 487 (51.5) and 534 (60.4) kg at 40, 80, 120 and 160 days post turnout, respectively, for calves grazing clean pasture at a conventional stocking density (5 calves/ha). In contrast, although parasitological inputs were normally or log-normally distributed, the frequency distribution of predicted WB and FEC became increasingly right-skewed over time, as demonstrated for FEC in figure 3.2.

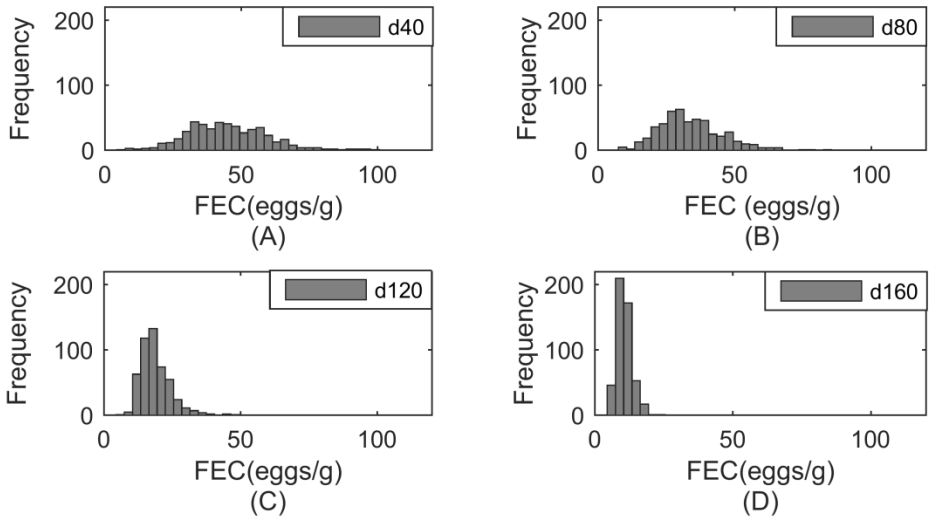

Figure 3.2: Frequency distribution of faecal egg counts (FEC, eggs/g faeces) of 500 calves grazed at a conventional stocking density of 5 calves/ha on a pasture initially contaminated with 200 *Ostertagia ostertagi* L_3/kg DM grass, on day: A) 40, B) 80, C) 120 and D) 160.

3.4.1.2 Increasing initial contamination (IL_0)

3.4.1.2.1 Parasitological traits.

The population mean of WB and FEC for IL_0 levels of 100, 200 and 500 L_3/kg DM are given in figure 3.3(A,B). Whilst increasing IL_0 caused minor changes in the maximum predicted WB, the timing of peak WB was predicted to decrease with increasing IL_0. The maximum mean WB (and day of peak) for IL_0 levels of 100, 200 and 500 L_3/kg DM were 37,159 (114), 37,772(109) and 32,831 (103), respectively. For the highest IL_0 of 500 L_3/kg DM an additional small peak in WB was observed during the early stages of infection at approximately day 45.

Additionally all IL_0 levels showed a marked increase in the gradient of WB around day 80. Similar to WB, the day of peak FEC (eggs/g faeces) decreased with increasing IL_0, and caused minor changes in the maximum predicted FEC. The maximum FEC (and day of peak) for IL_0 levels of 100, 200 and 500 L_3/kg DM were 47 (95), 48 (43) and 67 (38), respectively. The intermediate IL_0 of 200 L3 larvae/kg DM was predicted to show a similar maximum FEC to the lowest IL_0 of 100 L3 larvae/kg DM; however, two peaks of approximately equal magnitude were observed. Ultimately, FEC reached similar final levels irrespective of IL_0.

3.4.1.2.2 Performance traits.

The population mean for feed intake and relative reductions in bodyweight gain are given in figure 3.3(C,D). Increasing IL_0 resulted in an increased maximum reduction and earlier achievement of maximum reduction in feed intake, and a faster rate of recovery towards the feed intake of an uninfected calf. Across the duration of the grazing season the average feed intake for control calves on clean pasture was 7.64 kg DM/day: the average relative reductions were 5% for all IL_0 levels. Consistent with the predicted patterns for feed intake, reductions in bodyweight gain were greater for higher IL_0 in the early stages of infection; however, in the latter stages the magnitude of differences between IL_0 became negligible. The average relative reductions in average daily bodyweight gain across the season were 0.12, 0.12 and 0.10 kg/day for IL_0 levels of 100, 200 and 500 L_3/kg DM, respectively.

3.4.1.2.3 Pasture contamination.

Predictions for PC (L_3/kg DM) are given in figure 3.3(E). Similar patterns were observed for all IL_0 with PC decreasing up until day 52 when PC began to increase towards a peak. Increasing IL_0 resulted in an earlier peak, however the maximum predicted PC did not relate directly to IL_0. The intermediate IL_0 of 200 L_3/kg DM showed the lowest peak PC. The maximum predicted PC (and day of maximum) for IL_0 levels of 100, 200 and 500 L_3/kg DM were 903 (116), 825 (82) and 901 (77) L_3/kg DM, respectively. Upon reaching the peak, PC then declined to similar levels, irrespective of IL_0.

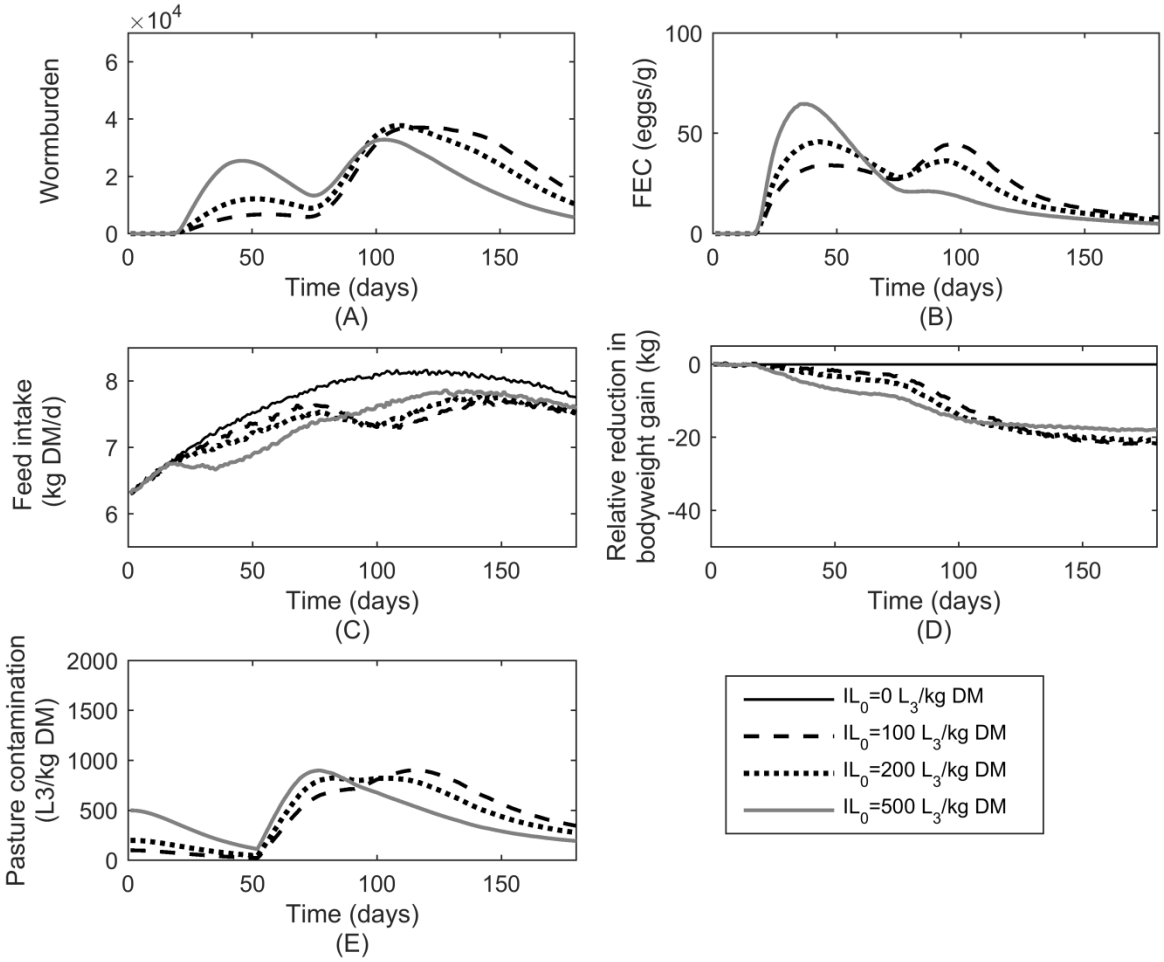

Figure 3.3: The mean parasitological and performance traits for 500 calves, at a conventional stocking rate of 5 calves/ha, grazing pasture initially contaminated (IL_0) with either 0, 100, 200 or 500 *Ostertagia ostertagi* L_3/kg DM grass. The parasitological traits provided are: A) mean worm burden, and B) mean faecal egg count (eggs/g faeces) for the population. The performance traits provided are: C) mean feed intake (kg DM), and D) mean relative bodyweight gain (kg) in relation to the un-infected calf population. The epidemiological trait provided is: E) pasture larval contamination (L_3/kg DM grass).

3.4.1.3 Stocking rate

3.4.1.3.1 Parasitological traits.

The population mean for WB and FEC for three stocking rates are given in figure 3.4(A,B). Calf stocking rates had no effect on WB until day 78, at which point WB increased with increasing stocking rates as a reflection of patterns in PC. Higher stocking rates resulted in increased maximum WB. The maximum WB (and day of peak) for low, conventional and high stocking rates were 20,749 (110), 37,772 (109) and 61,508 (109) respectively. Maximum FEC was similar for all stocking rates as was the day of FEC peak. The maximum FEC (and day of maximum) for low, conventional and high stocking rates were 48 (44), 48 (43) and 48 (38), respectively. A second peak in FEC was observed for conventional and high stocking rates; the second peak (and day of peak) for conventional and high stocking rates were at 38 (94) and 44 (90) respectively.

3.4.1.3.2 Performance traits.

The population mean for feed intake and relative reduction in bodyweight gain are given in figure 3.4(C,D). As with the parasitological outputs, there was no divergence between stocking rates for either of the performance traits until day 78. The maximum reduction in feed intake increased with increasing stocking rates, and feed intake remained compromised in relation to un-infected calves for all stocking rates throughout the simulated grazing period. Across the duration of the grazing season, the average feed intake for control calves on clean pasture was 7.64 kg DM/day, and the average comparative feed intake were reduced by 4%, 5% and 5% for low, conventional and high stocking rates, respectively. The relative reduction in bodyweight gain increased for increasing stocking rates. The average daily bodyweight gain across the season were reduced in comparison to uninfected calves by 0.07, 0.12 and 0.24 kg/day for low, conventional and high stocking rates, respectively.

3.4.1.3.3 Pasture contamination.

Predictions for PC (L_3/kg DM) are given in figure 3.4(E). Initially, similar patterns were observed for all stocking rates with PC decreasing until day 52, at which point L_3 from eggs deposited on pasture eggs first appear and PC increased to a peak and then declined. Increasing stocking rates resulted in an increased maximum PC. The maximum predicted PC (and day of maximum) for low, conventional and high stocking rates were 409 (82), 825 (82) and 1722 (108) L_3/kg DM, respectively. It was therefore observed that IL_0 did not affect performance or infestation significantly.

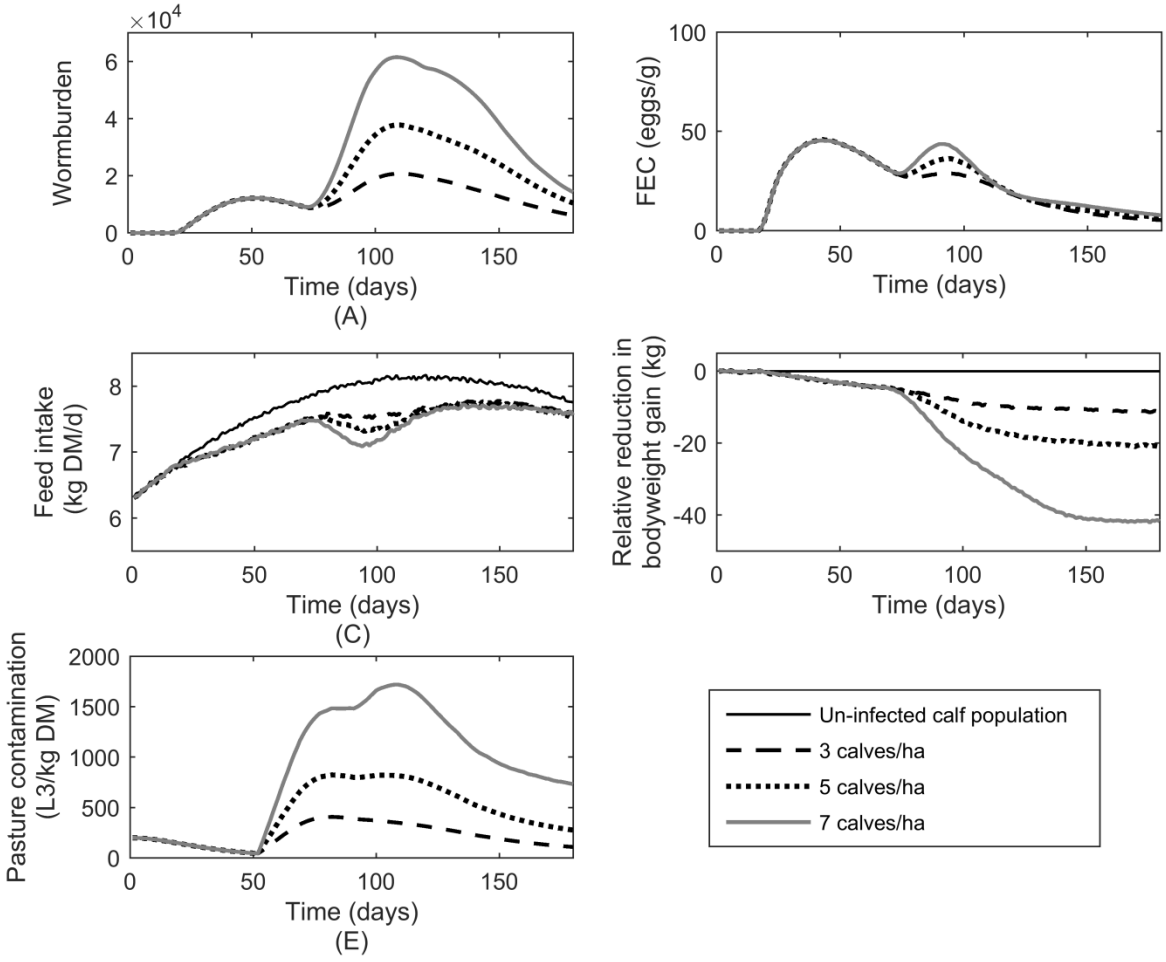

Figure 3.4: The mean parasitological and performance traits for 500 calves grazing pasture initially contaminated with 200 *Ostertagia ostertagi* L_3/kg DM grass, and kept at stocking rates of either 3, 5 or 7 calves/ha. The parasitological traits provided are: (A) mean worm burden, and (B) mean faecal egg count (eggs/g faeces) for the population. The performance traits provided are: (C) mean feed intake (kg DM), and (D) mean relative body weight gain (kg) in relation to the un-infected calf population. The epidemiological trait provided is: (E) pasture larval contamination (L_3/kg DM grass). The group of untreated calves showed no differences in feed intake and growth due to the assumption of optimal grass availability at the start of the grazing season.

3.4.2 ***Validation***

The following sections detail model outputs for the validation simulations.

3.4.2.1 Stocking rates.

Graphical comparisons of FEC between the model and the experiments conducted by Nansen et al. (1988) are provided in figure 3.5 (A-D). In general, model predictions showed similar patterns to the observed data. FEC increased steadily to a peak and then began to decline, with the exception of observations made on calves kept at high stocking rates on the same pasture (figure 3.5 C), for which a high FEC was observed at the final measurement. The majority of data were close to the predicted population mean, and all observations except one were between the estimated lower and upper extreme values of the modelled population.

A graphical comparison for observed and predicted levels of PC is provided in figure 3.5 (E-H). For calves remaining on the same pasture throughout the study (figure 3.5 E,G), the model predicted PC to increase to a peak and then decline. A slight dip was predicted on day 60 when the stocking rate was halved. For the calves moved to clean pasture on day 60 post-turnout (figure 3.5 F,H), the model predicted an increase in PC up until day 60 when PC was reset to low levels; after which PC increased to a peak then slowly declined. For both comparisons of PC, a more pronounced effect was seen at the higher stocking rate. Although there was some lack of consistency in the patterns of observed values the model predictions appear to show a reasonable likeness to individual observed points upon graphical comparison, with the exception of the final measurements taken for calves remaining on the same pasture for both stocking rates; the latter appears to be an outlier among the other observations.

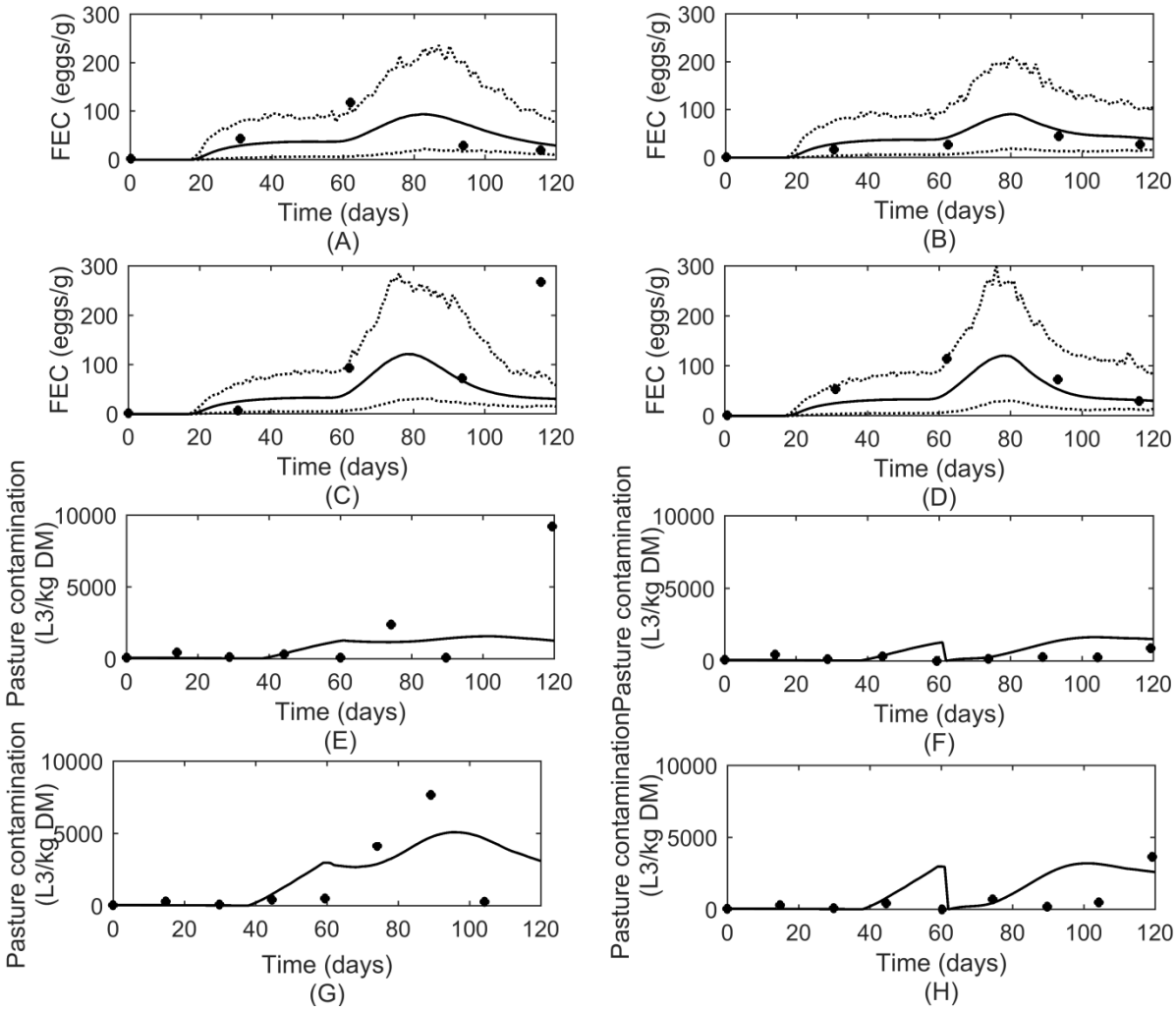

Figure 3.5: Comparison of experimental observations (●) of Nansen et al. (1988) to simulated mean prediction (-) for faecal egg count (*FEC*, eggs/g faeces) (A-D) and pasture contamination (L_3/kg DM grass) (E-H), along with the lower and upper extreme values (¨) for individuals within the simulated population. Calves were kept at a moderate stocking rate (11.7 calves/ha) for the first half of the grazing season , and on day 60, split into two equal groups (5.8 calves/ha) and either: (A) remained on the same pasture, or (B) moved to a cleaner pasture (10 L_3/kg DM grass). This was repeated for a high stocking rate (17.5 calves/ha), and on day 60, groups of calves (8.8 calves/ha) either: (C) remained on the same pasture, or (D) moved to a cleaner pasture (10 L_3/kg DM grass).

3.4.2.2 'Dose and move'.

A graphical comparison of PC was made for the three 'dose and move' experiments conducted in successive years (Michel and Lancaster, 1970). For calves remaining on the same pasture (figure 3.6 A,C,E) similar patterns were seen for observed and predicted outputs with an increase in PC up to a peak followed by a decline. The calves moved mid-July (figure 3.6 B,D,F) showed a reduced Contamination from the move date with only a small increase in PC on the new pasture.

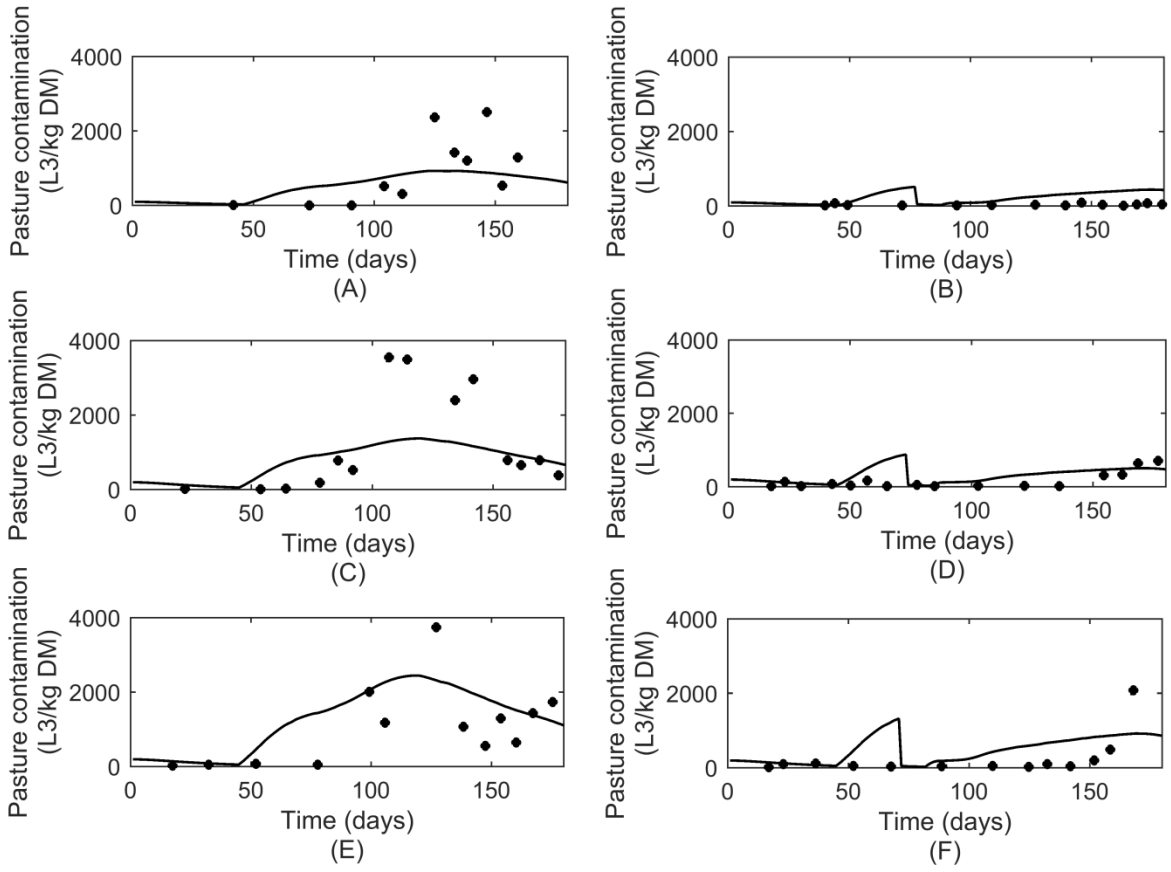

Figure 3.6: Comparison of experimental observations (●) of Michel and Lancaster (1970) to simulated predictions (-) for pasture contamination (L_3/kg DM grass). For untreated control calves grazed on pasture in: (A) 1965, (C) 1966, and (E) 1967. For calves given thiabendazole on day 70 and moved to 'clean' pasture (50 L_3/kg DM grass) in: (B) 1965, (D) 1966, and (F) 1967.

3.4.2.2 Strategic dosing.

Graphical comparisons of FEC for each of the six previously identified strategic anthelmintic dosing studies are presented in figure 3.7(A-F). Predicted FEC in the untreated groups were similar to observed FEC. Observed FEC increased as time progressed, and in studies conducted for a sufficient time period (>150 d) FEC reached a peak and began to decline (Jacobs et al. 1989; Taylor et al. 1995; Satrija et al. 1996) although rebounded later. Model predictions were consistently similar to observations made for the ivermectin treated groups (figure 3.7(G-L)) which showed low FEC across time, with the exception of data from Fisher and Jacobs (1995). For all comparisons, the majority of data were close to the predicted population mean for FEC, falling between the estimated lower and upper extreme values for individuals of the modelled population. Additional graphical comparisons were made for PC for five of the studies; a graphical comparison for untreated calves is given in figure 3.8(A-E), both observed and predicted patterns showed initially an increase in PC as time progressed. Congruent with FEC, PC also reached a peak and began to decline (Taylor et al. 1995). However, this was not supported by Satrija et al. (1996), where predictions diverged from observed PC from day 100. For the graphical comparisons of ivermectin treated groups (figure 3.8(F-J)), all observations and predictions showed a low level of PC, with the exception of Satrija et al. (1996) where a notable increase in PC was observed at the latter stages of the experiment.

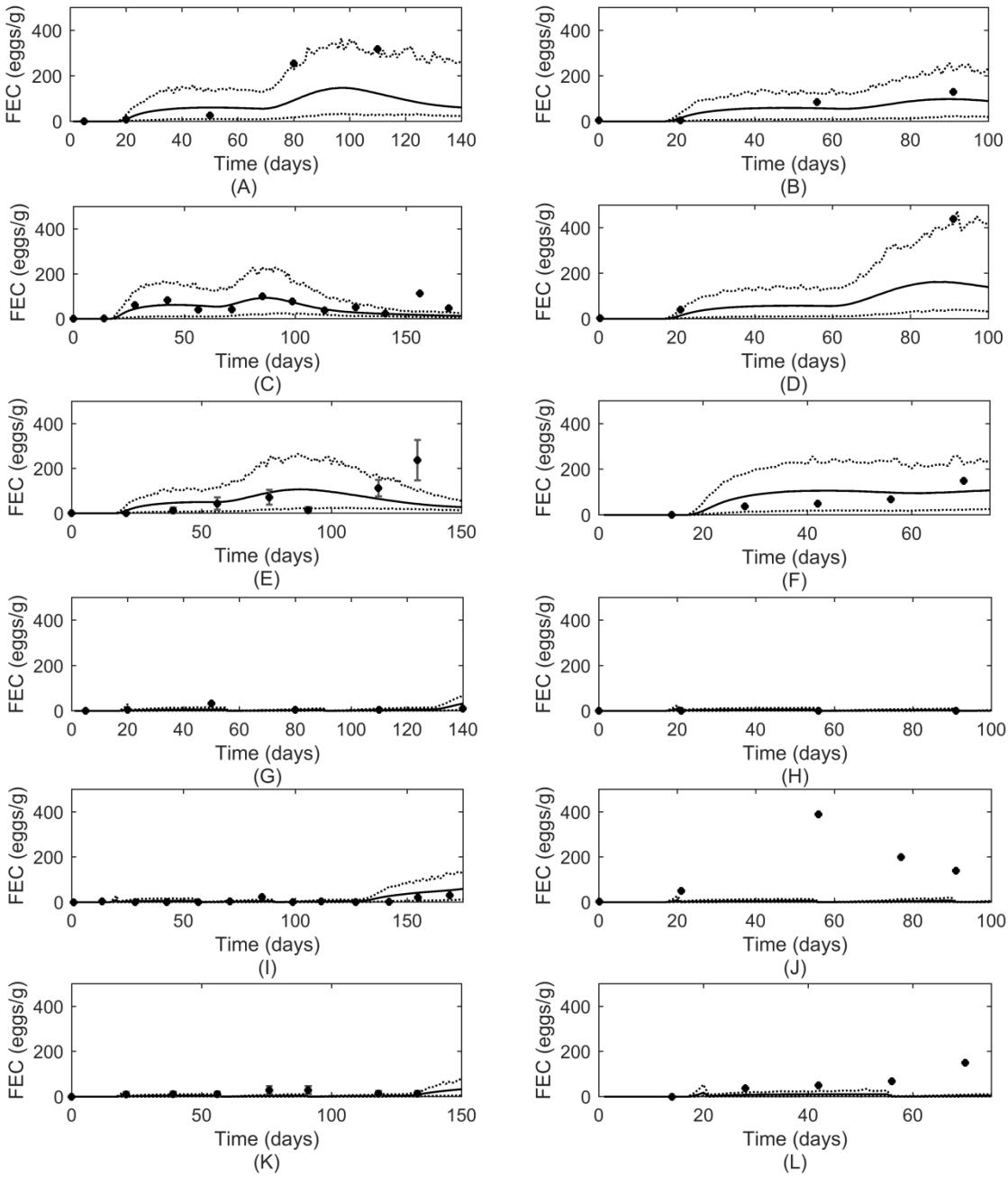

Figure 3.7: Comparison of experimental observations (●) to simulated mean prediction (-) for faecal egg count (*FEC*, eggs/g faeces), along with the predicted lower and upper extreme values (¨) for individuals within the simulated population. Predictions were made for the group of calves receiving no anthelmintic treatment for experimental data from: (A) Taylor et al. 1995; (B) Vercruysse et al. 1995; (C) Satrija et al. 1996; (D) Fisher and Jacobs, 1995; (E) Jacobs et al. 1989; and (F) Sarkũnas et al. 1999. Comparisons were also made for calves receiving ivermectin on weeks 3, 8 and 13 post-turnout (G-L).

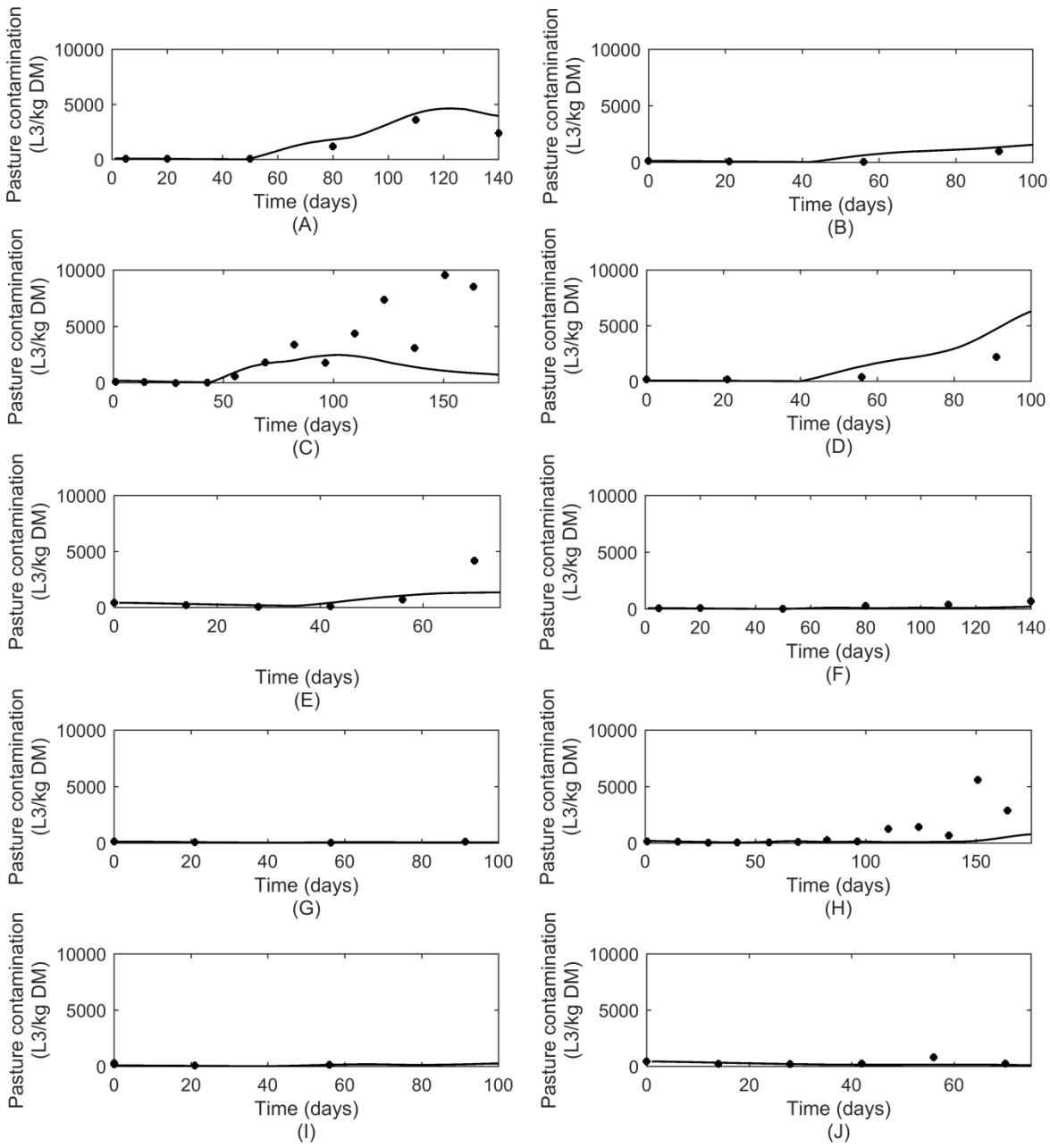

Figure 3.8: Comparison of experimental observations (●) to simulated mean prediction (-) for pasture contamination (L_3/kg DM grass) in the group of calves receiving no anthelmintic treatment. The experimental data are from: (A) Taylor et al. 1995; (B) Vercruysse et al. 1995; (C) Satrija et al. 1996; (D) Fisher and Jacobs, 1995; and (E) Sarkũnas et al. 1999. Comparisons were also made for calves receiving ivermectin on weeks 3, 8 and 13 post-turnout (F-J).

3.5 Discussion

A stochastic model was developed to account for the impacts of variation between calves in their ability to deal with *O. ostertagi*, under management conditions that have the potential to affect parasite infra- and supra-populations. Previous comparable studies where calves received the same, or similar, levels of parasite challenge indicated disparities in the immune response exhibited by individuals (Michel, 1969b; Michel and Sinclair, 1969). A recent meta-analysis on *O. ostertagi* infections of calves (Verschave et al. 2014) found large variations between studies when predicting immune responses. Thus introducing such variation in simulation models is necessary, as individuals affect parasite epidemiology and can influence the effectiveness of controls. This cannot be captured by models that assume that all individuals within a group are alike and deal with an 'average' animal, as is the case for deterministic models (Smith and Guerrero, 1993; Grenfell et al. 1995; Fox et al. 2013).

Stochastic models enable to address uncertainty and variability in the various factors believed to be important in the behaviour of the system, which in this case comprises of the cattle herd, the parasites and their environment. The major issues explored here was variation within the herd and how the distribution of parameter values could affect herd performance and parasitological outputs. The mean characteristics of the system reflect complex interactions of the model parameters, which were defined as probabilistic distributions rather than fixed values. Beyond the mean characteristics, the model also predicted the expected range of outcomes for FEC, such as those shown in figures 3.5 and 3.7. For the purposes of comparability, the simulations presented here were performed on a fixed number of calves (n=500) while stocking density values were set by specifying different values for the grazing area; hence it was possible to compare directly the predicted averages and extremes. It would be possible to model smaller, more typical, herd sizes, but in this case would be necessary to perform multiple simulations to obtain a proper statistical description of herd characteristics. The emphasis in this work has been on describing variation within the calf population, but the approach can be extended to capture uncertainties in other factors. For example, the historical average temperature profile used here could be replaced by a stochastic representation; multiple simulations over time would then give insights into the range of possible outcomes.

Converting our deterministic model into a stochastic one presented us with two major challenges. The first one was to introduce variation between the individuals of a herd. Values

that enable parameterisation of the variation between individual calves in growth characteristics exist or at least can be deduced **(**Ferreira et al. 1999; Laurenson et al. 2011; Mc Hugh et al. 2011). This, however, is not the case for traits that are associated with the ability of hosts to deal with the parasite. For this reason, we resorted to values that have been assumed for sheep (Vagenas et al. 2007c; Laurenson et al. 2011). As there is an increased requirement for characterising animals for a number of phenotypic and genetic traits (Goddard and Hayes, 2009), the hope is that animal breeders will provide such information for health-related traits, in a manner already done for other animals, such as for resistance to mastitis in dairy cattle (Gernand et al. 2012).

The second challenge was to introduce an epidemiological component to the model. Previous attempts to quantify free-living stages of *O. ostertagi* have become increasingly complex (Gettinby and Paton, 1981; Grenfell et al. 1987b; Smith et al. 1987b; Chaparro et al. 2013; Rose et al. 2015b). As our focus was on host-parasite interactions we kept this aspect relatively simple. Moisture was assumed to be a non-limiting factor, although in reality rainfall and moisture levels may have a notable effect on aspects of parasite epidemiology (Young and Anderson, 1981). However, the net impact on PC levels can be considered to be small due to counteracting mechanisms. For example, heavy rainfall increases larval mortality and accelerates the passage of larvae from pasture downward into the soil reservoir (Al Saqur et al. 1982; Gruner et al. 1982; Grenfell et al. 1986), whilst increased moisture helps the transmission of larvae from faecal pats to herbage by translocation and by splash dispersal (Grønvold and Høgh-Schmidt, 1989; Stromberg, 1997). Only temperature was accounted for in the model, as being the most influential climatological feature on PC levels (Stromberg, 1997). Development time (*DT*) for eggs to reach infective L_3 larvae was dependent on the average daily temperature on the day of excretion alone. A cumulative measure of temperature was not used due to the non-linear relationship between temperature and development, and daily fluctuations in temperature. The sensitivity of the average *DT* to temperature was tested by adding random variation (CV=0.5) in temperature; however, there was little to no impact on the outputs generated suggesting this to be a fair assumption.

Additionally, demographic stochasticity was incorporated into the model in the form of variation in feed intake and random aggregated distribution of larvae in the pasture. Random variation in calf feed intake impacts on calf growth and the larval intake of an

individual, whilst aggregated variation in pasture larvae will influence the larval intake of an individual. Seasonal effects are perceived to impact upon the levels of larval aggregation across pasture; this is an almost ubiquitous feature of parasitic infections due to weather-dependent dispersal patterns of L_3 larvae from faecal pats. It has previously been observed that significant aggregation was only apparent during particular months, with the level of aggregation correlating to larval numbers (Flota-Bañuelos et al. 2013). High PC related to low aggregation and low PC to high aggregation (Flota-Bañuelos et al. 2013; Verschave et al. 2015). Although mitigating factors, such as passive dispersal or faecal avoidance behaviours (Hutchings et al. 2001; 2007) are recognised, an aggregated pasture is still expected (Grenfell et al. 1995) and accounted for. The negative binomial is known to provide a good empirical relationship for this over-dispersion (Barger, 1987; Boag et al. 1989; Fox et al. 2013); however to avoid model complexity the level of aggregation (*k*) was assumed the same for all PC levels.

Contrary to horizontal aggregation, distribution of larvae along the sward was assumed to be evenly distributed. Due to factors such as distance of larvae from the faeces, seasonal variations and vertical migration of larvae, modelling the vertical distribution would be incredibly complex (Pandey, 1974). Often greater proportions of larvae are found lower on herbage; this may have implications for calves kept at high stocking rates where calves graze closer to the base of thee sward. An exaggerated increase in larval uptake can be observed relative to lower stocking rates (Gruner and Sauve, 1982), inducing a more rapid immune acquisition.

An investigation of model behaviour highlighted the importance of interactions between immune acquisition and epidemiology. Parasitological burdens of those individuals that exhibited a slow immune acquisition began to recover earlier than might be expected due to the effect of immunocompetent calves within the herd, which produced fewer eggs, acting to reduce PC levels. Increasing levels of IL_0 resulted in earlier peaks in PC and parasitological outputs (WB and FEC) arising from higher parasitic exposure and hence a more rapid immune acquisition. Differences between peak values were marginal due to assumed density-dependent effects on parasite fecundity (Michel et al., 1978; Smith et al., 1987a) and the mid-summer rise in PC. The faster immune acquisition by calves exposed to high IL_0 enabled them to counteract the mid-summer rise in L_3 in comparison to a lower IL_0. This is supported by the hypothesis that turnout date, ultimately defining the degree of immune

acquisition prior to the mid-summer rise in PC, is perhaps more important than IL_0 (Eysker, 1986; Höglund et al. 2013b; Taylor et al. 2015). The final PC and net impact of parasitism on performance was similar for all IL_0 levels; this is in line with a meta-analysis which suggested the relationship between weight gain and IL_0 was insignificant (Shaw et al. 1998b). However, this is not to say IL_0 levels are not important to consider. When accompanied by different control strategies the IL_0 will likely have an impact on parasitological and performance outcomes.

Changes in stocking rate had comparatively greater parasitological and performance effects than changes in IL_0. The effect is generally inconsequential early in the season due to high grass growth and low PC; however as the season progresses grass growth subsides and a mid-summer rise in PC occurs (Henriksen et al. 1976; Nansen et al. 1988). At high stocking rates the intensity of hosts results in lower grass availability and increased total egg excretion, causing a more dramatic rise in PC. Consequently, the peak parasitological outputs increased with increased stocking rate, as observed experimentally (Hansen et al. 1989; Thamsborg et al. 1998). There was a significant difference predicted in the final net performance of calves kept at each stocking rate. Since it was assumed that pasture availability was non-limiting, this was purely a result of infection. This was in line with experimental work showing significant reductions in mean bodyweight gains for conventional and high stocking rates comparative to a low stocking rate (Hansen et al. 1989; Thamsborg et al. 1998). Although experimentally it is difficult to ascertain whether these losses resulted from parasitism or a lack of grass availability, Nansen et al. (1988) concluded that parasitism was the major cause of poor performance at high stocking rates. The model predicted a reduction in bodyweight gains of between 5 and 16%; interestingly meta-analyses conducted on a variety of breeds have shown average reduction in bodyweight gain of 5.4% (Shaw et al. 1997) and 22.7% (Shaw et al. 1998a) for sub-clinical infections. Although breed may affect observed reductions, it should also be noted these may be slightly larger as a result of concurrent *Cooperia* infections; this is discussed later.

To validate the model, the most common control strategies aiming to reduce the parasitic challenge and burden were identified; these included reduced stocking rate and the Weybridge 'dose and move' technique (Michel and Lancaster, 1970). 'Dose and move' incorporates a planned move coinciding with an anticipated peak in PC, generally mid-July for most of the UK (Smith, 2014). It has previously proved to be a successful control strategy

(Michel and Lancaster, 1970; Henriksen et al. 1976; Nansen et al. 1989; Eysker et al. 1998). However, lack of pasture availability has made it increasingly difficult to implement low stocking rates and 'dose and move' strategies (Herd, 1988; Shaw et al. 1997). The 'dose and move' strategy is also believed to accelerate the development of anthelmintic resistance by removing *refugia* on pasture (van Wyk, 2001). As a result, strategic anthelmintic dosing at specific time points has become critical to maintaining calf health. The objective is to prevent the build-up of PC by limiting faecal egg output during the early part of the grazing season (Vercruysse and Claerebout, 1997). This is achieved by strategic treatment with anthelmintics, which has been observed to be effective against parasitic gastroenteritis for a full season, under conditions where the parasitic challenge is large enough to induce severe parasitic gastroenteritis in controls (Hollanders et al. 1992; Vercruysse et al. 1995).

Previous quantitative evaluation of the deterministic model on which the current stochastic one was based, revealed the former model as reasonably proficient at estimating mean parasitological traits. This places a degree of confidence on the current model, provided that its sources of stochastic variation have been estimated accurately. Based on comparing observed and predicted FEC for the current, stochastic model in order to estimate parameter values for calf variation and parasite epidemiology, the model appeared to be proficient at estimating observed outputs under the specified scenarios. In cases where discrepancies between predicted and observed FEC were observed, contributory factors were identified. Some studies did not distinguish between parasite genera, stating only that *O. ostertagi* were the most prevalent species, whilst in others calves were treated with anthelmintics on clinical grounds following the final measurements used for validation suggesting disease may have been border-line clinical at the time of measurements.

Additional comparisons were made between observed and predicted values for average PC; in most cases the predictions provided a good fit, however a few discrepancies were apparent. As previously mentioned, the aggregated nature of larvae on pasture is likely to influence the sampling of PC; if sufficient repeated measures are not taken then an under or over-estimation of the PC level may occur (Verschave et al. 2015). Upon sampling PC some experimenters have opted to consciously avoid faecal pats, where the highest concentrations of larvae exist: this may have resulted in an under estimation of observed PC (Henriksen et al. 1976; Nansen et al. 1988). Poor grass growth causes a higher concentration of larvae on pasture (Vercruysse et al. 1995) and, as for FEC, the lack of distinction between

parasite genera may also result in discrepancies between observed and predicted PC. A clear example comes from Satrija et al. (1996) whereby PC switches from predominantly *O. ostertagi* to predominantly *Cooperia* in August; from this point onwards the model does not predict PC well.

Should these factors not account for the differences between observed and predicted PC it may be a result of a model over-simplification. These may result in inaccurate predictions made on PC which in turn would affect the larval intake due to the self-proliferating nature of the relationships defined in the model. If this is the case, explanations for why FEC still provide a good fit must be considered, implying that the within-host relationships may over or under compensate for these differences.

Monospecific and concurrent artificial infections of *O. ostertagi* and *Cooperia* suggested an absence of inter-species interactions (Kloosterman et al. 1984; Hilderson et al. 1995; Satrija and Nansen, 1993). Concurrent infections did, however, show greater than additive FEC in comparison to the two monospecific infections (Kloosterman et al. 1984; Hilderson et al. 1995; Satrija and Nansen, 1993), thought to be a consequence of enhanced pathological effects (Parkins et al. 1990). This has been suggested to reflect the fact that *Cooperia* increases the rate of protein loss leading to a reduced growth rate and growth requirements. Slower growth will be accompanied by lower feed intake, which will have a concentration effect on FEC due to lower output of faeces (Parkins et al. 1990). This is supported by reduced pepsinogen levels, reflecting abomasal damage (Parkins et al. 1990), and almost doubled plasma losses for concurrent infections comparative to monospecific *O. ostertagi* infections (Kloosterman et al. 1984; Parkins and Holmes, 1989).To account for a mixed infection the most comprehensive method would be to create a model component for predicting the effects of *Cooperia* on the host, and determine species interactions. Although some data exists on artificial *Cooperia* infections as has been summarised by Verschave et al. (2016b), there is very limited data on artificial mixed infections and hence it would be difficult to decipher species interactions for a full range of infection levels.

The development of a stochastic model to account for host-parasite interactions opens up a number of opportunities for future developments. Firstly, it enables the effectiveness of different control strategies to be assessed, including TST where specific individuals of a population are treated, as opposed to the whole population (Höglund et al. 2013a;

O'Shaughnessy et al. 2014a; 2015a; 2015b). This method has been advocated as a potential way to reduce parasite resistance to anthelmintics, but hard, non-confounded data to support this does not exist (Höglund et al. 2010). Introduction of potential parasite resistance mechanisms would allow for such *refugia*-based strategies to be assessed for effectivity and sustainability over short and long term periods; this would provide a useful tool considering the challenges of experimentally investigating long-term effects. Further to this, the addition of second grazing season calves would allow exploration of the impact of different control strategies on the immune acquisition of calves in their second grazing season and effects of hypobiosis. The model is also flexible enough to allow the investigation into the consequences of breeding for parasite resistance through the addition of a genetic component. Although breeding of resistant cattle stock would prove challenging (Kloosterman et al. 1978) there is large potential for genetic progress, more so than sheep (Kloosterman et al. 1992).

Chapter 4: Estimating the consequences of targeted selective treatment strategies on performance and emergence of anthelmintic resistance amongst grazing calves

4.1 Abstract

The development of anthelmintic resistance by helminths can be slowed by maintaining *refugia* on pasture or in untreated hosts. Targeted selective treatments (TST) may achieve this through the treatment only of individuals that would benefit most from anthelmintic, according to certain criteria. However TST consequences on cattle are uncertain, mainly due to difficulties of comparison between alternative strategies. We developed a mathematical model to compare: 1) the most 'beneficial' indicator for treatment selection and 2) the method of selection of calves exposed to *Ostertagia ostertagi* , i.e. treating a fixed percentage of the population with the lowest (or highest) indicator values versus treating individuals who exceed (or are below) a given indicator threshold. The indicators evaluated were average daily gain (ADG), faecal egg counts (FEC), plasma pepsinogen, combined FEC and pepsinogen, versus random selection of individuals. Treatment success was assessed in terms of benefit per R (BPR), the ratio of average benefit in weight gain to change in frequency of resistance alleles R (relative to an untreated population). The optimal indicator in terms of BPR for fixed percentages of calves treated was plasma pepsinogen and the worst ADG; in the latter case treatment was applied to some individuals who were not in need of treatment. The reverse was found when calves were treated according to threshold criteria, with ADG being the best target indicator for treatment. This was also the most beneficial strategy overall, with a substantially higher BPR value than any other strategy, but its degree of success depended on the chosen threshold of the indicator. The study shows strong support for TST, with all strategies showing improvements on calves treated selectively, compared with whole-herd treatment at 3, 8, 13 weeks post-turnout. The developed model appeared capable of assessing the consequences of other TST strategies on calf populations.

4.2 Introduction

The control of gastrointestinal parasitism for small ruminants has long been under threat from the development of anthelmintic resistance by parasite populations (Kaplan, 2004; Wolstenholme et al., 2004; Jabbar et al., 2006; Papadopoulos et al., 2012). However, in recent years it has become evident that this is also an emerging problem for cattle (Edmonds et al., 2010; Sutherland and Leathwick, 2011; O'Shaughnessy et al., 2014b; Rose et al., 2015a). With nematode resistance now present to all three of the broad spectrum anthelmintic classes (benzimidazoles, levamisole and macrocyclic lactones (MLs)) used on cattle (Sutherland and Leathwick, 2011), control strategies aiming to sustain effective parasitic control are of key importance.

Methodologies designed to maintain *refugia* within nematode populations can help to reduce the build-up of resistance by preserving susceptible nematode genotypes. A reservoir of susceptible genotypes on pasture helps to dilute the frequency of resistance alleles amongst nematodes and maintain anthelmintic efficacy (van Wyk, 2001; Gaba et al., 2010). One strategy that aims to achieve this is targeted selective treatment (TST), which involves the treatment of selected individuals that require, or will benefit from, treatment, as opposed to treatment of the entire group (van Wyk et al., 2006). Individuals are generally identified as needing to receive treatment on the basis of their level of parasitism or performance (Charlier et al., 2014). Although TST strategies have been developed and applied successfully in lambs (Greer et al., 2009; Kenyon et al. , 2009, 2013), there are considerably fewer studies on cattle, with the first insights into the application of TST having occurred relatively recently (Greer et al., 2010; McAnulty et al., 2011; Höglund et al., 2013a; O'Shaughnessy et al., 2014a; 2015a; 2015b). As there are important differences in host-parasite interactions and parasite epidemiology between cattle and sheep, differences in the methodology and application of TST in cattle can be expected.

Although TST strategies in sheep have been shown to be beneficial in reducing selection for anthelmintic resistance (Kenyon et al., 2013), it is difficult to know which of the various strategies would be most effective under various scenarios. At present there are no direct comparisons of TST strategies in cattle, in part due to difficulties arising from confounding variables (Höglund et al., 2013a; O'Shaughnessy et al., 2015a). Additionally, it is difficult and time consuming to test such strategies in the long-term. Simulation modelling on the other hand may offer an effective alternative, and be highly beneficial in assessing the feasibility of

novel control strategies. Here we address these gaps, by developing and using a simulation model that represents calf - *Ostertagia ostertagi* interactions and the epidemiology of the infection (Chapters 2 and 3), in order to test the effectiveness of different TST approaches. *O. ostertagi* is the parasite of greatest significance in cattle grazing in temperate climates, and as the developed model is stochastic, it allows us to make predictions for the application of TST in a population of calves.

4.3 Materials and methods

The current model was based on the simulation approach of Chapters 2 and 3, which aims to predict the effects of parasitism with *O. ostertagi* on a population of growing calves, taking into account host phenotype, host-parasite interactions and parasite epidemiology. The model has been further developed here to account for anthelmintic resistance amongst nematodes, by considering the susceptibility of each nematode genotype to anthelmintic treatment.

4.3.1 ***Host-parasite interactions***

Briefly, it was assumed that a healthy calf attempts to ingest sufficient nutrient resources to meet demands for growth and maintenance (Coop and Kyriazakis, 1999). In the presence of parasitism, resource requirements increase due to endogenous protein losses to the calf (Fox, 1993). It is further assumed that the calf acquires immunity to reduce the impact of infection (Claerebout and Vercruysse, 2000), and by doing so further increases resource (e.g. protein) requirements. In addition to the endogenous protein loss and the increased resource requirements, a reduction in appetite and feed intake accompanies infection (Fox et al., 1989b; Forbes et al., 2000; Kyriazakis, 2014). Although complex, the mechanism for inappetance in ostertagiosis was modelled as a function of the rate of immune acquisition, as it has been suggested that this reduction is associated with components of the immune response (e.g. cytokines), and related pathological and inflammatory responses (Fox et al., 1989b; Kyriazakis, 2010; 2014). Consequently, the calf consumes insufficient resources to fulfil its requirements. Ingested protein is usually the first limiting nutrient resource. Once the protein loss due to parasitism has been accounted for it was assumed that allocation of limited resources were prioritised towards maintenance and repair (Coop and Kyriazakis, 1999). Remaining resources were then allocated between growth and immunity, proportional to their requirements (Kahn et al., 2000; Doeschl-Wilson et al., 2008; Laurenson

et al., 2011). The model was parameterised such that the calf and its growth represented a weaned, castrated male (steer) Limousin x Holstein Friesian born in autumn (Chapter 2).

The individual calf model was extended to a stochastic model by considering between-animal variation in calf characteristics (Chapter 3); between-animal variation was assumed in intrinsic growth rate, body composition (expected protein and lipid content at maturity), maintenance requirements (protein and energy), and immune response traits (rate of acquisition, as well as initial and final rates for the immune traits of establishment, mortality and fecundity). The rates of acquisition in the three immune traits were assumed to follow a log-normal distribution, whereas all other traits were assumed to be normally distributed (Vagenas et al., 2007c; Laurenson et al., 2012b). Additionally the rates of immune acquisition for all 3 immune traits were assumed to be a function of overlapping effector mechanisms (Mihi et al., 2014); thus they were assumed to be strongly correlated (r = +0.5) (Laurenson et al., 2012b). Due to the nature of the defined relationships for establishment and mortality it was also necessary to assume a weak correlation (r = -0.2) between minimum mortality and maximum establishment (Chapter 3). All other traits were assumed to be uncorrelated (Doeschl-Wilson et al., 2008). Further, random variation in feed intake was included to achieve a phenotypic correlation between feed intake and growth rate of approximately 0.8 (Cammack et al., 2005).

4.3.2 *Epidemiological module*

In the epidemiological module of Chapter 3, the grazing pasture was defined by the number of hectares and pasture available for grazing (Sibbald et al., 2000), taking into account grass growth and grass consumption on a daily basis. Pasture was assumed to be initially contaminated with overwintered eggs and larvae; subsequent larval contamination of pasture was assumed to arise from eggs excreted by infected calves. The development period from eggs to larvae and the larval mortality were assumed to be temperature-dependent (Stromberg, 1997); the resultant larvae on pasture were considered to have an aggregated distribution. Calves were assumed to graze randomly across the pasture (Laurenson et al., 2011) and consume larvae, removing them from pasture, thus completing the parasitic lifecycle.

4.3.3 ***Parasite anthelmintic resistance***

The mechanism for the development of anthelmintic resistance by *O. ostertagi* to a wide spectrum of anthelmintics is currently not well understood; however there is growing evidence to support a polygenic mechanism (Wolstenholme et al., 2004; Gilleard and Beech, 2007; Prichard, 2007; Yazwinski et al., 2009; Kotze et al., 2014). In the first instance resistance to a single anthelmintic drug, ivermectin, was assumed to be controlled by two genes, each consisting of two alleles. Subsequently, nine possible allele combinations were identified (Barnes et al., 1995). Each allele was assumed to have equal expression within the phenotype (i.e. perfect gene and allele neutrality) hence conveying the same degree of either resistance (R) or susceptibility (S) (Barnes and Dobson, 1990). Ivermectin action was segregated into four key components; a) the degree of dominance of the resistance allele (R), b) drug efficacy against each nematode genotype, c) drug efficacy against each parasitic developmental phase and d) the persistent activity of the drug, which was assumed to be a pharmacokinetic trait of the drug and thus independent of resistance (Smith et al., 1999).

The nine possible genotypes constitute 4 different phenotypic expressions; these were assumed to show a graded response from susceptible ($S_1S_1S_2S_2$) to resistant ($R_1R_1R_2R_2$) (Barnes et al., 1995), dependent on the number of R alleles present as represented in figure 4.1. For example, the genotype combination S_1S_1 R_2R_2 would be considered to have the same phenotype as $S_1R_1S_2$ R_2. Additionally it has been observed that the efficacy of ivermectin is not the same across all stages of development (Eddi et al., 1997; Vercruysse et al., 2000; Yazwinski et al., 2009), hence the efficacy for each stage was defined according to Yazwinski et al. (2009).

Ivermectin is known to display persistent activity of between 1-4 weeks against gastrointestinal nematodes in cattle when administered subcutaneously at a rate of 200µg/kg bodyweight (Armour et al., 1985; Borgsteede and Hendriks, 1986; Williams and Broussard, 1995; Ranjan et al., 1997). This variation in the length of persistent activity can be explained by innate differences in sensitivity amongst various nematode species, environmental factors, such as level of infection (Vercruysse et al., 2000) and within and between differences in pharmacokinetics amongst cattle breeds (Toutain et al., 1997). A curve describing the decay of ivermectin efficacy as a declining sigmoidal function of time was adapted from the equation used by Smith et al. (1999) (equation 4.1). The efficacy of a given genotype *x* ($Efficacy_X$) at time *t* was defined, whereby efficacy falls between 0 and 1, 0 signifying the drug to have no effect and 1 signifying complete effectiveness.

$$Efficacy_{Xt} = \frac{w_X \cdot \exp(w_1 - w_2 t)}{1 + \exp(w_1 - w_2 t)} \quad (4.1)$$

where *t* is time, w_1 and w_2 are constants and w_X is a parameter that depends on parasite genotype (see below).

Parameters were fitted to published literature to show the expected persistence activity of ivermectin against *O. ostertagi* parasites (Armour et al., 1985; Borgsteede and Hendriks, 1986; Williams and Broussard, 1995; Toutain et al., 1997; Ranjan et al., 1997); as such, w_1=7.3, w_2=0.47 and w_X was dependent on the drug efficacy which was defined separately for each genotype according to the number of R alleles present. Drug efficacy against the susceptible genotype was defined according to Yazwinski et al. (2009); however, estimates do not exist for the resistant genotypes. It was therefore necessary to make assumptions about this; it was assumed that drug efficacy against the completely resistant genotype (RRRR) was 0.01 with each R allele assumed to contribute equally to reduction in efficacy (Leathwick et al. 1995; Laurenson et al. 2013a). As an example, drug activity against adult worm genotypes is demonstrated in figure 4.1. It was assumed that the initial concentration of anthelmintic increased so rapidly in the host tissues that it was possible to ignore the time taken to reach maximum drug efficacy (Toutain et al., 1997; Lifschitz et al., 2000). Previous versions of the model assumed a persistent activity of 3 weeks against *O. ostertagi*, followed by a decline in efficacy of 0.15 per day for simplicity (Chapter 3); this was considered a sufficient approximation to the defined curve for the specified treatment.

The resistance genotypes of the initial nematode population on pasture were assumed to arise from random mating, assuming Hardy-Weinberg equilibrium, from an initial frequency of the resistance (allele) assumed to be 0.001 (Barnes and Dobson, 1990). Subsequently, the frequency of R in the worm burden (WB) of each host was used to calculate the frequencies of each genotype in the excreted eggs, again assuming Hardy-Weinberg equilibria. Once the new eggs had hatched and developed into larvae their contribution to the genetic makeup of larvae on pasture was accounted for. It was assumed that all genotypes were equally fit on pasture, such that in the absence of anthelmintic drenching the frequency of R remains the same throughout the simulated grazing season. The total frequency of each genotype in hosts and on pasture was tracked on a daily basis, along with the frequency of R.

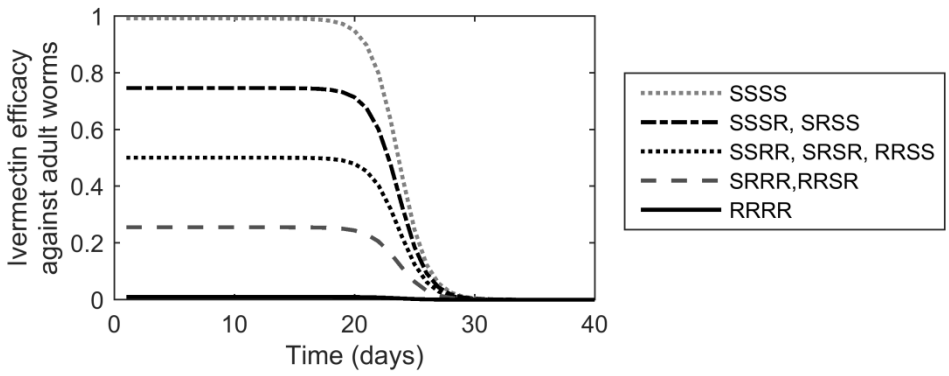

Figure 4.1: The assumed efficacy, i.e. the mortality success of the drug, over time for which a single treatment with ivermectin is effective against adult *Ostertagia ostertagi.* The efficacy is shown for corresponding worm genotypes with zero, one, two, three and four alleles for resistance; each R allele is assumed to decrease drug efficacy by equal amounts.

4.3.4 ***Treatment strategies***

4.3.4.1 Timing of treatments

The most appropriate timings for dosing with the antiparasitic drug were determined by simulating a population of untreated calves to predict nematode population (adult worms), pasture contamination (PC) (L_3/kg DM) and bodyweight gain (kg). It was observed that at approximately 8 weeks post-turnout both parasitic burden and PC began to increase and bodyweight gains were compromised; this coincides with experimental findings in which the majority of calves benefited from treatment at 8 weeks (Höglund et al., 2013a; O'Shaughnessy et al., 2015b). In line with O'Shaughnessy et al. (2015a) the simulations support a second treatment at approximately 16 weeks. An 8-week interval between ivermectin treatments in cattle is based on ~4 weeks of persistent activity against common gastrointestinal nematodes (NAOH, 2015), an average pre-patent period of three weeks and a week of limited exposure to infection (Shaw et al., 1998a). There are no recent studies into the changes in persistence activity due to the build-up of resistance.

4.3.4.2 Key quantifiable host features

Key quantifiable traits that can be observed non-invasively to provide an indication of the parasitic load (or resulting compromised performance) were identified. Performance can easily be quantified by average daily bodyweight gain (ADG) (kg/d). This was preferable to bodyweight (kg) as variation in initial bodyweights is accounted for; hence any reduction can be attributed to parasitism (Höglund et al., 2009). Direct measures of parasitic load are more complex; the most appropriate and widely used measures were concluded to be faecal egg counts (FEC) (eggs/g) and plasma pepsinogen (international units of tyrosine/ litre (IUT/l)), both having their own limitations. Elevated pepsinogen levels occur from around 2-3 weeks as young adult worms emerge from the gastric glands (Jennings et al., 1966; Ritchie et al., 1966). All these traits have shown promising outcomes for the success of TST in practice (Greer et al., 2010; McAnulty et al., 2011; Höglund et al., 2013a; O'Shaughnessy et al., 2014a, 2015a; 2015b), therefore relationships for these traits had to be defined within the model.

Pepsinogen concentrations are considered to be a good diagnostic tool for abomasal damage associated with *O. ostertagi* burdens in cattle for the duration of the first grazing season (Charlier et al., 2014); a strong correlation has been observed between adult WB and pepsinogen levels (Anderson et al., 1966; Allen et al., 1970; Baker and Gershwin, 1993; Dorny et al., 1999). Concurrent measurements of WB and pepsinogen were obtained from existing literature (Anderson et al., 1966; Snider et al., 1981; Williams et al., 1987; Baker and Gershwin, 1993; Almería et al., 1996; Szyszka and Kyriazakis, 2013). In the case of Szyszka and Kyriazakis (2013) only pepsinogen was recorded, however as the infections were artificially administered it was possible to replicate the experimental conditions within the model and hence simulate predicted WB associated with elevated pepsinogen levels. The model only considers sub-clinical infections, hence pepsinogen levels above 5 IUT/l were ignored, as these are considered to be clinical (Hilderson et al., 1989; Shaw et al., 1997; Vercruysse and Claerebout, 2001). Pepsinogen levels would be expected to increase as adult worms emerge from gastric glands, causing elevated abomasal pH and leakage due to increased mucosal permeability (Jennings et al., 1966; Allen et al., 1970; Fox et al., 1987); they will continue to increase with increasing WB until a plateau is achieved as no further changes in gastric function occur (Dorny et al., 1999). Consequently, a monomolecular growth function was fitted to the published data for concurrent WB and pepsinogen levels; this equation provided the best fit and mirrored the expected relationship between WB and pepsinogen:

$$Pepsinogen = Pep_{max} - (Pep_{max} - Pep_{min}) \cdot \exp(-C_p \cdot WB) \quad \text{(IUT/l)} \qquad (4.2)$$

where Pep_{max} is the maximum pepsinogen for a sub-clinical infection (3.8 IUT/l), Pep_{min} is the minimum pepsinogen, observed in a healthy calf (0.8 IUT/l) and $C_p$is a rate constant defining the relationship between WB and pepsinogen level (1.67×10^{-5}) (R=0.603, RMSE=0.636). Pepsinogen levels do not provide an exact description of the WB, for this reason random variation in pepsinogen was added and parameterised to mirror a correlation between WB and pepsinogen of approximately 0.7 (Anderson et al., 1966; Allen et al., 1970; Baker and Gershwin, 1993; Dorny et al., 1999).

4.3.4.3 Targeted selective treatment strategies

The aim of this study was to compare the consequences of control strategies and identify the most effective and sustainable method(s). To provide a baseline for comparison, treatment groups included calves administered no treatment and strategically treated calves with the whole group receiving anthelmintic dosing at the time points of 3, 8 and 13 weeks post-turnout; this has been shown to provide good control of parasitic gastroenteritis in set-stocked, first grazing season calves (Shaw et al., 1998a). Subsequently, a variety of TST strategies were simulated (detailed below) using the aforementioned traits of ADG, FEC and pepsinogen as determinant criteria for treatment. A summary of the different TST strategies investigated is provided in table 4.1.

Determinant criteria	Treating a fixed percentage of the population with the lowest (or highest) trait values	Treating individuals who exceed (or are below) a given trait threshold
ADG	✓	✓
FEC	✓	✓
Pepsinogen	✓	✓
FEC and Pepsinogen	-	✓
Random selection	✓	-

Table 4.1: A summary of the different control Targeted Selective Treatment (TST) strategies investigated for differing methods of selection for treatment; ADG = Average Daily Gain (kg/d); FEC= Faecal Egg Counts (eggs/g).

4.3.4.3.1 TST based on herd percentages.

One specification for TST is to dose a percentage of calves according to a pre-determined criterion (Laurenson et al., 2013a). In order to investigate a range of scenarios, treatments were assumed to occur for 10, 25, 50 and 100% of the host population, as indicated by each of the determinant criteria; 100% signifying whole group targeted treatment. Calves within a population were treated at the specified times, subject to a determinant criterion: for ADG the calves with the lowest gains were preferentially treated; for FEC and pepsinogen the calves with the highest values were preferentially treated. A total of 2 days was allowed for processing and analysis of the samples; ivermectin was then assumed to be administered the following day at 200µg/kg bodyweight (Höglund et al., 2013a; O'Shaughnessy et al., 2014a). An additional comparison group was included whereby calves were selected for treatment at random by generating random pseudo-numbers relating to calf ID numbers; as such, the other determinant criteria were evaluated in relation to this.

4.3.4.3.2 TST based on threshold values

In contrast to selecting a fixed percentage of the herd for treatment, TST can also be achieved by dosing calves when a determinant criterion reaches a threshold level (Charlier et al., 2014). The same 3 determinant criteria were investigated, with the addition of a group of calves treated according to a combination of FEC and pepsinogen, as this strategy has been investigated in the field (O'Shaughnessy et al., 2014a; 2015a; 2015b). Available literature was used to define threshold values for each determinant criterion. When using ADG as the determinant criterion, calves were treated when individual ADG was inferior to the ADG averaged over the poorest growing 50% of calves in a strategically treated group (3, 8 and 13 weeks) (Höglund et al., 2013a). The threshold for FECs was considered to be 80 eggs/g. A trigger of 200 eggs/g has been used previously, however this was defined for mixed infections (O'Shaughnessy et al., 2014a). Although seasonal variation in egg ratios is observed in temperate regions (Dorny et al., 1988; Vercruysse et al., 1988; Verschave et al., 2014), for simplicity it was assumed that an average proportion of 0.4 was *O. ostertagi* eggs (Dorny et al., 1988; Vercruysse et al., 1988; Hilderson et al., 1990; Ploeger and Kloosterman, 1993; Almería et al., 1996; Areskog et al., 2013b; Verschave et al., 2015). The threshold for pepsinogen levels was assumed to be 2 IUT/l and therefore the final group involved treating calves when both FECs greater than 80 eggs/g and pepsinogen levels greater than 2 IUT/l were attained by an individual. For all determinant criteria, trait measurements were assumed to be taken every 3 weeks starting from 8 weeks post-turnout (Greer et al., 2010; Höglund et al., 2013a; O'Shaughnessy et al., 2014a; 2015a; 2015b) and treatment applied to individuals presenting measurements above or below the specified threshold. The reduction in anthelmintic use was calculated as a percentage of the total anthelmintic applications administered in the strategically treated group.

4.3.5 *Simulation procedure and outputs*

A population of 500 calves was simulated on pasture over their first grazing season for a period of 6 months from weaning. All calves were assumed to be parasitologically naïve prior to turn-out to pasture at a conventional stocking rate of 5 calves/Ha (AHDB, 2016a) and an initial PC of 200 L_3/kg DM (Larsson et al., 2007). The same population was modelled for all treatment groups. All model simulations were programmed in Matlab (2015).

A population of calves was simulated for each of the selected strategies. Outputs were recorded on a daily basis and compared for the following: performance traits (population average of bodyweight (kg)), parasitological traits (population average of WB and FEC), epidemiological traits (PC (L_3/kg DM grass)) and anthelmintic resistance traits, such as the frequency of R in the nematode population on pasture and total number of anthelmintics administered over the grazing season.

Each modelled strategy was compared with the untreated group for its effect on average empty bodyweight (EBW) (providing a similar output to carcass weight) and R allele frequency at the end of the first grazing season (Laurenson et al., 2016). The average weight gain benefit arising from treatment (AWGB, kg) was calculated at the end of the first grazing season when animals were taken off pasture and moved indoors, which was defined as housing (*h*):

$$AWGB_h = EBW_{TST_h} - EBW_{C_h} \quad \text{(kg)} \quad (4.3)$$

where EBW_{TST_h} is the EBW at the time of housing (*h*) for a group of calves receiving a given TST strategy and EBW_{C_h} is the EBW at time of housing for a group of calves left untreated.

Similarly, for each treatment strategy the frequency of R allele was compared with the untreated control group to determine the impact upon anthelmintic resistance. The increase in R allele frequency ($IRAF_h$) from turnout to the end of the grazing season was calculated at housing:

$$IRAF_h = RAF_{TST_h} - RAF_{C_h} \quad (4.4)$$

where RAF_{TST_h} is the frequency of the R allele on pasture at time of housing (*h*) for a group of calves receiving a given TST strategy and RAF_{C_h} is the frequency of the R allele on pasture at time of housing for a group of calves left untreated.

In order to evaluate each of the simulated strategies, the 'benefit per R' (BPR) was calculated to account for production benefits and the impact on anthelmintic resistance such that equal weighting was given to both traits. BPR at time of housing was calculated according to Laurenson et al. (2016) as follows:

$$BPR_h = \frac{AWGB_h}{IRAF_h} \qquad \text{(kg/R)} \qquad (4.5)$$

As such, the best strategy will be the one displaying the highest value for BRP.

To make a comparison of the benefit gained from treating a percentage of calves according to each determinant criteria relative to random selection, a number of outputs were assessed in terms of their final predicted values at the end of the grazing season (day 180); these were: A) cumulative faecal egg counts as a measure of parasitism; B) relative reductions in bodyweight gain as a measure of performance; C) frequency of R on pasture as a measure of resistance and D) BPR value. A two-tailed Z test was carried out to assess the statistical significance of treatments according to each determinant criterion, with the exception of relative reductions in bodyweight gain which were assessed using the Mann-Whitney U test due to the skewed data distribution. For outputs related to resistance (frequency of R on pasture and BPR) the output was a single measure for the complete pasture and therefore variation was estimated by simulating 10 populations for each treatment group. In each simulation, all stochastic parameters describing individuals and their environment were assigned based on a different unique sequence of computer-generated random numbers. The statistical tests revealed whether treatments according to determinant criteria produced outputs different from what might be obtained by random selection. All statistical comparisons were carried out to the 95% confidence level. Additionally, the model recorded which individuals were treated at each assessment, from this the number of treatments shared between groups treated according to different determinant criteria was calculated along with the number of repeat treatments made within each treatment group, i.e. percentage of the individuals receiving treatment at the first assessment to also receive treatment at the second assessment. A comparison of determinant criteria used for the threshold treatments was made for BPR using the same methods, there was no standard control to compare all treatments to and therefore they were compared with one another.

4.4 Results

4.4.1 *TST based on herd percentages*

4.4.1.1 Comparison of treatment percentages

The impact of different percentages of treated calves was investigated for determinant criteria of ADG, FEC and pepsinogen. The pattern of outcomes for different percentages of the population treated was similar for all determinant criteria and for this reason the outputs for the determinant criterion ADG are shown on figure 4.2. The impact of treatments on the parasitological output of average WB (figure 4.2A) over one grazing season showed a reduction in peak WB, remaining below that of the untreated group throughout the grazing season. The larger the percentage of calves treated the lower the average WB. This pattern was reflected in the average FEC (figure 4.2B); average FEC was reduced from the first anthelmintic treatment on 56 day post-turnout (dpt) until approximately 105 dpt, when all groups showed an increase in FEC to values equal to or greater than those of an untreated group of calves. The effects were more pronounced when a greater percentage of calves were treated. Following the second anthelmintic treatment, FECs were again reduced relative to the percentage treated; at approximately 155 dpt all treated groups showed an increase to levels above the untreated group. The observed increase was larger when a greater percentage of calves were treated, with the 100% treated group showing the largest final FEC.

PC expressed as L_3/kg grass DM (figure 4.2C) was reduced by the treatments relative to the untreated herd, the extent of the reduction was higher the greater the percentage of calves treated. For the group treated at 100%, PC continued to rise for 2-3 weeks following the first treatment due to developing eggs already present on pasture pre-treatment. A subsequent trough in PC was observed. The final PC was approximately the same for all treated and untreated groups. As a result of lower WB and PC prompted by anthelmintic treatments the impacts of parasitism on the relative reduction in bodyweight gain (compared with a healthy control population) was less for the groups with the highest percentage of calves treated for any of the determinant criteria (figure 4.2D).

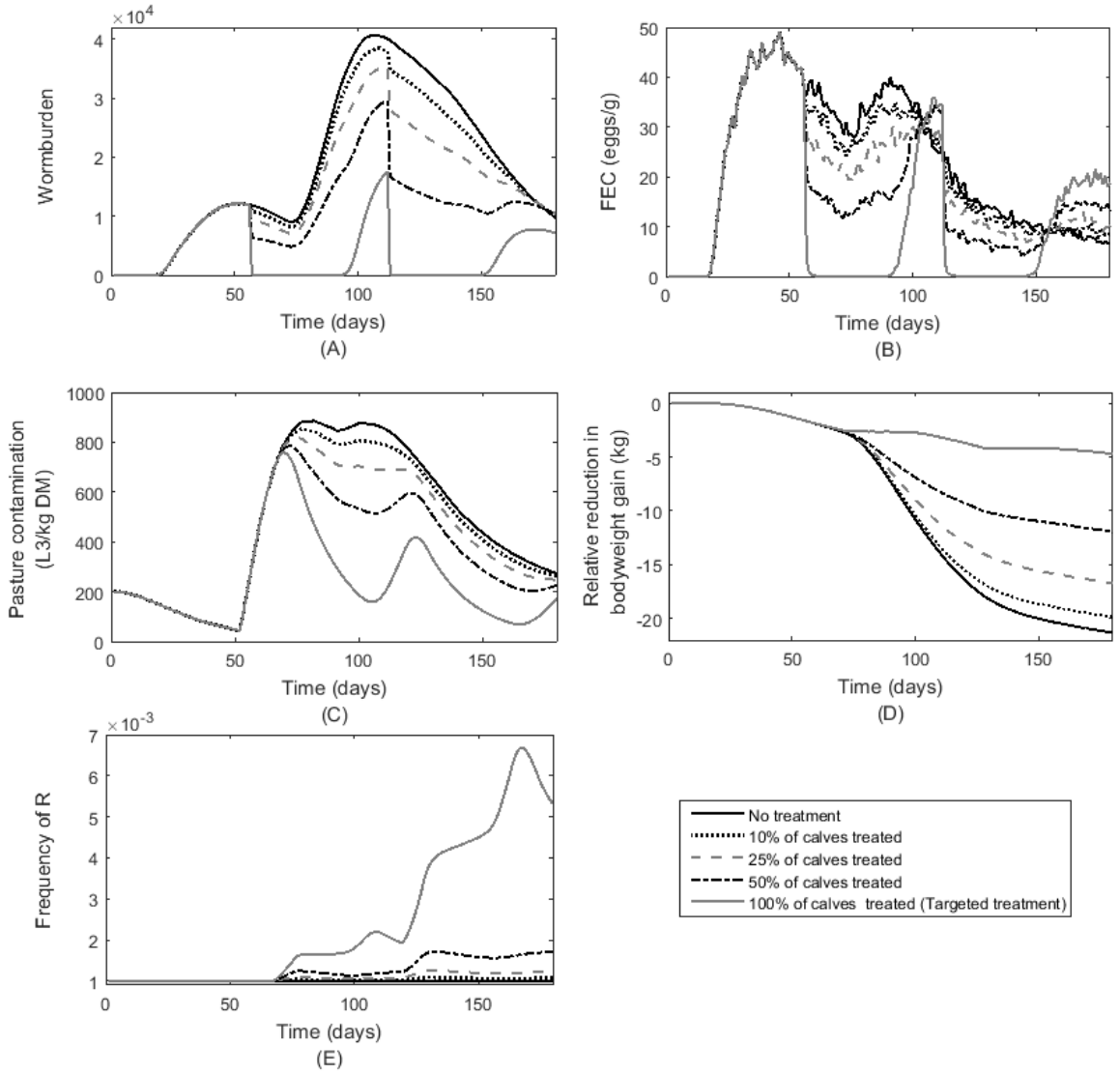

Figure 4.2: Predictions for groups of calves either left untreated or treated at weeks 8 and 16 according to lowest average daily bodyweight gain (ADG, kg/d) when a percentage of 10, 25, 50 and 100% of a herd of 500 calves grazing on pasture initially contaminated with 200L_3/kg DM grass were treated with ivermectin; the population averages are presented for outputs of A) worm burden; B) faecal egg output (FEC) (eggs/g); C) pasture contamination (L_3/kg DM grass); D) relative reduction in bodyweight gain relative to a non-parasitised population (kg) and E) the frequency of resistant parasite genotype R on pasture.

Predictably, the treatments most successful at reducing parasitological burdens and reductions in bodyweight gain were also most likely to result in a high frequency of resistant (R) alleles in the nematode population at pasture. Figure 4.2E shows the change in frequency of R allele on pasture; as would be expected the larger the percentage of treated calves the greater the increase in R allele frequency, with disproportionally large increases observed when the percentage treated was increased. For example, the increase in frequency of R was 0.0007 and 0.0043 when 50 and 100% of the population were treated. In all cases the frequency of R increased following each anthelmintic treatment. Increasing the percentage of the population treated increased the impact upon the R allele frequency. As such, the pattern predicted for the largest treatment percentage of 100% (whole-herd treatment) was the most exaggerated as a consequence of a reduction in S alleles in eggs deposited onto pasture and a reduction in PC. Following this initial increase, the R allele frequency continued to vary as a consequence of the impact of treatment upon PC coupled with the continued persistent activity of ivermectin. This effect was most notable as a secondary peak in R allele frequency on pasture, prior to the impact of the second anthelmintic, for the whole-herd treatment group. This secondary peak in R allele frequency decreased around 115 dpt reflecting the increase in PC as the persistent effect of ivermectin reduced.

4.4.1.2 Comparison between determinant criteria

Figure 4.3 provides a comparison of population averages for cumulative FEC, final relative reduction in bodyweight gain (in comparison to uninfected controls), final frequency of R on pasture and BPR value for groups of calves drenched at different percentages according to the different determinant criterion traits of ADG, FEC, pepsinogen or random selection. The optimal determinant criterion would be the one that offers a small change in the frequency of R whilst preventing extreme reductions in bodyweight gains. A statistical comparison of the benefits to cumulative FEC, reduction in final bodyweight gain, final frequency of R on pasture and BPR of treating according to each determinant criterion was made in relation to treating according to random selection.

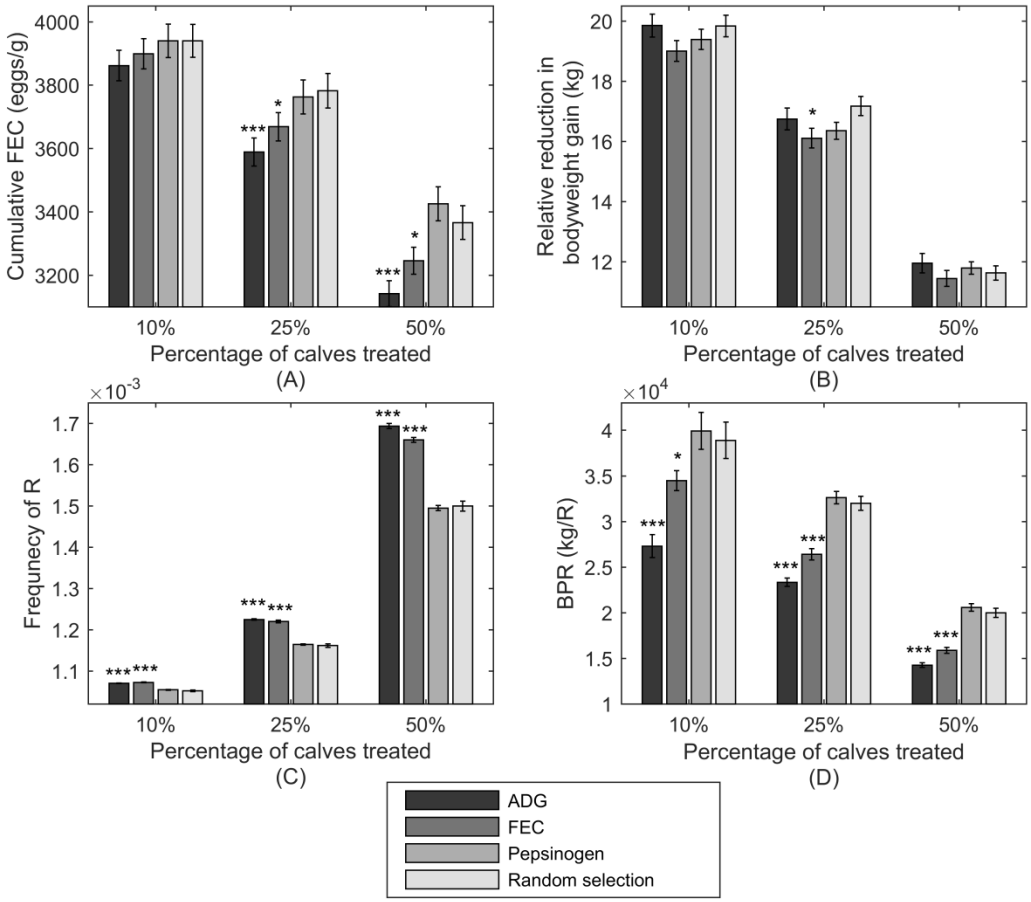

Figure 4.3: End of season (day 180) predictions for: A) cumulative faecal egg count (eggs/g), B) relative reduction in bodyweight gains (kg) in comparison to a non-parasitised population, C) frequency of R on pasture, and D) benefit per R (BPR) representing the benefit in bodyweight gain (kg) per change in frequency of R; for 500 calves grazing on pasture initially contaminated with 200L_3/kg DM grass. Anthelmintic treatment was administered at weeks 8 and 16 to either 10, 25 or 50% of the population according to lowest average daily bodyweight gain (ADG, kg/d), highest faecal egg count (FEC, eggs/g), highest pepsinogen (IUT/I) or selected at random. Predictions for frequency of R on pasture and benefit per R (BPR) are provided as an average of ten simulations. Statistical indications are provided for each treatment group in comparison to those selected for treatment at random.(* $p<0.05$; ** $p<0.01$; *** $p<0.001$)

Fig 3A shows the population average and standard error of cumulative FEC, which was used as an indicator of parasitism. Treating calves according to pepsinogen levels showed similar effects to random selection, whereas treatment according to the determinant criteria of FEC or ADG was more effective at reducing FEC, with ADG being predicted to have the greatest improvement over random selection for groups where 25% and 50% of calves treated ($P<0.001$). The differences between determinant criteria increased with increasing percentages of treated calves, with the FEC treated group also showing significant improvements over the random selection group when 25% and 50% of the population was treated ($P<0.05$).

Figure 4.3B shows the consequences of parasitism on performance; all groups showed similar reductions in final bodyweight gain. Groups treated according to FEC yielded marginally greater improvements in bodyweight gain (i.e. smallest relative reduction in bodyweight gain in comparison to a non-parasitised group), with the difference being significant when 25% of the population was treated ($P<0.05$). In contrast, groups treated according to ADG showed the least improvement (i.e. largest relative reduction in bodyweight gain in comparison to a non-parasitised group), whilst being accompanied by the largest range of values within the population. In contrast to cumulative FEC outcomes, the final frequency of R shown in figure 4.3C was highest for groups treated according to FEC and ADG with a significant increase observed relative to calves treated according to random selection for all treatment percentages ($P<0.001$),whereas there was no significant difference between calves treated according pepsinogen and random selection. Again, this effect was clearer for greater percentages of treated calves. This was conveyed in the BPR values (figure 4.3D): calves treated according to ADG and FEC showed a significantly lower value than predicted for random selection ($P<0.001$, $P<0.05$), whereas when treated according to pepsinogen there was no statistical difference. The largest differences between determinant criteria were observed when a smaller percentage of calves were treated, along with the largest variation between populations. When 100% of the herd was treated there was no difference between determinant criteria and therefore it was not possible to conduct a statistical comparison, however it should be noted the average BPR value was 4,012 (181) which is notably lower than the value observed for any of the other treatment percentages described.

Treatment strategies were further compared by examining the individuals selected at each treatment stage. Table 4.2 gives the percentage of total treatments that were shared between populations treated according to different determinant criteria. As can be seen, treatment according to ADG or FEC shared more individual treatments than would be expected by random probability whereas ADG and pepsinogen shared fewer. It was also possible to examine whether individuals treated on the first occasion are more or less likely to be selected on the second occasion; this statistic is also shown in Table 4.2. Both ADG and FEC showed a greater number of repeat treatments than would be expected at random with ADG showing the largest number of repeat treatments. Conversely, groups treated according to pepsinogen showed fewer repeat treatments than would be expected at random.

	Determinant criteria	Percentage of herd treated		
		10%	**25%**	**50%**
% reduction in anthelmintic use[a]	-	93%	83%	67%
% of shared treatments between determinant criteria	Random	10.0%	25.0%	50.0%
	ADG-FEC	20.0%	32.0%	55.6%
	ADG -Pepsinogen	4.0%	16.4%	42.2%
	FEC-Pepsinogen	7.0%	27.6%	48.6%
% of first treated group to be selected for second dose	Random	10.0%	25.0%	50.0%
	ADG	84.0%	87.2%	90.0%
	FEC	26.0%	39.2%	70.0%
	Pepsinogen	2.0%	18.4%	40.4%

a. comparative to strategically treated calves (3, 8 and 13 weeks)

Table 4.2: A comparison of TST strategies whereby 10, 25 and 50% of calves were treated at 8 and 16 weeks either at random or according to lowest average daily bodyweight gain (ADG, kg/d), highest faecal egg count (FEC, eggs/g) or plasma pepsinogen (IUT/l). Values provided represent the percentage reduction in anthelmintic use relative to a population of calves treated strategically at 3, 8 and 13 weeks post-turnout. Additionally, the number of treatments shared between groups treated according to ADG, FEC or pepsinogen are provided. Within each treatment group a record was made of the number of individuals that had been treated at the first assessment that were also treated at the second assessment. The expectation of each occurring at random is provided as a comparison.

4.4.2 *TST based on threshold values*

The impact of defining a threshold level for treatment for the different determinant criteria of ADG, FEC, pepsinogen and the combination of FEC and pepsinogen was assessed in terms of the parasitological outputs of WB (figure 4.4A) and FEC (figure 4.4B). Following the first assessment for treatment, all groups showed a lower peak in WB and FEC than that of an untreated group of calves, although the reductions observed were minimal in the group treated according to a combination of FEC and pepsinogen. The largest reductions in WB, and consequently FEC, were observed when the determinant criterion was pepsinogen; this was then followed by groups where the determinant criterion was ADG and finally FEC. Reductions in WB and FEC started earlier when ADG and FEC were used as determinant criteria, compared with the other groups with notable sudden decreases in WB for the pepsinogen group from 98 dpt. For the remaining determinant criteria the decline in WB and FEC was smoother across the grazing period. The strategically treated group in which the whole-herd treatments were applied at 3, 8 and 13 weeks post-turnout showed very low burdens for the duration of the season, with a clear increase observed at the end of the season (from 130 dpt).

As per parasitological traits there was a reduction in peak PC relative to an untreated control for all treatment groups (figure 4.4C); again the decrease predicted for combined FEC and pepsinogen showed minimal reductions. The PC predictions for determinant criteria largely mirrored the predictions in WB and FEC with the reduction occurring more rapidly when pepsinogen was used as the determinant criterion. All treatment groups showed an improvement upon the untreated group for relative reduction in body weight gain in comparison to a non-parasitised population (figure 4.4D). Consistent with reduced parasitological burdens and PC, the groups treated strategically showed bodyweight close to that expected of a healthy (non-parasitised) calf. The groups treated according to pepsinogen and ADG showed the least reductions in bodyweight relative to a healthy (non-parasitised) population of calves, followed by FEC, and then the combination of FEC and pepsinogen which showed minimal improvements compared with an untreated groups of calves.

However, upon comparing the frequency of R in the group administered strategic treatment (whole-herd treated at 3, 8 and 13 weeks post-turnout) an increase in the frequency of R (figure 4.4E) compared with all other strategies was evident, with large increases observed

up until approximately 135 dpt, coinciding with the increase in eggs excreted to pasture. For this reason outputs for this treatment are shown separately. Figure 4.4(F) shows the frequency of R for the different TST groups. The group treated according to the determinant criterion of pepsinogen alone was seen to give the largest increase in R, followed by ADG and FEC treated groups both of which showed an increase in frequency less than half that of the pepsinogen group. In agreement with other outputs, the group treated according to both FEC and pepsinogen showed minimal changes in the frequency of R.

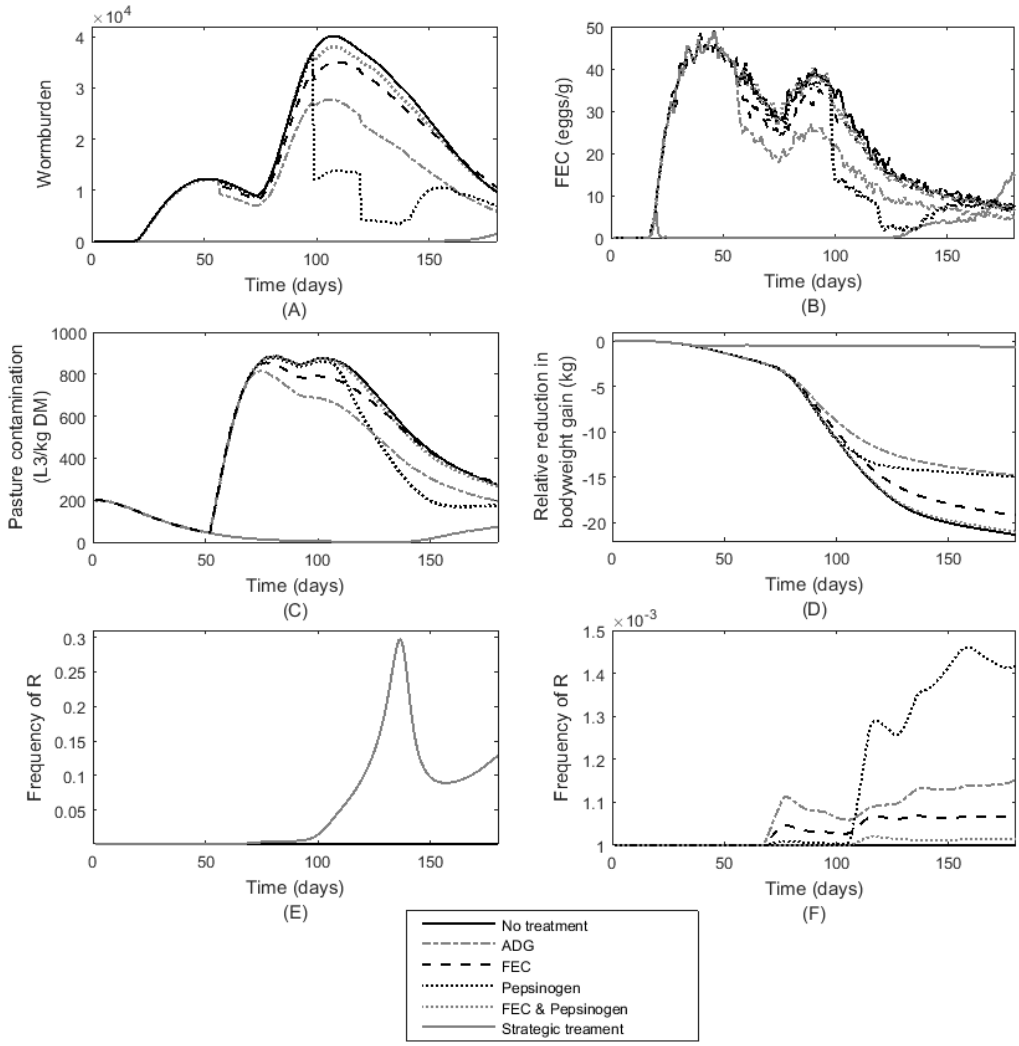

Figure 4.4: Predictions for groups of calves either left untreated, strategically treated with ivermectin at 3, 8 and 13 weeks post-turnout, or treated with ivermectin according to threshold values for different determinant criteria of average daily bodyweight gain (ADG, kg/d), faecal egg count (FEC, eggs/g), pepsinogen (IUT/l) or the combination of values for FEC and pepsinogen; were made for a herd of 500 calves grazing on pasture initially contaminated with 200L_3/kg DM grass. The population averages are presented for outputs of A) worm burden; B) FEC (eggs/g); C) pasture contamination (L_3/kg DM grass); D) relative reduction in bodyweight gain relative to a non-parasitised population (kg); E) the frequency of R on pasture for the strategically treated group and F) the frequency of R on pasture for the remaining strategies.

Figure 4.5 represents the BPR values for each of the strategies; the highest value and therefore most beneficial was attributed to the group treated according to ADG. FEC was the next best strategy, closely followed by those treated according a combination of FEC and pepsinogen, then pepsinogen alone. Strategically treated groups were predicted to have a dramatically lower BPR value. The difference between each treatment group was observed to be substantial in all cases. Additionally, the reductions in anthelmintic applications for each strategy compared with strategic treatment were calculated and revealed that the combination of FEC and pepsinogen showed reductions of 98.3%, closely followed by treatment according to FEC for which a 93.4% reduction was observed. Considerably more treatments were applied for ADG and pepsinogen treated groups with reductions of 47.0% and 68.4% respectively.

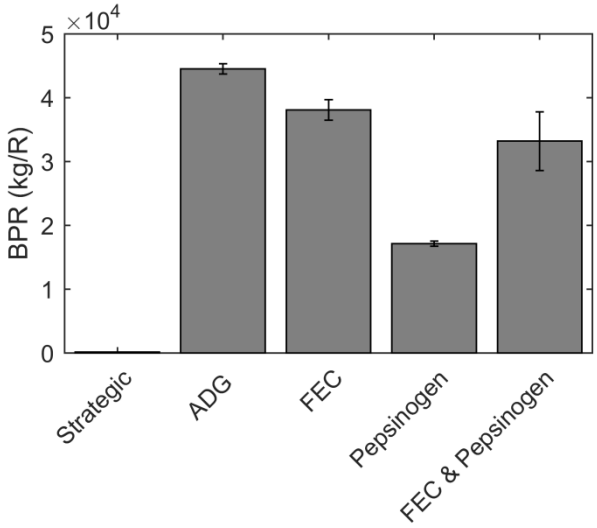

Figure 4.5: Benefit per R (BPR) simulated at the end of the grazing season (day 180) on a population basis for each of the simulated control strategies; BPR represents the benefit in bodyweight gain (kg) per change in frequency of R on pasture, so the higher the value the more beneficial the strategy is perceived to be. Ten discrete populations of calves were simulated on pasture initially contaminated with 200L_3/kg DM grass for calves treated strategically with ivermectin at 3, 8 and 13 weeks post-turnout or according to threshold values of average daily bodyweight gain (ADG, kg/d), faecal egg count (FEC, eggs/g), pepsinogen or a combination of FEC and pepsinogen. Statistical comparisons were made between groups and are reported within the text.

4.5 Discussion

With the emergence of anthelmintic resistance in gastrointestinal parasites of cattle (Edmonds et al., 2010; Sutherland and Leathwick, 2011; O'Shaughnessy et al., 2014b; Rose et al., 2015a) there have been attempts towards developing TST strategies for cattle. This is important, as although resistance has been slow to develop amongst cattle parasites, it appears that multi-drug resistance for multiple parasite species is developing more rapidly than expected (Sutherland and Leathwick, 2011). There are a number of challenges to address when developing and assessing such strategies. The first is the basis upon which these strategies are developed. Secondly, it is difficult to make direct comparisons on the effectiveness of such strategies through field studies due to confounding variables, such as climatic conditions or management techniques (O'Shaughnessy et al., 2015a). These will have consequences on the underlying infection levels and subsequently affect the perceived success of any treatment strategy. It is therefore unclear from the literature as to which strategy might be most beneficial in treating the effects of parasitism whilst delaying the development of resistance. Finally, in practice it is difficult to assess the development of resistance, especially over a short time-scale (Besier, 2012; Sutherland and Bullen, 2014), which is usually the case with experimentation. Currently faecal egg count reduction tests (FECRT) are used to assess this, however this technique has only been validated for sheep and not cattle nematodes (Sutherland and Bullen, 2014). Compared with sheep nematodes, *O. ostertagi* tends to show less aggregation between hosts, excrete fewer eggs (Demeler et al., 2010; El-abdellati et al., 2010; Yazwinski et al., 2013) and FEC are generally less reflective of WB as a result of density-dependent effects on parasite fecundity (Michel et al., 1978; Smith et al., 1987a). As a result, the limited numbers of studies conducted on cattle TST have focused on performance and total number of anthelmintic applications. In this paper, the relative success of different TST applied here was evaluated on the basis of BPR, the ratio of average benefit in weight gain to change in frequency of R (relative to an untreated population).

With these difficulties in mind we embarked upon further developing a recently published population model to predict the consequences of different TST strategies on cattle and their *O. ostertagi* populations (Chapter 3). We were particularly interested in the consequences of: 1) the most appropriate determinant criteria for treatment selection and 2) the method of selecting animals for treatment, the contrast being treating a fixed percentage of the

population with the lowest (or highest) trait values versus treating individuals who exceed (or are below) a given trait threshold for treatment. As the model was population-based, it allowed us to trace individual animals within a group and select individuals on the basis of the different methods. The model was applied to first season grazing calves infected with *O. ostertagi*, the most important parasite affecting health and productivity in temperate climates. Strategies were selected on the basis of literature; however different methods of defining threshold triggers have been proposed, in particular for ADG. Höglund et al. (2009) suggested that an ADG below 0.75kg/d would provide a good trigger threshold for treatment; however a set value does not account for the sigmoidal nature of growth or indeed for variability in intrinsic growth between and within genotypes. For example, healthy calves that are close to their maximal weights and hence show slower growth would be considered to require treatment. However, the risk of this misinterpretation may not be a major concern for the time interval considers, as calves were probably in the linear growth phase. Greer et al. (2010) and McAnulty et al. (2011) proposed determining the expected ADG for individuals at any given time point dependent on individual calf bodyweight measured at the previous time point. Although a significant improvement to the previous method, there are also problems associated with this, which arise from the natural uncertainty associated with a single body weight measurement. The selected method, based on the mean ADG of the poorest 50% of calves in a strategically treated population, was assumed to provide conservative estimates of the expected ADG in a healthy population hence indicating individuals who showed an ADG below this expectation.

As expected, all of the simulated TST regimens improved weight gains and reduced most (but not all) measures of parasitism compared with an untreated herd. The methods used for selection and their determinant criteria predicted different outcomes and therefore each is addressed separately.

4.5.1 *TST based on herd percentages*

A comparison of the impact (upon various traits) of treating 10, 25, 50 or 100% of calves according to the determinant trait ADG is provided in figure 4.2. Upon treating 100% of the population individuals experienced a temporary elimination of parasitological burden, but later displayed a rebound effect – a steep rise in infection. This rebound effect was a consequence of a reduced rate of immune acquisition due to a reduction in antigen

exposure in the treated individuals. Results for partial treatment of the herd were essentially weighted averages of the untreated and treated predictions. As a consequence of reduced WBs the treated individuals experienced reduced protein loss and increased feed intake (due to reduced immune acquisition), hence resulting in greater average bodyweights when a greater percentage of calves were treated. Larger calves with larger feed intake requirements consumed higher quantities of grass, and therefore a greater proportion of the larvae on pasture. Consequently, large numbers of susceptible larvae were removed from pasture and killed by anthelmintic activity, whereas resistant larvae removed from pasture survived within the host to produce eggs. This selective process tended to reduce PC but enriched the fraction of resistant eggs excreted to pasture. Therefore the frequency of R alleles increased as a greater percentage of calves was selected for anthelmintic treatment. Overall it was predicted that treating fewer calves provided the greatest overall benefit as reflected in BPR (figure 4.3). This finding is similar to what has been found when modelling similar TST for lambs (Gaba et al., 2010; Laurenson et al., 2013a).

Selection for treatment according to each determinant criterion was compared with random selection on the basis of cumulative FEC, reduction in bodyweight gain (relative to non-parasitised group), frequency of R on pasture and BPR (figure 4.3). Determinant criteria of ADG and FEC resulted in reduced cumulative FEC compared with random selection, either through a direct effect on eggs or via the impact of calf size on volume of faeces produced (hence concentration of eggs in faeces). Little absolute difference in reduction in bodyweight gain (relative to non-parasitised group) was observed between different determinant criteria; however ADG resulted in the largest average reduction in bodyweight gain. Dissection of the model components revealed this to be a result of varied intrinsic growth rates within the population; a portion of those selected for treatment on the basis of low ADG were intrinsically slow growers and not impeded by parasitism. Additionally a portion of the calves experiencing large reductions in ADG did not receive treatment as they were intrinsically fast growers and their ADG did not fall below that of non-parasitised intrinsically slow growers. Although determinant criteria of ADG and FEC resulted in treatment to many of the same individuals, FEC showed the greatest improvements in bodyweight gain. This was due to treatment of intrinsically fast growers impeded by parasitism and a lack of treatments to intrinsically slow growers showing few signs of parasitism. Unlike bodyweight gain the determinant criteria substantially impacted on the frequency of R, and therefore

BPR. The most efficient strategy was to treat calves with the greatest WB, which suffer the greatest parasite-related loss of productivity, whilst due to density-dependent effects and immune response are contributing less to the aggregate herd excretion of eggs (Michel et al., 1978; Smith et al., 1987a). Calves with lower WB may nevertheless have high FEC, and it is advantageous to allow them to continue producing susceptible eggs while their performance is not as severely affected by WB. According to this rationale, pepsinogen selection was the best method to identify the optimal treatment group, whereas ADG and FEC tend to exclude optimal candidates: ADG by selecting intrinsic slow-growers with low WB, and FEC by selecting low to moderately infected calves showing high FECs.

Interactions between the percentage of calves treated and the determinant criteria used for selection were predicted for BPR (figure 4.3). The largest difference in BPR value between determinant criteria was observed when a smaller percentage of calves were treated. For all determinant criteria, treating 10% of the population resulted in the largest variation in BPR values across different calf populations. This implies a greater range of possible outcomes associated with treating fewer calves. Interactions were not observed between treatment percentage and determinant criteria, simulations suggest that these selection criteria of FEC and ADG are counter-productive compared with random or pepsinogen based selection because of their more detrimental effect on *refugia* for reasons discussed above.

4.5.2 ***TST based on threshold values***

TST based on threshold triggers appeared to show the reverse pattern in terms of the most beneficial determinant criterion compared with treating a fixed percentage of the population (figure 4.5). Treating calves according to thresholds for ADG showed by far the greatest benefit; this was followed by FEC, combined FEC and pepsinogen, and pepsinogen alone. This pattern can be explained by the observations of figure 4.4. Although the modelled treatment for selection according to ADG required the highest number of treatments, the development of resistance remained low. This is explained by a combination of factors: first, the tendency for this method to select calves with an intrinsically slow growth genotype that do not necessarily have high WB. Second, the method does not select tolerant individuals (i.e. individuals in which infection is not limited but negative fitness consequences are offset), experiencing large WBs without showing clear signs of poor performance due to parasitism. The former resulted in only small numbers of resistant

alleles contributing to pasture, whereas the latter allowed large numbers of susceptible eggs contributing to pasture.

Treatment according to FEC had similar effects on PC. Simulations showed FEC was highest early in the grazing season, meaning that selection according to FEC resulted in the majority of treatments administered early in the season, preventing the build-up of PC. Conversely, WBs began to rise towards the latter stages of the grazing season in tandem with the expected mid-season rise in PC, causing elevated pepsinogen levels. This resulted in large numbers of treatments administered in unison, therefore causing sudden reductions in WB and PC. Although bodyweight gain recovered, this had significant implications for the frequency of R on pasture. Due to a lack of correlation between WBs (represented here by pepsinogen levels) and FEC there were very few individuals selected for treatment based on the combined criteria of pepsinogen and FEC. However, in this case, greater variation was observed in the BPR between simulated populations than for other determinant criteria.

4.5.3 *Comparison of strategies*

Upon assessing the best determinant criterion for the two described methods of selection for treatment contrasting patterns were observed. ADG was the best determinant criterion for treating individuals who cross a given threshold for treatment, in accordance with previous work on sheep (Cabaret et al., 2006; Greer et al., 2009; Chylinski et al., 2015). However, ADG was the worst determinant criterion when treating a fixed percentage of the population, in accordance with Laurenson et al. (2013a). This paradoxical difference between methods can be explained by the frequency and timing of treatment assessments. When treating calves according to threshold triggers more frequent assessments were made, ADG was a good early indicator of infection and hence by assessing individuals more frequently infection can be caught in the early stages preventing further reductions in ADG or the accumulation of PC. Only two assessments were made when treating fixed percentages of the population; by the second assessment treating calves that displayed the largest reductions in ADG had in general developed a strong immunity, implying little benefit was gained from treatment. Alternatively pepsinogen was the best criterion when treating a fixed percentage of calves, but the worst when treating individuals according to a threshold trigger. Pepsinogen relates closely to WB and abomasal damage providing a good indicator of individuals that are heavily parasitised and display a lack of immunity, and would

therefore benefit from treatment (Jennings et al., 1966; Armour and Bruce, 1974; Armour et al., 1979). However, this made pepsinogen a poor indicator when treating according to threshold triggers, being less effective than other determinant criteria at preventing a build-up of PC.

4.5.4 *Qualitative validation*

Where possible, comparisons were made between model predictions and reported experimental studies. Threshold trigger values for the determinant criteria of ADG and combined FEC and pepsinogen have been tested experimentally (Greer et al., 2010; McAnulty et al., 2011; Höglund et al., 2013a; O'Shaughnessy et al., 2014a; 2015a; 2015b). The model predicted treating calves according to threshold triggers for ADG to be the most beneficial strategy, in agreement with Höglund et al. (2009) who conducted a retrospective study on the feasibility of different TST determinant criteria and concluded ADG to be the most promising. In subsequent studies conducted to corroborate this prediction, Greer et al. (2010) made comparisons of two farms of dairy calves treated according to threshold triggers of ADG versus calves receiving routine treatment, with assessments made at monthly intervals. For groups treated according to TST, an average of 0.83 and 1.76 anthelmintic treatments per calf were required for the two farms respectively, representing an 84% and 65% reduction in anthelmintic usage compared to the control group. On both farms the TST groups showed larger within-group variations in bodyweight along with a reduction in ADG of 6% and 4% comparative to the control group routinely treated at monthly intervals. These observations relate well to model predictions: the simulated TST using threshold triggers of ADG required 1.72 treatments per calf and showed a 5% reduction in ADG relative to a non-parasitised calf. To make these comparisons on reduction in ADG it was necessary to assume that the experimental control group (given routine monthly treatment) showed similar ADG to what would be expected of a healthy calf. The method of Greer et al. (2010) was repeated by McAnulty et al. (2011) for two herds. Comparable to Greer et al. (2010) the first herd required 1.4 treatments per calf resulting in a 74% reduction in anthelmintic usage and a 5% reduction in ADG relative to the control group of calves (given routine monthly treatment), supporting model outputs. However, the outcomes on the second herd was less agreeable with model predictions; 3.7 treatments were required per calf representing a 47% reduction in anthelmintic usage and reductions in

ADG of 2% relative to the control group were achieved, emphasising the difficulty of making quantitative comparisons even when the same strategy is applied.

Further studies using ADG as a threshold trigger have been conducted for beef cattle. Höglund et al. (2013a) compared first grazing season bull calves subject to different treatment strategies. Calves were left untreated, routinely treated every 4 weeks, or treated by TST when the ADG was inferior to the ADG averaged over the poorest growing 50% of calves in the group receiving routine treatment every 4 weeks. A total of 0.6 treatments per calf were required, a 92% reduction in anthelmintic usage when compare with the control group. In general the experimental TST group showed bodyweight gains intermediate to those of untreated and routinely treated groups, but similar FEC to the untreated group. Similar patterns were also predicted by the model; when compared with untreated calves the TST group showed very similar FECs but an improved ADG, although the simulated reductions in bodyweight gain were not always as extreme as those observed in the experiment.

No studies exist investigating the sole use of FEC or pepsinogen as a trait for TST. Recent studies by O'Shaughnessy (2014a; 2015a; 2015b) have looked at implementing TST using combined pepsinogen and FEC thresholds, often with a third condition for treatment based on the presence of lungworm. In all studies a control group treated three times was included for comparison. O'Shaughnessy et al. (2014a; 2015b) found that no individuals reached both FEC and pepsinogen levels large enough to trigger threshold treatment. Similar to these studies the model predicted very low numbers of treatments required with 0.05 treatments needed per calf. However, O'Shaughnessy et al. (2015a) found 1.5 treatments were required per calf, a 50% reduction in anthelmintic usage of the control group, although only 0.5 were as a result of *O. ostertagi* markers with the majority due to lungworm. Although the reported studies are in good general agreement, there are many confounding variables and only qualitative comparison can be made. Model predictions are subject to the influence of factors such as climatic conditions, nutrition, management practices, presence of other infectious agents and the level of drug resistance, not all of which are described in the reported studies. For example, the low number of treatments required in the studies by O'Shaughnessy et al. (2014a; 2015b) was hypothesised to be a result of the low level PC experienced throughout the field trials. Additionally, many of the control groups used in

these studies represented more frequent treatments than would be recommended in practice (Höglund et al., 2013a).

4.5.5 ***Perspectives***

The developed model gives a detailed analysis of various control strategies formulated to ensure continued effective control of parasitism in the future, providing valuable insights that were previously absent in literature and considerable support for treating calves according to TST. Support was provided for treating fewer calves to help maintain *refugia* and more strongly for treating calves according to threshold trigger values, in particular for the determinant criterion ADG. Trigger thresholds may be considered more applicable across infection levels. For example, over-treatment of herds exposed to very low levels infections may be reduced. Treating according to ADG is beneficial not only in terms of treatment success, but also for ease of practical implementation. However, the modelled trigger threshold for ADG was calculated based on growth rates of their strategically treated counterparts. In practice a group of strategically treated calves would not be kept to calculate this threshold level. One way of overcoming this is by looking at growth trajectories of individual animals and treat animals that deviate from their own trajectory.

Our model focused on a first grazing season over 6 months however, many calves are kept for a second grazing season or more. Extension of the model to simulate calves over multiple grazing seasons would provide insights into the implementation of these strategies over a longer period. At the end of the first grazing season treatment strategies will have different effects on factors such as final PC, hypobiosis and immunity (Claerebout et al., 1999). All these have important implications for second grazing season calves in terms of infection dynamics, making this an important issue to address in terms of the sustainability of different control strategies.

In the model we developed a relationship between ivermectin activity, an anthelmintic widely used in cattle in the UK (Barton et al., 2006), and different *O. ostertagi* genotypes. This was required to determine the effect of treatment on the frequency of resistance alleles (R) within the nematode population. There is now strong evidence that the mechanism for ivermectin is complex and controlled by many alleles at separate loci (Gilleard, 2006; Prichard, 2007; Kotze et al., 2014). To avoid model complexity anthelmintic resistance was assumed to be conferred by two independent genes. There are many unknown factors

influencing the rate of anthelmintic resistance, for example the number of relevant alleles, the relative importance of various alleles (on drug efficacy and persistence activity), level of pre-existing alleles and the relative fitness of alleles on pasture (or within an untreated host), amongst others. Should alterations be made to these parameters it would be expected that the rate at which anthelmintic resistance develops would be affected (Barnes et al., 1995; Leathwick, 2013), although the same general principles and patterns would be expected to apply. For example, little indication exists in the literature as to the fitness of each genotype either on pasture or against anthelmintic treatment. Upon modelling a fitness cost associated with R alleles (either on pasture or within an untreated host) it was observed that the development of resistance was slowed, however the same general patterns were observed. Ultimately, the aim of the model was not to accurately predict the rate at which resistance occurs, but rather to compare the relative effect of a range of control strategies.

In conclusion, we have developed a simulation model that appears to be capable of predicting the consequences of TST on the performance and development of nematode resistance amongst calf populations. We suggest that the utility of the model is such that allows it to be extended to consider other strategies for reduction of the development of resistance, including different parasite species and host genotypes and variation in climatic influences on larval availability and grass growth.

Chapter 5: Modelling the impacts of different conditions on the development of targeted selective treatment strategies to control *Ostertagia ostertagi* infection in calves

5.1 Abstract

Targeted selective treatments (TST) aim to reduce the development of anthelmintic resistance by only treating those individuals that would benefit most from anthelmintic, according to certain phenotypic trait criteria. However, the consequences of TST have not been investigated under varied conditions. A previously developed mathematical model was applied to investigate the effects of varied initial pasture contamination and different stocking rates on: 1) the most 'beneficial' phenotypic trait used as an indicator for treatment selection and 2) the method of selection of calves exposed to *Ostertagia ostertagi*, i.e. treating a fixed percentage of the population with the lowest (or highest) indicator values versus treating individuals who exceed (or are below) a given indicator threshold. The indicators evaluated as determinant criteria were average daily gain (ADG), faecal egg counts (FEC) and plasma pepsinogen, versus random selection of individuals. Treatment success was assessed in terms of benefit per R (BPR), the ratio of average benefit in weight gain to change in frequency of resistance alleles R (relative to an untreated population). When treating a fixed percentage of the herd, plasma pepsinogen was the most beneficial determinant criterion under all conditions; in some cases this was found to be significantly better than random selection of individuals but in others significantly worse. When treating calves according to threshold values, ADG was found to be the most effective determinant criterion under all conditions, and also the most beneficial strategy overall. By increasing initial pasture contamination the benefits gained from treatment were less for all TST strategies, except treatments according to threshold values of FEC due to density-dependence effects. In general increasing stocking rates resulted in a greater BPR, up to a point whereby the extra benefit to bodyweight gain was not large enough to warrant the increase in resistance build-up. The point at which this was reached was largely dependent on the TST strategy in question. Overall, the model simulations support TST according to threshold values for ADG under all conditions, although further work is required before this recommendation can be made in practice.

5.2 Introduction

The recent emergence of anthelmintic resistance in gastrointestinal parasites of cattle (Sutherland and Leathwick 2011; Rose et al. 2015a) has resulted in an increased focus on alternative or complimentary methods to control parasitism and maintain or prolong anthelmintic efficacy. Targeted selective treatment (TST) has previously been proposed as a means to reduce selective pressure for drug resistance by only administering anthelmintics to those individuals that would most benefit from treatment, as opposed to treatment of the entire group (van Wyk et al., 2006). Individuals are identified for treatment using phenotypic traits indicative of their parasitic burden or parasitic tolerance. For example, individuals identified as having a high level of parasitism, or reduced performance as a result of parasitism, may be considered to require treatment. As such, untreated individuals will harbor parasitic burdens which will continue to contribute susceptible parasite genotypes to pasture (i.e. increasing *refugia*) and thus prolong anthelmintic efficacy by reducing selection pressure for anthelmintic resistance.

Two differing methods have previously been proposed for implementing TST strategies: 1) treatment of a fixed percentage of the herd according to a given phenotypic trait (Laurenson et al., 2013a), or 2) treatment of individuals that exceed a threshold value for a given phenotypic trait (Charlier et al., 2014). Experimental studies on TST in cattle have previously investigated the use of various phenotypic traits as determinant criteria for treatment. These include performance traits such as average daily bodyweight gain (ADG, kg/d) (Greer et al., 2010; McAnulty et al., 2011; Höglund et al., 2013a), and parasitic traits such as faecal egg count (FEC, eggs/g) and plasma pepsinogen (international units of tyrosine/litre) (O'Shaughnessy et al., 2014a; 2015a; 2015b).

To date no experimental studies have been conducted to directly compare the methods for implementing TST strategies or the phenotypic traits used as determinant criteria for treatment. A previous simulation study concluded treatment according to threshold values of ADG was most beneficial in maximising performance whilst minimising the build-up of resistance (Chapter 4). However, these simulations were only carried out for a conventional stocking rate of 5 calves/ha (AHDB, 2016a) and an initial pasture contamination (IL_0) of 200 infective L_3 larvae/kg DM (Larsson et al., 2007) which were assumed to be representative of 'average' conditions (i.e. most likely to occur). As previously demonstrated (Chapters 2 and 3), variation in IL_0 or stocking rate (as a key factor in herd management) have significant

consequences upon parasitological and epidemiological outputs. These outputs, in particular worm burden (WB) and pasture contamination (PC), are important factors expected to heavily influence the build-up of resistance. Hence, in order to conclude any one strategy as the most beneficial, a range of potential TST strategies must be tested over a multitude of conditions. Although this would prove experimentally difficult due to confounding variables, including weather conditions, a modelling approach allows us to make such comparisons.

Within TST strategies, comparisons between selection methods (fixed percentage or threshold treatments) and determinant criteria can be made by determining the benefit per R (BPR, kg/R) (Chapter 4; Laurenson et al., 2016). BPR is calculated as the ratio of the average benefit in weight gain resulting from treatment, relative to the change in frequency of R (resistance allele) resulting from treatment. BPR can thereby identify the selection method and determinant criterion resulting in the greatest productive gain per impact upon anthelmintic efficacy. As such, the aim of the current simulation study was to evaluate whether variations in the IL_0 or stocking rate had a significant effect on the best selection method for TST, or the best determinant criterion (phenotypic trait) on which to base treatment, as assessed by BPR.

It was hypothesized that there would be a greater benefit to TST treatment strategies when calves were exposed to a lower IL_0; this would be due to the fact that the immune response develops slower and therefore treatment may provide a greater benefit in weight gain with little difference in the build-up of R alleles on pasture. It was also hypothesised that only small differences would be observed in the relative benefit of each phenotypic trait used to determine treatment. When considering stocking rates, it was hypothesised that as stocking rate increased the greater potential for improvement in weight gain would outweigh the larger increase in frequency of resistance alleles on pasture. Again it was hypothesised that only small differences would be observed in the relative benefit of phenotypic traits used to define treatment.

5.3 Material and Methods

A mathematical model previously developed in Chapter 4 describes the impacts of *O. ostertagi* on a population of growing calves, taking into account variation in host phenotypes, host-parasite interactions, parasite epidemiology and anthelmintic resistance amongst nematode populations. In the current chapter this model was used to evaluate whether variations in IL_0 or stocking rate impacted upon recommendations in regards to the best selection method (fixed percentage or threshold treatments) and determinant criterion (phenotypic trait) for use in a TST strategy.

5.3.1 *Host-Parasite interactions*

An individual calf model was developed (Chapter 2). Between-animal variation was considered in calf intrinsic growth rate, body composition, maintenance requirements and calf immune reponse traits (rate of acquisition, as well as initial and final rates for the immune traits of establishment, mortality and fecundity) (Chapter 3).

5.3.2 *Epidemiological module*

An IL_0 was assumed as a result of overwintered eggs and larvae; subsequent larval contamination of pasture was assumed to arise from eggs excreted by infected calves. Development of excreted eggs into L_3 larvae was assumed to be temperature-dependent, as was larval mortality (Stromberg, 1997). The resulting larvae were assumed to be aggregated across pasture and consumed by calves, hence completing the parasitic lifecycle.

5.3.3 *Parasite anthelmintic resistance*

Anthelmintic resistance was considered for a single anthelmintic drug, ivermectin. Resistance was assumed to be controlled by 2 genes, assuming perfect gene and allele neutrality. Drug efficacy against susceptible nematodes was defined according to Yazwinski et al. (2009) and assumed to show a sigmoidal decay of ivermectin over time. Drug efficacy against the resistant genotype was assumed to be 0.01. The frequency of each genotype over time was calculated using the Hardy-Weinberg equilibria and all genotypes were assumed to be equally fit on pasture.

5.3.4 ***Treatment strategies***

Two methods of selection for TST were investigated: 1) dosing a fixed percentage of calves according to a given determinant criterion and 2) dosing calves that cross a threshold value for given determinant criterion. A summary of TST strategies investigated in provided in table 5.1.

Determinant criterion	Fixed percentage treated (10%, 25%)	Threshold treatments
ADG	✓	✓
FEC	✓	✓
Pepsinogen	✓	✓
Random	✓	-

Table 5.1: a summary of the different targeted selective treatment strategies included in model comparison.

5.3.4.1 TST based on fixed herd percentages

In a previous chapter (Chapter 4) it was predicted that the frequency of R on pasture increased exponentially with the percentage of calves treated. This resulted in a decreasing BPR for increasing percentages of the calves treated, meaning that the rapid build-up in frequency of R was not justified by the accompanying improvement in weight gain. For this reason the current study only investigated the consequences of treating 10% or 25% of the host population. Individual calves were assessed for treatment at 8 and 16 weeks post-turnout. Three determinant criteria for treatment were investigated; ADG, FEC and pepsinogen. These determinant criteria were selected as they are relatively non-invasive and provide a good indication of parasite load, or resulting compromised performance. As such, calves were preferentially treated according to lowest ADG, highest FEC or highest pepsinogen level. An additional comparison group was included whereby a fixed percentage of calves were selected for treatment at random; the relative success of each determinant criterion was subsequently evaluated in contrast to this.

5.3.4.2 TST based on threshold values

For treatments triggered by threshold values, the same three determinant criteria of ADG, FEC and pepsinogen were investigated. Assessment for treatment was made every 3 weeks from 8 weeks post-turnout. When ADG was used as the determinant criterion, a threshold value was calculated on the basis of the average ADG taken for the poorest growing 50% of the population of strategically treated calves (whole-herd treatment at 3, 8 and 13 weeks). Calves were treated when individual ADG was inferior to this threshold value. FEC threshold values of 200 eggs/g have previously been investigated, however these were for mixed infections (O'Shaughnessy et al., 2014a). For simplicity it was assumed that *O. ostertagi* eggs comprised a proportion of 0.4 (Hilderson et al., 1990; Ploeger and Kloosterman, 1993; Areskog et al., 2013b) hence the threshold was considered to be 80 eggs/g, where individuals displaying FECs above this were treated with anthelmintic. Populations treated according to threshold values for plasma pepsinogen were treated when levels exceeded 2 IUT/l (O'Shaughnessy et al., 2015b).

5.3.5 ***Simulation procedure and outputs***

Simulations were based on a herd of weaned and castrated male (steer) Limousin ×Holstein Friesians, a common cross of beef cattle reared in the UK (Todd et al., 2011). It was assumed the calves were autumn-born and capable of utilising grass in early spring; hence calves were turned out at 6 months of age and left on pasture for a further 6 months until housing in late autumn (Phillips, 2010). A population of 500 calves was simulated over the first grazing season; the same population was modelled for all treatment groups. All calves were assumed to have no prior exposure to parasites. The initial frequency of the recessive allele conveying anthelmintic resistance (R) was assumed to be 0.001 on pasture (Barnes and Dobson, 1990). All model simulations were programmed in Matlab (2015).

To investigate the impact of IL_0, starting values of 100, 200 or 500 *O. ostertagi* L_3/kg DM (Chapter 3) were simulated, and the grazing area was set to 100ha representing a conventional stocking rate of 5 calves/ha (AHDB, 2016a). These values were selected to cover the range of possible IL_0 levels that are likely to occur. For each IL_0 separate comparisons were made for each of the methods of selection for treatment. When treating a fixed percentage of calves, a 3x4x2 factorial design was used to compare the effects of IL_0 level, determinant criteria and the percentage of calves treated. When treating calves according to threshold values a 3x3 factorial design was used to compare the effects of IL_0 and determinant criteria.

To investigate TST strategies under differing stocking rates, IL_0 was set to 200 *O. ostertagi* L_3/kg DM, and the grazing area adjusted for low (3calves/ha), conventional (5 calves/ha) and high (7 calves/ha) stocking rates as defined by AHDB (2016a). As per the investigation into IL_0 separate comparisons were made for each method of selection for treatment. When treating a fixed percentage of calves a 3x4x2 factorial design was used to compare the effects of stocking rate, determinant criteria and the percentage of calves treated. When treating calves according to threshold values a 3x3 factorial design was used to compare the effects of IL_0 level and determinant criteria. The experimental design is summarised in table 5.2.

	Initial pasture contamination		
Stocking rate	Small	Medium	Large
Low	-	✓	-
Conventional	✓	✓	✓
High	-	✓	-

Table 5.2: Summary of the different conditions under which targeted selective treatment strategies were compared. These combinations were selected such that any changes predicted in the outputs could be attributed to a single factor in comparison to a population of calves kept at conventional stocking rates and exposed to a medium level of initial pasture contamination.

Model outputs were recorded on a daily basis. These included performance traits (population average of bodyweight (kg)), parasitological traits (population average of WB and FEC), epidemiological traits (PC (L_3/kg DM grass)) and anthelmintic resistance traits (Frequency of R). An overall assessment was made in terms of benefit per R (BPR). The patterns observed for populations of calves exposed to 200L_3/kg DM at a conventional stocking rate of 5 calves/ha have previously been described (Chapter 4) and therefore throughout the results section the focus of this chapter was to comment on any deviations from these patterns for differing IL_0 and stocking rates. These outputs were provided for both methods of selection (treatment according to fixed percentages and treatment according to threshold triggers). All determinant criteria showed broadly similar patterns when comparing the effect of treatment percentage, hence treatment percentages were compared on the basis of the determinant criterion of pepsinogen only for clarity. Pepsinogen was selected as previous simulations showed this to be the most beneficial determinant criterion (Chapter 4).

To make a comparison of the benefits gained from each determinant criteria when treating a fixed percentage of calves, a number of outputs were assessed in terms of their final predicted values at the end of the grazing season (day 180); these were: A) cumulative faecal egg counts as a measure of parasitism; B) relative reductions in bodyweight gain as a measure of performance; C) frequency of R on pasture as a measure of resistance and D) BPR value. When making a comparison of the benefits from each determinant criteria according to threshold values only BPR was considered. For outputs related to resistance (frequency of R on pasture and BPR) the output was a single measure for the complete pasture and therefore variation was estimated by simulating 10 populations for each treatment group. For each output a statistical comparison was conducted to assess whether determinant criteria showed a significantly different outcome to what would be expected by random selection. With the exception of relative reduction in bodyweight gain all outputs were normally distributed and therefore a two-tailed Z test was carried out. Relative reductions in bodyweight gains were assessed using the Mann-Whitney U test. Analyses were conducted for each of the experimental design groups as summarised in table 5.2. The P values for all statistical tests are provided in appendix E.

5.4 Results

5.4.1 *Initial Pasture Contamination*

5.4.1.1 TST based on fixed herd percentages

5.4.1.1.1 Comparison of treatment percentages

The impact of treating different percentages of calves was investigated for determinant criteria of ADG, FEC and pepsinogen and for selection at random for populations exposed to IL_0 of 100, 200 and 500 L_3/kg DM. At each IL_0 level the effect of treating a fixed percentage of calves appeared to be similar when compared for all determinant criteria, therefore in order to compare the effects of different treatment percentages the outputs are only shown for the determinant criterion of plasma pepsinogen (figure 5.1). The impact of treatment on reducing parasitological burdens was similar for all IL_0 levels. In each case greater reductions were observed when a larger percentage of calves were treated. When comparing the effects of treating 10% or 25% of the population it was predicted that the reduction in WB relative to the untreated population was smaller as IL_0 increased (figure 5.1A-C). These general patterns were reflected in FEC (figure 5.1D-F). For all IL_0 levels greater reductions in FEC were predicted when a larger percentage of calves were treated, but ultimately the final FEC achieved on day 180 by both treatment percentages was the same as for the untreated group. When assessing the effect of IL_0 following the immediate reduction in FEC a steeper gradient of increase in FEC was predicted for lower IL_0; this was consistent for both percentages of calves treated. It was also predicted that at the largest IL_0 the reduction in FEC relative to the untreated group was notably smaller than for other IL_0 levels.

Patterns of parasitological burdens were conveyed directly onto PC (figure 5.1G-I), expressed in terms of L_3/kg grass. It was predicted that treatment reduced PC in all cases with greater reductions predicted when a larger percentage of calves were treated, irrespective of IL_0. As per FEC, the final PC was the same for all treatment groups when compared within IL_0groups. When comparing the effects of each treatment percentage at differing IL_0 levels it was predicted that the magnitude of the reduction at high IL_0 was smaller than at low and medium IL_0. When assessing the effects on performance it was predicted that the relative reduction in bodyweight gain in comparison to a healthy (non-parasitised) population was smaller when a larger percentage of calves were treated (figure 5.1J-L). This prediction was seen for all IL_0 levels. When comparing the effect of IL_0 it was predicted that similar final

reductions in bodyweight gain compared to a healthy (non-parasitised) population were achieved for each treatment percentage, irrespective of IL_0. However, a greater improvement in performance was predicted compared with the untreated group of calves as IL_0 decreased. This was consistent for both treatment percentages.

The frequency of resistant (R) alleles in the nematode population on pasture (figure 5.1M-O) increased when a larger percentage of calves were treated. IL_0 was found to have little effect on the frequency of R with minimal differences in the magnitude of change when compared at either treatment percentage.

5.4.1.1.2 Comparison between determinant criteria

The determinant criteria on which fixed percentages of the herd were selected for treatment were compared for populations exposed to IL_0 levels of 100, 200 and 500 L_3/kg DM. Comparisons were made on the basis of cumulative FEC, reduction in bodyweight (relative to non-parasitised group), frequency of R on pasture and BPR (figure 5.2) when 10 or 25% of the population was treated. A statistical comparison was made of the benefits of treatment for each of the outputs by comparing treatment according to each determinant criterion to treatment according to random selection.

Figure 5.2A-C shows the population average and standard error of cumulative FEC; upon comparing determinant criteria when 10% of calves were treated, similar patterns were predicted for all IL_0. In all cases the determinant criterion of ADG resulted in the smallest cumulative FECs, followed by FEC and finally pepsinogen and random selection which were predicted to show similar values. However, none of these predictions were significant. When treating 25% of the population, determinant criteria of ADG and subsequently FEC resulted in the smallest cumulative FEC for all IL_0 levels. Treatment according to pepsinogen and random selection resulted in the largest cumulative FECs; as IL_0 increased, random selection became progressively worse at reducing cumulative FEC relative to other determinant criteria. Consequently when comparing each determinant criterion to random selection for treatment, significantly lower cumulative FECs were predicted for determinant criteria of ADG ($P<0.001$) and FEC ($P<0.05$) for medium and large IL_0, but only for ADG ($P<0.01$) at high IL_0.

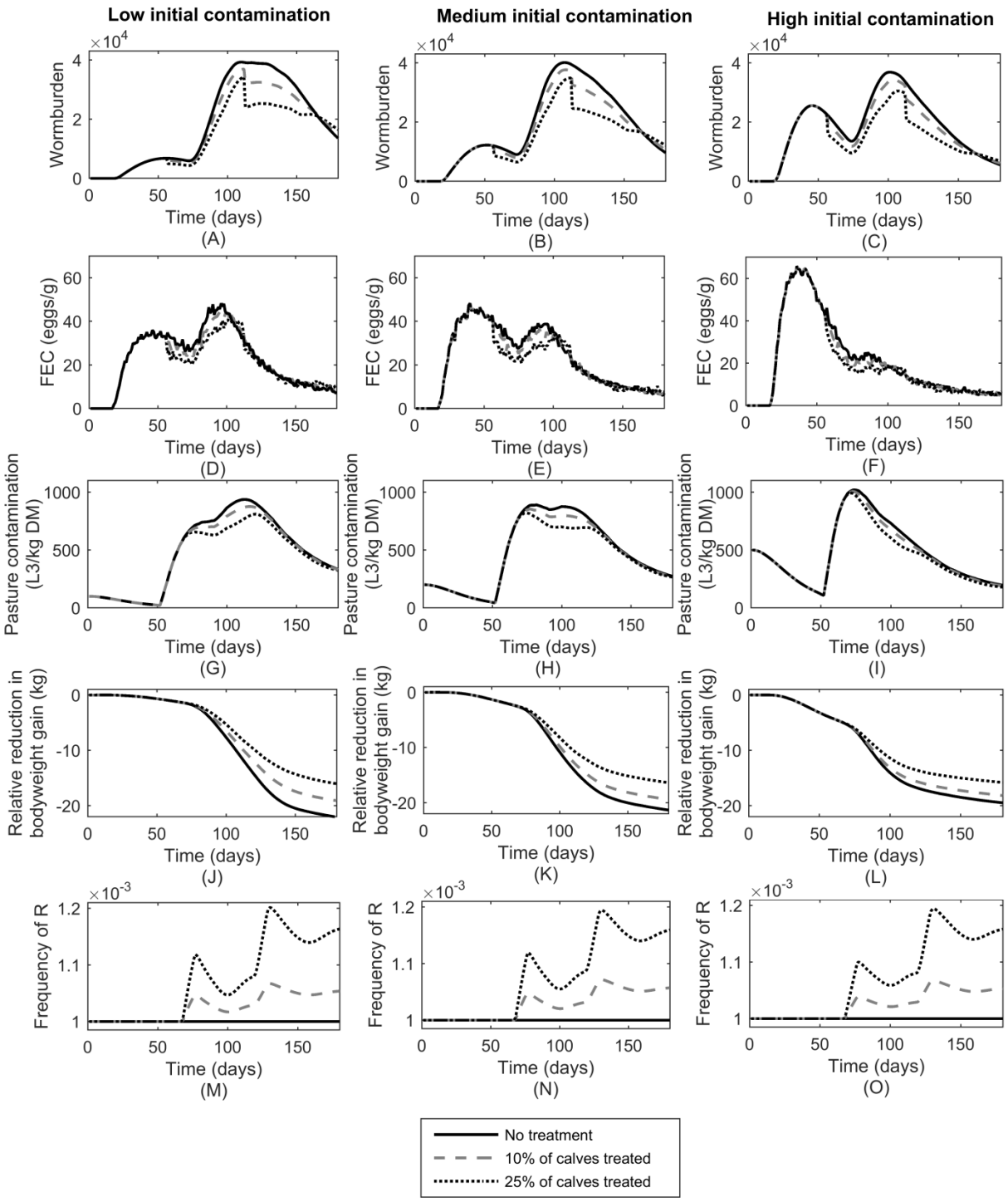

Figure 5.1: The effect of initial pasture contamination on targeted selective treatments (TST) of populations of calves. TST Predictions for groups of calves either left untreated, or treated at weeks 8 and 16 according to highest plasma pepsinogen level (IUT/l) when a percentage of 10% or 25% of a herd of 500 calves grazing on pasture initially contaminated with 100, 200 and 500 L_3/kg DM grass were treated with ivermectin. The population averages are presented for outputs of worm burden (A-C); FEC (eggs/g) (D-F); pasture contamination (G-I); reduction in bodyweight gain relative to a non-parasitised population (kg) (J-L) and the frequency of R on pasture (M-O).

The consequences of parasitism on performance in terms of final reductions in bodyweight gain relative to a healthy (non-parasitised) population was assessed for each treatment percentage at different IL_0 levels as shown in figure 5.2(D-F). When treating 10% of the population it was predicted that under low and medium IL_0 treatment according to FEC resulted in the smallest reduction in bodyweight gain relative to a healthy (non-parasitised) population, followed by treatment according to pepsinogen. As IL_0 increased treatment according to ADG became progressively better at preventing reductions in bodyweight gain comparative to other determinant criteria; however none of these trends were statistically significant. When treating 25% of the population IL_0 had a greater impact on reductions in bodyweight gain. In contrast to cumulative FEC predictions, treatment according to random selection showed smaller reductions in bodyweight gain (relative to a non-parasitised population) than pepsinogen at low IL_0 levels; however pepsinogen became progressively better at preventing reductions as IL_0 increased. Conversely the determinant criterion of FEC became progressively worse at preventing reductions in bodyweight gain relative to other determinant criteria. This was reflected in pepsinogen being significantly better than random selection when calves were exposed to the largest IL_0 ($P<0.05$) and FEC at medium IL_0 ($P<0.05$).

Figure 5.2 (G-I) shows the final changes in frequency of R at the end of the grazing season for each treatment percentage at different IL_0 levels. Under all conditions (i.e. all IL_0 levels and treatment percentages) the determinant criteria of ADG and FEC resulted in significantly higher frequencies of R than random selection ($P<0.001$). When treating 10% of the population, pepsinogen was found to show a greater increase in frequency of R than random selection, although this was only significant for the largest IL_0 ($P<0.05$). When treating 25% of the population, again it was observed that pepsinogen resulted in a greater increase in frequency of R than random selection for medium and large IL_0. However in contrast to treating 10% of the population, when treating 25% of the population according to pepsinogen significant reductions in the frequency of R (in comparison to random selection) were only predicted for low IL_0 ($P<0.05$). Although significant in some cases, these differences between pepsinogen and random selection were marginal.

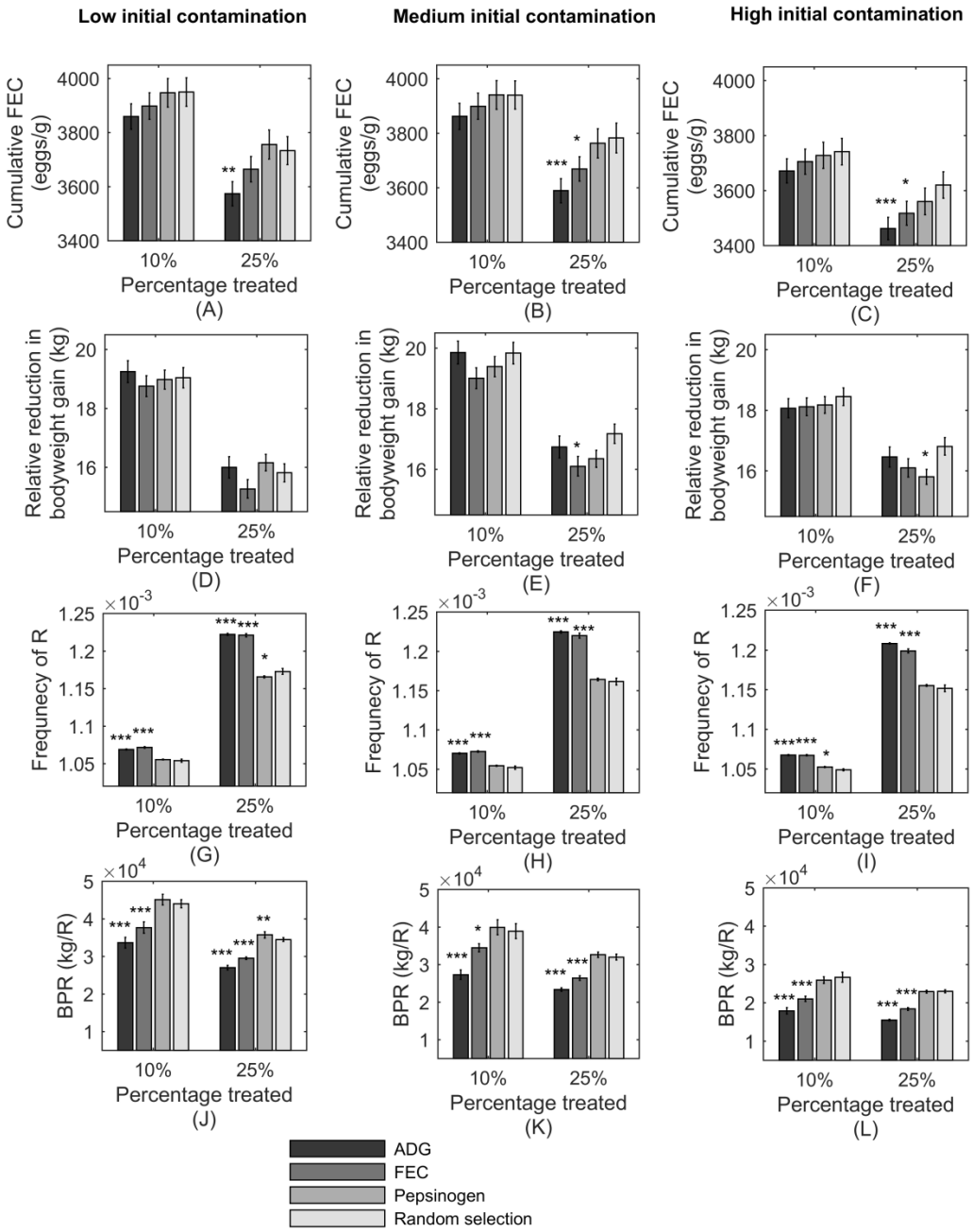

Figure 5.2: End of season (day 180) predictions for populations of 500 calves grazing on pasture initially contaminated with 100, 200 and 500 L_3/kg DM grass for cumulative faecal egg count (eggs/g) (A-C), relative reduction in bodyweight gains (kg) in comparison to a non-parasitised population (D-F), frequency of R on pasture (G-I), and benefit per R (BPR) representing the benefit in bodyweight gain (kg) per change in frequency of R(J-L). Anthelmintic treatment was administered at weeks 8 and 16 to either 10 or 25% of the population according to lowest average daily bodyweight gain (ADG, kg/d), highest faecal egg count (FEC, eggs/g), highest pepsinogen (IUT/l) or selected at random. Predictions for frequency of R on pasture are provided as an average of ten simulations.
(* $p<0.05$; ** $p<0.01$; *** $p<0.001$)

The benefit in average weight gain per change in frequency of R (BPR (kg/R) is presented in figure 5.2 (J-L). BPR values were compared for both treatment percentages at different IL_0 levels. Similar patterns were observed for both treatment percentages. When treating 10% of the population, patterns were similar in all cases with the determinant criteria of ADG ($P<0.001$) and FEC ($P<0.001$; $P<0.05$) being significantly worse than random selection. In general there was no significant difference between pepsinogen and random selection; however it was predicted that as IL_0 increased pepsinogen became less beneficial when compared to random selection. This was highlighted by pepsinogen only showing significantly better BPR than random selection when 25% of the population was treated at low IL_0. When treating 25% of the population the same patterns were observed as for 10% of the population.

5.4.1.2 TST based on threshold values

5.4.1.2.1 Comparison of determinant criteria

The impact of defining a threshold level for treatment for the different determinant criteria of ADG, FEC and pepsinogen was assessed when calves were exposed to IL_0 levels of 100, 200 and 500 L_3/kg DM. The outputs are given in figure 5.3. Parasitological measures of WB (figure 5.3A-C) and FEC (figure 5.2D-F) were broadly similar across IL_0 levels, however some differences were evident. For the untreated groups it was predicted that the high IL_0 resulted in a large initial increase in infection; however, the second wave of infection was reduced more rapidly as a result of a more strongly developed immune response. Consequently, PC at 100 days was less for high IL_0 than the low and medium IL_0 resulting in differences in the patterns predicted. For all IL_0 levels treatments administered according to threshold values for pepsinogen showed the largest reductions in WB, followed by determinant criteria of ADG and finally FEC. The determinant criterion of ADG showed similar peak WBs for all IL_0 levels; however, the other determinant criteria were affected more strongly. When comparing treatment according to threshold values of FEC, smaller reductions in WB were predicted as IL_0 increased. Treating calves according to threshold triggers of pepsinogen showed similar reductions in WB for low and medium IL_0; high IL_0 on the other hand, resulted in a greater frequency of treatments administered early on in infection, hence preventing large WBs being achieved.

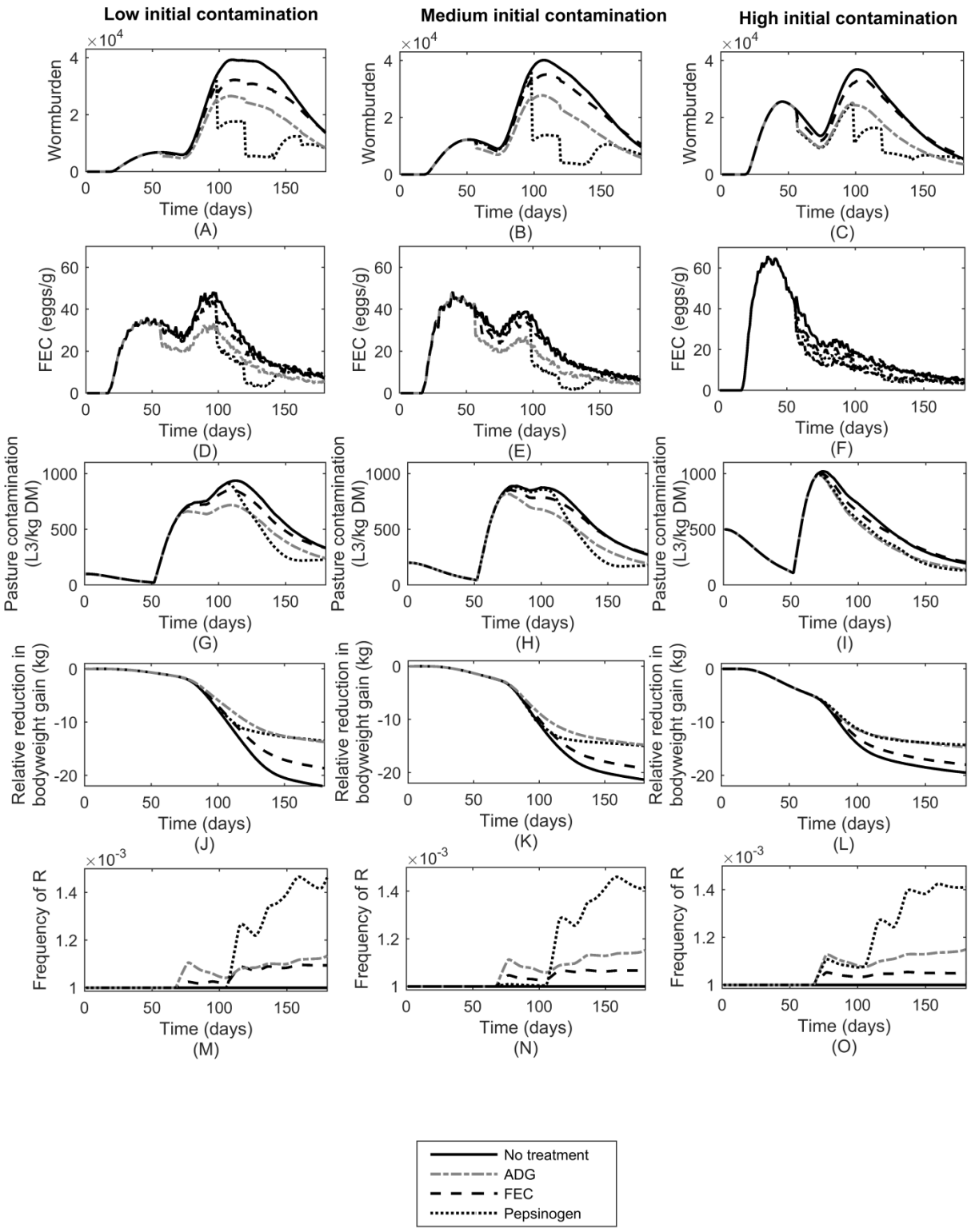

Figure 5.3: The effect of initial pasture contamination on targeted selective treatments (TST) of populations of calves. TST Predictions for groups of calves either left untreated, or treated with ivermectin according to threshold values for different determinant criteria of average daily bodyweight gain (ADG, kg/d), faecal egg count (FEC, eggs/g) or pepsinogen (IUT/l); were made for a herd of 500 calves grazing on pasture initially contaminated with 100, 200 and 500 L_3/kg DM grass. The population averages are presented for outputs of worm burden (A-C); FEC (eggs/g) (D-F); pasture contamination (G-I); reduction in bodyweight gain relative to a non-parasitised population (kg) (J-L) and the frequency of R on pasture (M-O).

The patterns described for WB were directly reflected in FEC. Low and medium IL_0 showed similar patterns; the determinant criterion of ADG showed reductions in FEC from early on in the course of infection hence reducing the second peak. Treatment according to threshold values for FEC showed only small reductions in FEC throughout infection. No reductions in FEC were predicted for populations treated according to threshold pepsinogen values until 98 days post-turnout at which point large reductions occurred. At the end of the grazing season all determinant criteria resulted in the same final FEC. Populations exposed to high IL_0 showed broadly similar patterns, however smaller reductions in FEC due to treatment were predicted for all determinant criteria. It was also observed that FEC were reduced from 56 days post-turnout as a result of threshold treatments according to pepsinogen.

Parasitological patterns were reflected directly onto PC (figure 5.3G-I); all determinant criteria resulted in a reduction in PC at all IL_0 levels. Low and medium IL_0 were again predicted to show similar patterns; in both cases treatment according to FEC resulted in small decreases in PC. Treatment according to threshold ADG showed the largest reduction in PC following the first assessment for treatment at 8weeks post-turnout, until approximately 110 days post-turnout. At this point, groups treated according to pepsinogen thresholds showed a sudden large decrease in PC, resulting in the lowest PC until the final stages of the grazing season. Ultimately, treatment according to threshold values of ADG and pepsinogen resulted in the same final PC. Populations exposed to high IL_0 on the other hand experienced smaller reductions in PC as a result of treatment when compared for all determinant criteria, in particular for FEC. The effects of the determinant criteria ADG and pepsinogen were indistinguishable from one another for high IL_0.

The effect of each determinant criterion on performance was assessed in terms of the reduction in bodyweight gain relative to a healthy (non-parasitised) population (figure 5.3J-L). As per parasitological and epidemiological patterns the improvements in bodyweight gain was similar for low and medium IL_0 levels. Of all the determinant criteria, FEC resulted in the largest reductions in bodyweight gain compared to a non-parasitised population. Treatment according to pepsinogen showed large reductions in the early stages of infection, however in the latter stages ADG and pepsinogen showed similar reductions in bodyweight gain relative to a non-parasitised population. When assessing the benefit to bodyweight gain from treatment by comparing the bodyweight reduction for untreated and treated groups it was predicted that populations exposed to high IL_0 showed less benefit than compared to

treatment under all determinant criteria with a medium and large IL_0. Treatment according to FEC showed the largest reduction in bodyweight gain compared to a non-parasitised population. As per PC it was difficult to distinguish between the effects of the determinant criteria of pepsinogen and ADG during the course of infection, with both ultimately resulting in the same final reduction in bodyweight gain.

Similar patterns were observed across all IL_0 levels for the build-up of frequency of R on pasture (figure 5.3M-O); the determinant criteria of pepsinogen showed the largest frequency of R, followed by ADG and finally FEC. The final frequencies of R were similar across IL_0 for all determinant criteria, with the exception of FEC. When treating calves according to threshold values of FEC, a smaller increase in frequency of R was predicted as IL_0 increased, this was highlighted at high IL_0.

Finally, BPR was evaluated for all IL_0 levels (figure 5.4). It was evident that there was less benefit to treatment as IL_0 increased, largely a result of the impacts of treatment on bodyweight gain. In all cases it was predicted that ADG was the most beneficial determinant criteria, followed by FEC and finally pepsinogen. Although treating populations according to ADG thresholds appeared to be the most beneficial strategy at all IL_0 levels, the relative advantage compared to the next best determinant criteria of FEC was smaller as IL_0 increased.

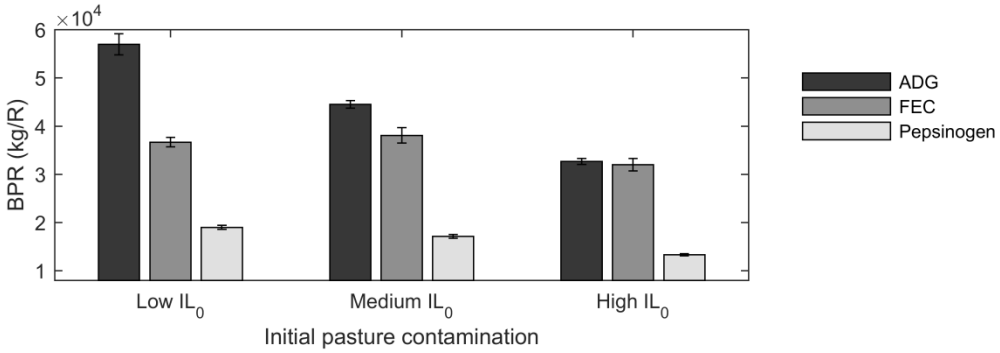

Figure 5.4: Benefit per R (BPR) simulated at the end of the grazing season (day 180) on a population basis for each of the simulated control strategies; BPR represents the benefit in bodyweight gain (kg) per change in frequency of R on pasture, so the higher the value the more beneficial the strategy is perceived to be. Ten discrete populations of calves were simulated on pasture initially contaminated with (A) 100, (B) 200 or (C) 500 L_3/kg DM grass for calves treated according to threshold values according to determinant criteria of average daily bodyweight gain (ADG, kg/d), faecal egg count (FEC, eggs/g) and pepsinogen.

5.4.2 ***Stocking Rate***

5.4.2.1 TST based on fixed herd percentages

5.4.2.1.1 Comparison of treatment percentages

The impact of treating different percentages of calves (10% or 25%) was investigated for determinant criteria of ADG, FEC and pepsinogen and random selection for calves kept at stocking rates of 3, 5 and 7 calves/ha. At all stocking rates the outcomes for different treatment percentages were similar for all determinant criteria; for this reason outputs are given only for plasma pepsinogen (figure 5.5). In all cases the parasitological traits of WB (figure 5.5A-C) and FEC (figure 5.5D-F) were reduced by treatment to a greater extent when a larger percentage of calves were treated. When comparing the effect of stocking rates on the outcome of treating a fixed percentage of calves, greater reductions in peak WB were predicted as stocking rates increased, albeit proportional to the greater size of the WB in the untreated populations at their respective stocking rates. When assessing the effect of treatment on FEC it was predicted that treating a larger percentage of calves resulted in greater reductions. This was the case for all stocking rates; similar FEC were predicted for untreated groups at each stocking rate, therefore treatment groups tended to show similar FECs when compared across stocking rates. However when treating 10% of the population, FECs appeared to be reduced to a greater extent at high stocking rates when compared with low and conventional stocking rates.

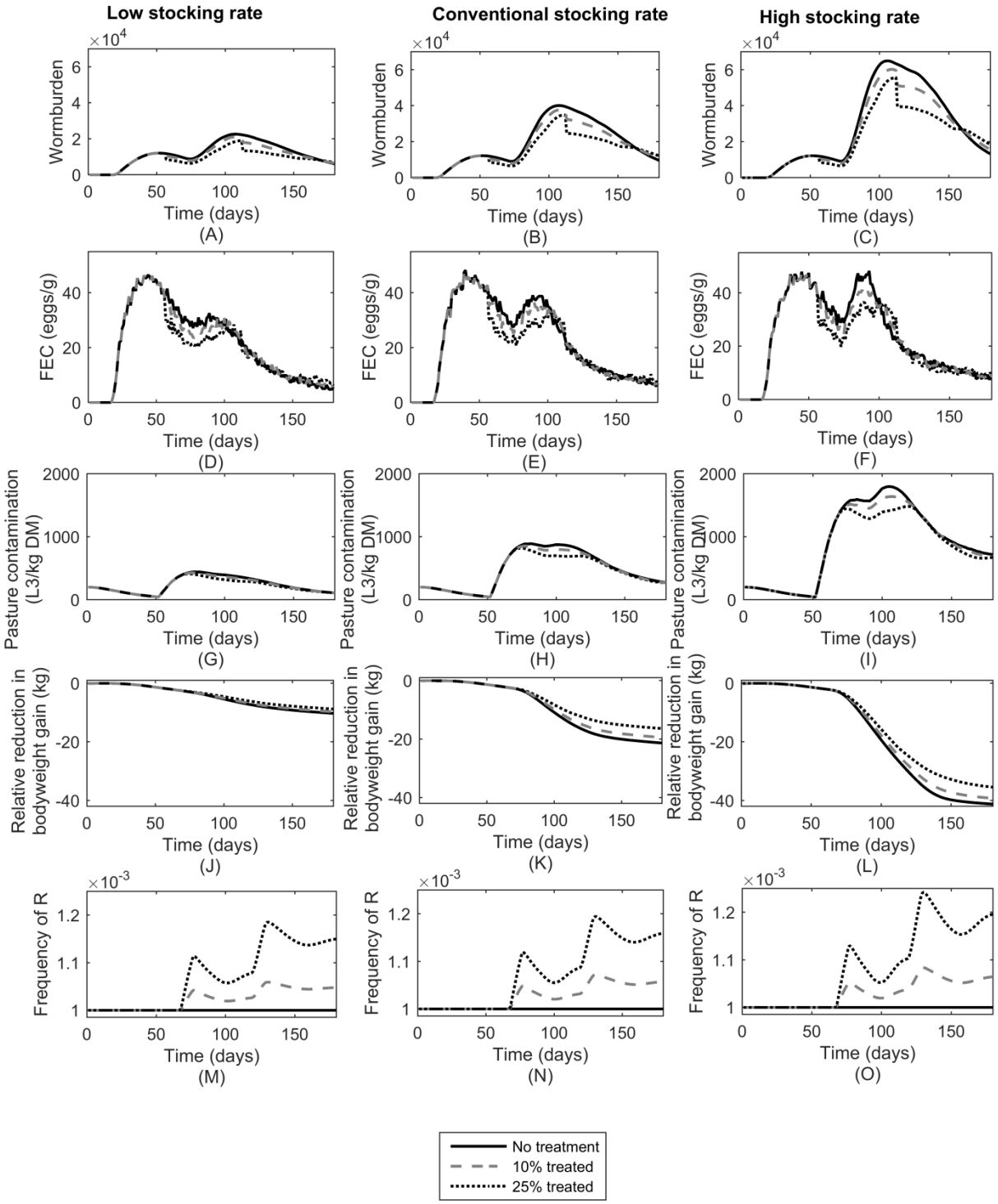

Figure 5.5: The effect of stocking rates on targeted selective treatments (TST) of populations of calves. TST Predictions for groups of calves either left untreated, or treated at weeks 8 and 16 according to highest plasma pepsinogen level (IUT/l) when a percentage of 10% and 25% of a herd of 500 calves grazing on pasture initially contaminated with 200 L_3/kg DM grass were treated with ivermectin. Calves were kept at a stocking rate of 3, 5 and 7 calves per hectare, the population averages are presented for outputs of A) worm burden (A-C); FEC (eggs/g) (D-F); pasture contamination (G-I); reduction in bodyweight gain relative to a non-parasitised population (kg) (J-L) and the frequency of R on pasture (M-O).

The patterns predicted for PC (figure 5.4G-I) reflected those described for WBs; greater reductions in PC were predicted when a larger percentage of calves were treated. When comparing the effect of stocking rate on PC, greater reductions were predicted as stocking rate increased, although again these were proportional to the peak PC for untreated groups of calves at their respective stocking rates. Patterns of parasitological and epidemiological traits influenced those of performance, assessed in terms of the relative reduction in bodyweight gains compared with a healthy (non-parasitised) population (figure 5.3J-L). When comparing treatment percentages, it was predicted that treating a smaller percentage resulted in greater reductions in bodyweight gain. Upon assessing the effects of stocking rate it was predicted that when comparing the relative reductions in bodyweight gain for a group of untreated calves to those treated according to fixed percentages, there was a larger improvement as a result of treatment for increasing stocking rates; however this was a result of the greater potential for improvement.

Frequency of resistant (R) alleles in the nematode population at pasture (figure 5.5M-O) increased when a larger percentage of calves were treated. When comparing each treatment percentage at different stocking rates, it was predicted that as stocking rate increased the frequency of R also increased.

5.4.2.1.2 Comparison of determinant criteria

Comparisons between determinant criteria were made on the basis of cumulative FEC, reduction in bodyweight relative to a healthy (non-parasitised) population, frequency of R on pasture and BPR (figure 5.6). A statistical comparison was made of the benefits of treatment for each of the outputs by comparing treatment according to each determinant criterion to treatment according to random selection.

Figure 5.6A-C shows the population average and standard error of cumulative FEC; upon comparing the determinant criteria when 10% of the population was treated it was predicted that ADG showed the largest reduction in cumulative FEC, followed by treatment according to FEC for all stocking rates. At low stocking rates treatment according to the determinant criterion of pepsinogen appeared to show the largest cumulative FEC, however treatment according to pepsinogen resulted in progressively smaller cumulative FEC as stocking rate increased when compared with random selection. None of these patterns were significant. When treating 25% of the population at conventional and high stocking rates the determinant criteria of ADG ($P<0.001$) and FEC ($P<0.05$) showed significantly lower

cumulative FECs than would be expected by random selection. Although not significant, this was also the case for treatment according to pepsinogen. However at the low stocking rate, random selection showed smaller cumulative FECs than groups treated according to pepsinogen; consequently only ADG showed a significantly larger reduction than random selection for treatment ($P<0.001$).

Figure 5.6(D-F) shows the consequences of parasitism on performance, in terms of the final reduction in bodyweight gain compared with a healthy (non-parasitised) population, for different stocking rates. Upon comparing determinant criteria when 10% of the population was treated there were no distinguishable differences between determinant criteria at the low stocking rate due to the small potential for improvement in bodyweight gain. When comparing the remaining two stocking rates similar patterns were predicted; calves treated according to FEC appeared to show the smallest reductions in bodyweight gain and random selection the largest, however these differences were not significant. Upon comparing determinant criteria when 25% of the population were treated a significant difference from random selection was observed for FEC and pepsinogen ($P<0.05$) at high stocking rates, and for FEC ($P<0.05$) at the conventional stocking rate. Differences in the overall bodyweight were marginal; it is likely that these were only considered significant due to the distributions of bodyweight improvements within the population picked up by a non-parametric test.

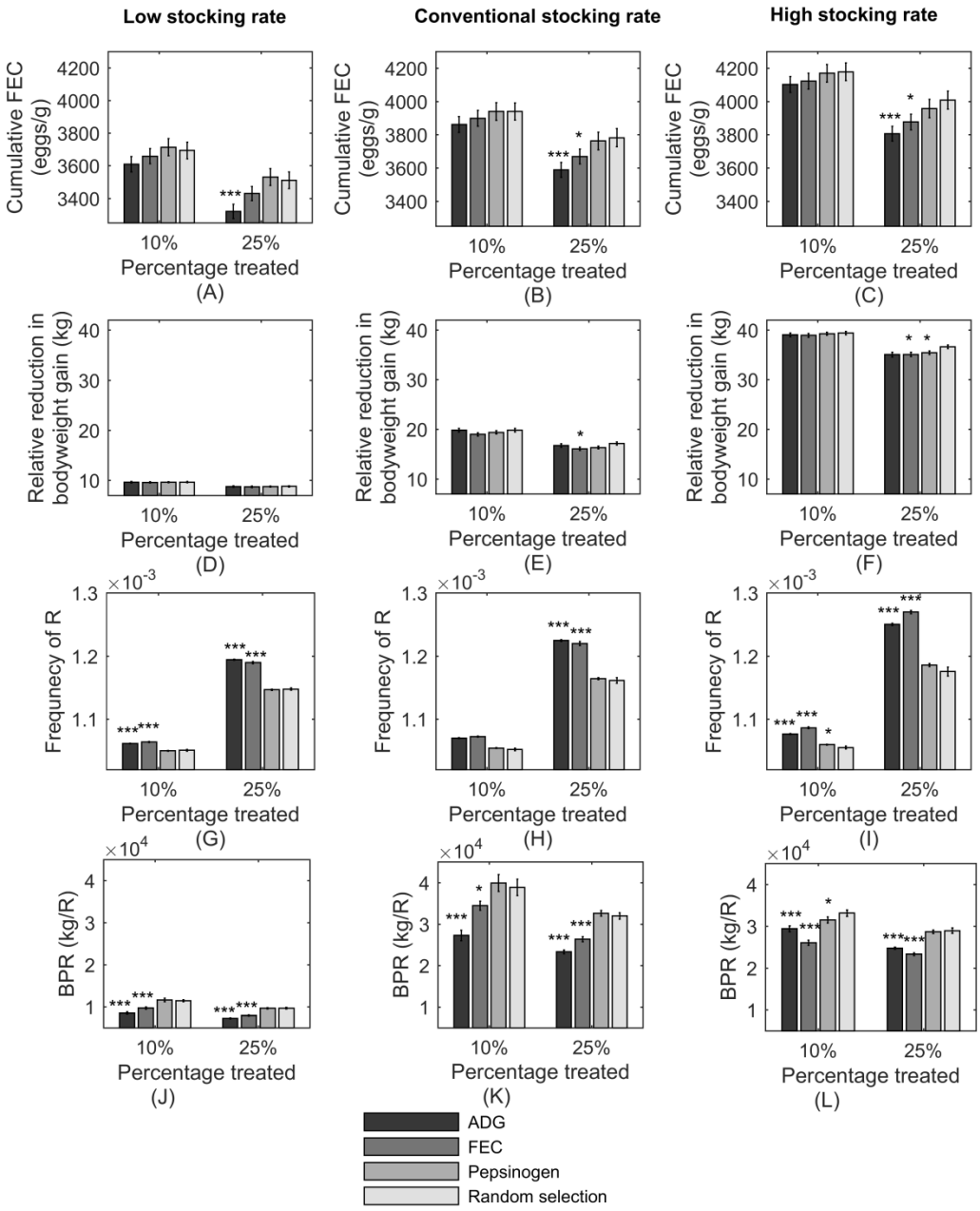

Figure 5.6: End of season (day 180) predictions for populations of 500 calves kept at stocking rates of 3, 5 and 7 calves/ha and grazing on pasture initially contaminated with 200 L_3/kg DM grass for cumulative faecal egg count (eggs/g) (A-C), relative reduction in bodyweight gains (kg) in comparison to a non-parasitised population (D-F) and frequency of R on pasture (G-I). Anthelmintic treatment was administered at weeks 8 and 16 to either 10 or 25% of the population according to lowest average daily bodyweight gain (ADG, kg/d), highest faecal egg count (FEC, eggs/g), highest pepsinogen (IUT/l) or selected at random. Predictions for frequency of R on pasture are provided as an average of ten simulations. (* $p<0.05$; ** $p<0.01$; *** $p<0.001$)

Figure 5.6 (G-I) shows the changes in the final frequency of R at the end of the grazing season for treated populations at different stocking rates. Determinant criteria were compared when 10% of the population was treated; ADG and FEC showed significantly greater frequencies of R than random selection ($P<0.001$) for all stocking rates. The determinant criterion FEC appeared to show the largest frequencies of R across all stocking rates. Pepsinogen and random selection showed similar final frequencies of R with the exception of high stocking rates where pepsinogen showed a significantly greater frequency of R than random selection ($P<0.05$). When treating 25% of calves determinant criteria ADG and FEC were again predicted to show a significantly greater frequency of R than random selection ($P<0.001$) for all stocking rates. However at low and conventional stocking rates ADG resulted in the largest frequency of R whereas at high stocking rate FEC resulted in the greatest frequency of R. Similar to patterns predicted in cumulative FEC, treatment according to pepsinogen showed increasingly larger frequencies of R compared to random selection as stocking rate increased, however these patterns were not significant.

Finally, BPR was assessed (figure 5.6J-L); upon comparing determinant criteria when 10% of the population was treated it was predicted that the determinant criteria of ADG ($P<0.001$) and FEC ($P<0.001$; $P<0.05$) were significantly worse than random selection for all stocking rates. At low and conventional stocking rates treatment according to FEC appeared to be more beneficial than ADG, however the converse was true at the high stocking rate. When comparing treatment according to pepsinogen to random selection it appeared that at low stocking rate differences were negligible, at conventional stocking rate pepsinogen was more beneficial and at high stocking rate random selection was more beneficial. However, the difference was only significant at high stocking rates ($P<0.05$). Upon comparing determinant criteria when 25% of the population was treated the same patterns were predicted, however pepsinogen was not significantly different from random selection for any stocking rate.

5.4.2.2 TST based on threshold values

5.4.2.2.1 Comparison of determinant criteria

The impact of defining a threshold level for treatment for the different determinant criteria of ADG, FEC and pepsinogen was assessed for calves kept at stocking rates of 3, 5 and 7 calves/ha. The outputs are given in figure 5.7. Parasitological traits of WB (figure 5.7A-C) and FEC (figure 5.7D-F) were reduced by all determinant criteria used for threshold treatments. In general, treating calves according to threshold values of pepsinogen was found to show the largest reductions in WB, followed by treatment according to ADG and finally FEC. However, for calves kept at low stocking rates the determinant criterion of pepsinogen showed smaller reductions relative to other determinant criteria. This was due to very few individuals in the low stocking rate group crossing the pepsinogen threshold for treatment (due to low WBs), whereas in the high stocking rate group large numbers of individuals crossed the threshold. For all determinant criteria it was clear that as stocking rate increased more treatments were required, and therefore larger reductions in WB were predicted. However, this was in proportion to the larger parasitological burdens experienced with increasing stocking rate.

Patterns of WB were reflected in FEC, low stocking rates showed the largest reduction for determinant criteria of ADG followed by FEC and pepsinogen. Ultimately, all determinant criteria reached similar final FECs. Conventional and high stocking rates shared a similar pattern; the determinant criterion of FEC showed the smallest reduction in FEC whilst the determinant criterion of ADG showed the largest reductions in FEC up until approximately 98 days post-turnout. At this point groups treated according to threshold values for pepsinogen showed dramatic reductions in FEC. The observed patterns in PC (figure 5.7 G-I) were also very similar between conventional and high stocking rates, mirroring the pattern observed in FEC. At low stocking rates little difference in PC was observed between any of the determinant criteria due to the low numbers of treatments administered.

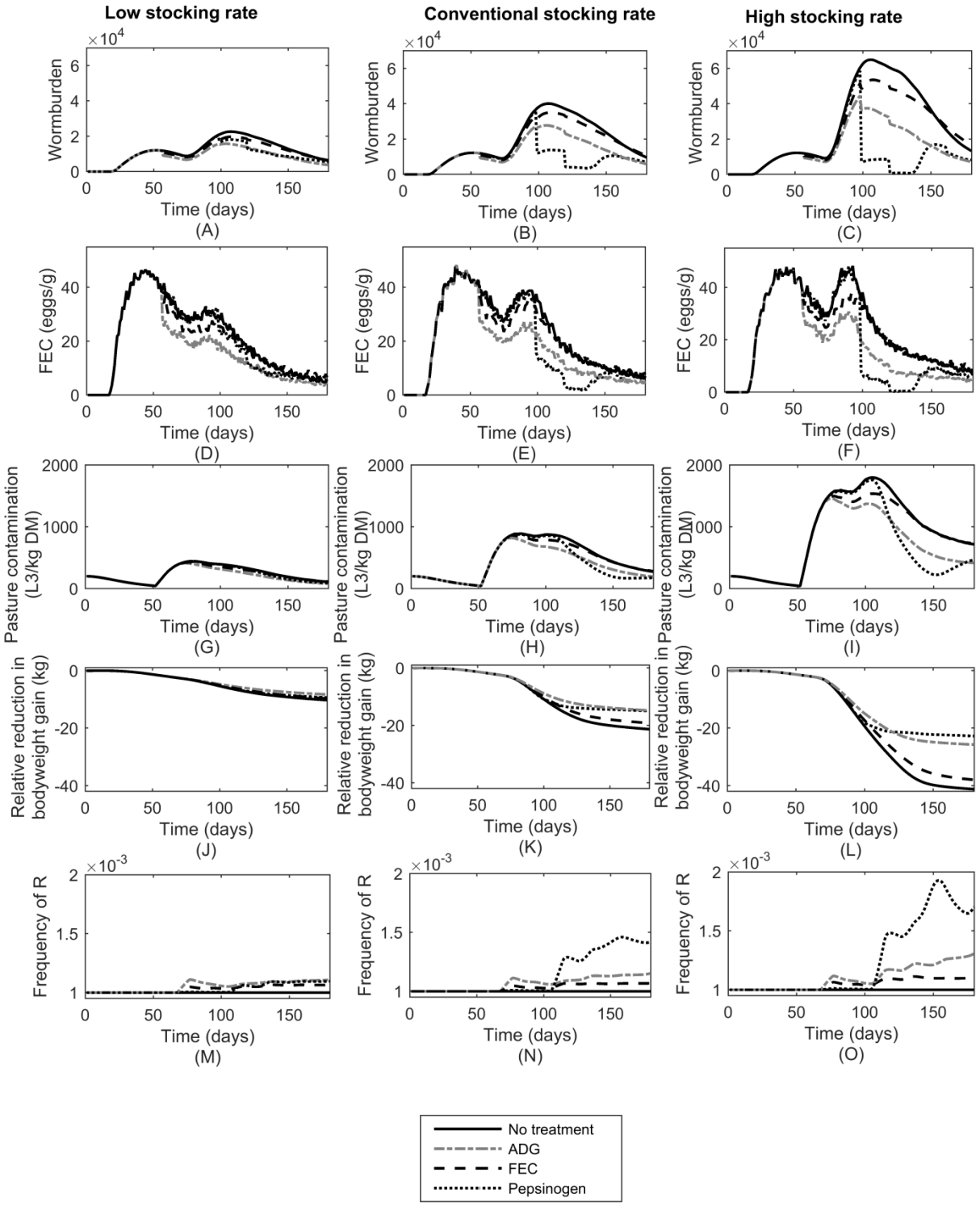

Figure 5.7: The effect of stocking rates on targeted selective treatments (TST) of populations of calves. TST Predictions for groups of calves either left untreated, or treated with ivermectin according to threshold values for different determinant criteria of average daily bodyweight gain (ADG, kg/d), faecal egg count (FEC, eggs/g) or pepsinogen (IUT/l); were made for a herd of 500 calves grazing on pasture initially contaminated with 200 L_3/kg DM grass respectively. Calves were kept at a stocking rate of 3, 5 and 7 calves per hectare, the population averages are presented for outputs of worm burden (A-C); FEC (eggs/g) (D-F); pasture contamination (G-I); reduction in bodyweight gain relative to a non-parasitised population (kg) (J-L) and the frequency of R on pasture (M-O).

The effect of parasitism on the reduction in bodyweight gain relative to a healthy (non-parasitised) population was assessed for different stocking rates. In all cases the determinant criterion of FEC showed the largest reduction in bodyweight gain, whilst the determinant criteria of ADG and pepsinogen showed similar outcomes. It was evident that the effect of stocking rates on treatment according to pepsinogen was disproportionately large compared to other determinant criteria; pepsinogen became progressively better than other determinant criteria at improving bodyweight gain as stocking rate increased. Upon assessing the improvement in bodyweight gained from treatment when compared to reduction in bodyweight gain achieved by an untreated population it was evident that larger stocking rates were accompanied by greater improvements; however there was also greater potential for improvement.

As would be expected the calves kept at higher stocking rates required more treatments when threshold levels were used. Consequently as stocking rate increased the frequency of R alleles increased, as did the relative differences between determinant traits. In general the determinant criterion of pepsinogen showed the largest frequency of R, followed by the determinant criteria of ADG and finally FEC. However, at low stocking rate the final frequency of R resulting from treatment according to pepsinogen and ADG was indistinguishable. As per the reduction in bodyweight gain compared to a healthy (non-parasitised) population it was observed that pepsinogen was affected more severely by stocking rates than other determinant criteria; the magnitude of the increase was disproportionately large as stocking rates increased.

Subsequently BPR was evaluated for determinant criteria across stocking rates (figure 5.8). In all cases treating populations according to the determinant criteria of ADG was most beneficial, followed by FEC and finally pepsinogen. For the determinant criteria of ADG and pepsinogen it was evident that there was a greater benefit to treatment as stocking rate increased. However, threshold treatments according to FEC showed the greatest benefit when applied at conventional stocking rates.

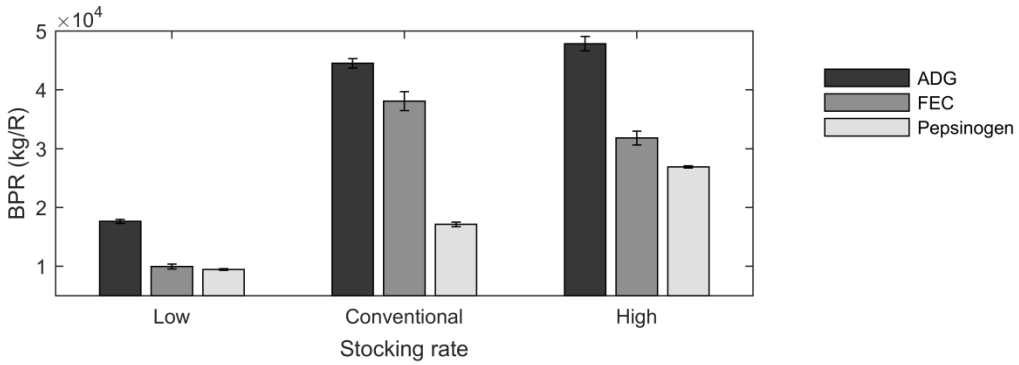

Figure 5.8: Benefit per R (BPR) simulated at the end of the grazing season (day 180) on a population basis for each of the simulated control strategies; BPR represents the benefit in bodyweight gain (kg) per change in frequency of R on pasture, so the higher the value the more beneficial the strategy is perceived to be. Ten discrete populations of calves were simulated on pasture initially contaminated with 200 L_3/kg DM grass an kept at stocking rates of (A) 3, (B) 5 or (C)7 calves/ha for calves treated according to threshold values according to determinant criteria of average daily bodyweight gain (ADG, kg/d), faecal egg count (FEC, eggs/g) and pepsinogen.

5.5 Discussion

Previously an assessment was made on a number of TST strategies under 'average' conditions (Chapter 4). Two methods of selection were proposed; 1) treating a fixed percentage of calves dependent on the values of a determinant criterion or 2) treating individuals that cross a threshold level for a given determinant criterion. When selecting the first option it was predicted that treating fewer individuals resulted in a greater BPR value; although a smaller improvement in bodyweight gain was observed this was vindicated by a small increase in frequency of R (as an indication of resistance). When assessing the different determinant criteria in terms of BPR, pepsinogen was the best, followed by FEC and finally ADG. It was predicted that ADG was the worst for improving weight gain, due to treatment of calves with slow intrinsic growth rates, and FEC the best. However, ADG and FEC also had the most detrimental effects on *refugia* due to direct targeting of large FECs. As a consequence, the determinant criterion pepsinogen was the most beneficial trait in terms of BPR; by treating calves with large WBs those individuals with low WBs but expressing large FECs were allowed to contribute towards *refugia* on pasture.

Alternatively, when treating calves according to threshold values the opposite pattern was predicted for determinant criteria with ADG the best in terms of BPR, followed by FEC and finally pepsinogen. Treatments according to ADG prevented a build-up of PC, hence reducing the effects of parasitism on bodyweight gain at an early stage whilst allowing tolerant

individuals to remain untreated and contribute towards *refugia*. Pepsinogen was weakest determinant criterion as large WBs were not achieved until the latter stages of the grazing season when PC had built up, hence requiring large numbers of treatments and consequently a large frequency of resistance.

These simulations point towards a best TST strategy of threshold treatments to ADG, however it may be that the most beneficial method of selection for treatment or determinant criterion for treatment may be affected by IL_0 levels or management practices such as herd stocking rates. These options were chosen in relation to other factors as they are both expected to strongly influence seasonal patterns of PC (Chapter 3; Nansen et al., 1988), and consequently influence the rate at which resistance builds-up, as well as the effects of treatment on performance. Additionally, stocking rates are a controllable aspect of herd management that must be considered on all farms, and therefore of key importance when considering any treatment strategy. As a result, TST strategies were tested for a range of IL_0 levels and stocking rates in a fashion that would otherwise prove challenging under experimental conditions. The consequences of altering these parameters and deviations from patterns observed under 'average' conditions are addressed in the following sections.

As previously stated, the availability of literature on this area is limited and only two such strategies have been investigated experimentally. The first of these is threshold treatments to ADG (Greer et al., 2010; McAnulty et al., 2011; Höglund et al., 2013a). This strategy has shown promising results both in previous simulation studies and field experiments, and hence was included in the current study. The second strategy investigated was TST whereby calves were treated when the threshold for both pepsinogen and FEC were exceeded (O'Shaughnessy et al., 2014a; 2015a; 2105b), however this strategy was excluded from the current study. This was due to a lack of correlation between high WBs (represented by plasma pepsinogen levels) and FEC (Chapter 3), but also due to impracticalities associated with conducting the sampling. Although no studies have looked at the sole use of pepsinogen or FEC for treatment, these appear to be more successful than the combined use and were therefore investigated as determinant criteria within the current study.

5.5.1 ***Initial pasture contamination***

5.5.1.1 TST based on herd percentages

A comparison of the impacts of IL_0 on treatment percentage (treating 10% or 25% of the herd) according to the determinant trait pepsinogen was made (figure 5.1). The patterns were similar for both percentages, but as would be expected more pronounced upon treating 25% of the population. In general, herds exposed to smaller IL_0 showed greater reductions in parasitological burdens from treatment as a result of the timing of peak burdens. Upon the second assessment for treatment, following the mid-summer rise in PC, calves exposed to high IL_0 had already achieved large burdens which were naturally decreasing as a result of a strongly developed immune response. Calves exposed to low IL_0 on the other hand had not developed such a strong immune response and therefore treatment had a greater reducing effect on parasitic measures. This was also the case for PC, reflecting FEC with a lag time of 2-3 weeks accounted for by temperature-dependent larval development of eggs to L3 larvae on pasture. Upon assessment for treatment, calves exposed to higher IL_0 had already experienced larger reductions in bodyweight gain as a result of reduced feed intake associated with immune development and protein loss (Chapter 3; Fox et al., 1989b; Fox, 1993); consequently there was less potential for improvement when compared to an untreated group. Interestingly, all groups showed a similar final frequency of R on pasture; in the case of low and medium IL_0 this was a result of similar effects of treatment on parasitological burdens, and consequently output of R alleles, whilst only small differences in PC were observed as a result of similar numbers of eggs excreted. Although high IL_0 resulted in a lower PC (hence a smaller reservoir of susceptible genotypes), this was counteracted by fewer resistant eggs excreted to pasture.

Slight variations in the effect of IL_0 on patterns of determinant criteria were evident when treating 10% or 25% of the population; hence treatment percentages were assessed separately. When treating 10% of the population, IL_0 had little effect on patterns of determinant criteria for BPR, in all cases ADG was the worst followed by FEC due to detrimental effects on *refugia* (Chapter 4). As expected pepsinogen appeared to be most beneficial for low and medium IL_0, however random selection was most beneficial at high IL_0. This was largely a result of a greater build-up in frequency of R with a significantly greater frequency of R observed for pepsinogen at high IL_0. This was due to parasitological burdens

and PC decreasing naturally as a result of immune development, meaning treatments targeting large WBs resulted in large numbers of R alleles contributing to a decreasing reservoir of susceptible alleles on pasture. Additionally, the patterns in bodyweight gain also differed for high IL_0 for which the determinant criterion of ADG was best at improving bodyweight gain. The greater magnitude of reduction in bodyweight gain achieved prior to assessment for treatment lessened the risk of selecting calves with intrinsically slow growth rates over parasitised calves showing poor performance. This did not apply when a larger percentage of calves were treated due to increased selection of slow intrinsic growers.

When treating 25% of calves the same general patterns in determinant criteria were observed as described for 10%, ADG was consistently the worst determinant criteria followed by FEC whilst pepsinogen was the best. However, as IL_0 increased the relative benefits of selection for treatment according to pepsinogen compared to random selection decreased. As IL_0 increased random selection became progressively worse at reducing cumulative FECs in comparison to using any of the determinant criteria. Although the smallest immediate reduction in FEC as a result of treatments was predicted for random selection, this allowed for greater antigenic exposure early in infection meaning calves were less vulnerable by the mid-summer rise in PC. The effect of this was greater at lower IL_0 due to the slower development of immunity; this was not observed for the smaller treatment percentage (10%) due to the lesser effects on parasitological burdens. As a result, the frequency of R was significantly lower for pepsinogen compared to random selection at low IL_0, due to the greater mid-summer rise in PC providing a larger number of susceptible genotypes on pasture. However, this was accompanied by the largest reduction in bodyweight gain. Upon assessment for treatment at low IL_0 calves are less immunocompetent (Ploeger et al., 1994) and therefore the majority is suffering reduced weight gain as a result of reduced voluntary feed intake and protein loss with a sudden influx of larvae due to mid-summer rise in PC (Fox et al., 2007). At high IL_0 the majority of calves have developed a strong immune response and therefore suffering reductions due to protein loss, largely a result of high WBs. As a result treatments according to pepsinogen (a marker for high WBs) become progressively better at preventing reductions in bodyweight gain as IL_0 increases.

5.5.1.2 TST based on threshold values

The effect of IL_0 on determinant criteria used for threshold triggers was assessed; in all cases ADG was predicted to be the most beneficial determinant criterion, followed by FEC and finally pepsinogen in terms of BPR (figure 5.4). As IL_0 increased, the BPR decreased for the determinant criterion of ADG; the effect of IL_0 on outputs was minimal with similar patterns predicted for build-up in frequency of R and final reductions in bodyweight gain. However, a greater improvement in bodyweight gain compared with an untreated group of calves was predicted at lower IL_0, eliciting a greater BPR. For similar reasons as IL_0 increased BPR decreased, but to a lesser extent, for the determinant criterion of pepsinogen. However, high IL_0 showed variations in the underlying mechanisms for build-up of R alleles to other IL_0 levels. Due to high WBs experienced early in infection for the high IL_0 treatments were administered from an earlier time point, however this did not prevent the build-up of PC as levels were already declining as a result of acquired immunity. Larger numbers of treatments resulted in a greater build-up of resistance in the early stages of infection. However, in the latter stages of the grazing season large WBs were prevented from occurring hence resulting in fewer treatments and ultimately a similar final frequency of R to the other IL_0 levels. The determinant criterion of FEC was impacted differently to other determinant criteria; although high IL_0 still resulted in the lowest BPR calves exposed to low and medium IL_0 showed similar BPR values. As per other determinant criteria this was partially attributable to patterns of bodyweight gain, but differences between low and medium IL_0 were primarily a result of changes in frequency of R. As IL_0 increased a greater effect of immunity on FEC was observed, hence fewer treatments were required resulting in a lesser build-up of R.

5.5.2 ***Stocking rates***

5.5.2.1 TST based on fixed herd percentages

A comparison of the impacts of stocking rate on treatment percentage (treating 10% or 25% of the herd) according to the determinant criterion of pepsinogen was made (figure 5.5). As would be expected the effects were more pronounced at the 25% level. At higher stocking rates, treatment resulted in a greater removal of WBs; however, this did not impact hugely on FEC due to density-dependent effects (Michel, 1978). Due to a smaller grazing area at high stocking rates the excreted eggs were more concentrated on pasture, resulting in higher PC. As a consequence calves at high stocking rates had the largest parasitological

burdens resulting in the greatest reduction in bodyweight gain, although also the greatest improvement from treatment.

The effect of stocking rate on determinant criteria was assessed (figure 5.6), there was little effect of treatment percentage on determinant criteria. Determinant criteria showed similar patterns in BPR at low and medium stocking rates, however high stocking rates showed slight variations. ADG and FEC were the least beneficial determinant criteria in all cases; however, at high stocking rates ADG appeared more beneficial than FEC. This was partially due to bodyweight gain, but more significantly the frequency of R. At high stocking rates a larger reduction in bodyweight gain is observed, mainly due to protein loss, and consequently the chance of selecting calves with intrinsically slow growth rates is reduced. Protein loss correlates well to WBs (Parkins and Holmes, 1989; Charlier et al., 2014), consequently selection according to ADG acts in a similar fashion to the determinant criterion of pepsinogen (by selecting against individuals with large WBs, whilst allowing those with small WBs but large FECs to continue to contribute susceptible genotypes to pasture). Similarly, for all stocking rates pepsinogen was most beneficial but did not differ significantly from random selection, with the exception of high stocking rate upon treating 10% of the population whereby random selection appeared to be significantly better. Under these conditions the greatest density-dependent effects are perceived; treatment according to pepsinogen has a small effect on the total FEC, however results in a larger proportion of resistant alleles than random selection.

5.5.2.2 TST based on threshold values

The effect of stocking rate on determinant criteria used for threshold triggers was assessed; in all cases ADG was predicted to be the most beneficial determinant criterion, followed by FEC and finally pepsinogen in terms of BPR (figure 5.8). As stocking rate increased BPR also increased for the determinant criteria of ADG and pepsinogen. This can be explained by the greater improvements in bodyweight gain outweighing the larger increases in frequency of R. Although larger reductions in parasitological burdens and PC were observed at higher stocking rates, and thus greater improvements in bodyweight gain, PC was still larger. Consequently a greater number of treatments resulted in a higher frequency of R, only partially counteracted by the high PC providing a reservoir of susceptible genotypes on pasture. The increase with stocking rate was disproportionately large for the determinant

criterion of pepsinogen; at high stocking rates the proportion of the reduction in bodyweight gain attributable to protein loss (associated with large WBs) increases, and hence pepsinogen became more effective at preventing reductions in bodyweight gain. Similar patterns were not conveyed in BPR values generated by the determinant criterion of FEC; only small increases in treatment numbers were predicted as stocking rates increased due to larger density-dependent effects. At low stocking rate there were minimal improvements in bodyweight gain, resulting in a small BPR. At high stocking rates the improvement in bodyweight gain was similar to that of conventional stocking rates, however there was a slightly greater build-up of R due to the larger number of treatment administered in the latter stages of the grazing season hence also resulting in a slightly smaller BPR.

5.5.3 ***Perspectives***

When assessing the best determinant criterion for each of the described methods of selection for treatment, contrasting patterns were predicted. Upon assessing determinant criteria when treating a fixed percentage of the population, treating calves according to pepsinogen or random selection was most beneficial under all conditions, with little difference predicted between the perceived benefits for each. However, under all conditions the highest BPR, and consequently the most beneficial TST strategy, was treating calves according to threshold values of ADG. This was due to the ability to prevent large reductions in bodyweight gain by preventing the build-up of PC, whilst simultaneously maintaining *refugia* by allowing tolerant calves (i.e. calves with high WB but little effect on performance) to contribute large numbers of susceptible genotypes to pasture. As a result, the best strategy to recommend to farmers would in fact be treatment according to threshold values of ADG.

It was hypothesised that lower IL_0 would result in greater benefits from TST strategies; this was found to be the case for all TST strategies, with the exception of treating calves according to threshold values of FEC. At medium and low IL_0 similar patterns in FEC were predicted, partially a result of density-dependence effects, resulting in similar treatment patterns and effects on bodyweight gain and resistance. As a result, similar BPR values were predicted. It was hypothesised that as stocking rate increased the greater potential for improvement in weight gain would outweigh the larger increase in frequency of resistance alleles on pasture. This was not always the case; although the hypothesis holds true when a

fixed percentage of the population were treated according to ADG, the remaining determinant criteria showed conventional stocking rates to show the greatest BPR, followed by high then low stocking rates. At higher stocking rates the improvement in bodyweight gain was not as great as expected, hence outweighed by the larger build-up of resistance alleles. Similarly to IL_0, the hypothesis holds true for threshold treatments according to ADG and pepsinogen but not for treatment according to FEC, again for density-dependence related reasons.

To address these questions experimentally would be near impossible for many reasons. Firstly, the large number of confounding variables, and for example weather conditions, management aspects and difficulties in controlling and measuring the IL_0 levels (O'Shaughnessy 2015a). Secondly, the difficulty associated with assessing resistance in cattle, especially over a short time period (Besier et al, 2012; Sutherland and Bullen, 2014), with no formally validated methods for detecting resistance in cattle.

The application of TST strategies in practice requires considerably more work and a change in farmer attitudes. The most feasible determinant criterion is ADG due to the lower costs associated with testing and instant side-crush measurements. Although weigh-scales are expensive other easy to measure representative traits, such as heart girth, have proved successful at accurately estimating liveweight (Jackson, 2013). However, threshold treatments require more frequent handling of cattle and consequently are more labour intensive. As a result, it may be more beneficial to treat fixed percentages at a fixed time point. In all cases random selection and pepsinogen were most beneficial, showing similar values under all conditions. It could be concluded that in all cases the extra costs associated with diagnostic tests and stress related weight loss from pepsinogen measurements would outweigh any advantage gained over random selection. Additionally, random selection would be viewed favourably by farmers due to ease of application and therefore convincing them to adopt such a strategy would be an easier feat.

Application of the model gives a detailed analysis of the effect of varied IL_0 and stocking rate on various TST control strategies designed to help ensure continued effective control of parasitism in the future. Such comparisons are currently absent from the literature and, although small variations in patterns were observed, the model currently supports a universal best method of TST according to threshold ADG values across all tested scenarios.

This is a positive outcome meaning measures of IL_0 or management practices will not need to be taken into consideration when applying treatment. However, to convince cattle farmers of the long-term benefits of such TST strategies a cost-benefit analysis must be conducted to determine whether the added cost associated with a given strategy is justified in the overall profitability.

Chapter 6: General Discussion

6.1 Introduction

The emergence of anthelmintic resistance in cattle helminths threatens the sustainability of beef cattle systems globally (Edmonds et al., 2010; Sutherland and Leathwick, 2011; O'Shaughnessy et al., 2014b; Rose et al., 2015a). Cases of multi-drug and multi-species resistance are now apparent in several countries across the globe (Sutherland and Leathwick, 2011). As a consequence, current practices of parasite control involving chemotherapeutic treatments are under threat. There is a growing need to develop alternative control methods of helminths aiming to avoid, or reduce, the number of anthelmintic treatments applied to cattle. A prominent, *refugia*-based strategy is targeted selective treatment (TST), whereby a select few individuals are treated, according to certain phenotypic traits (van Wyk, 2001). TST aims to slow the development of anthelmintic resistance, whilst maintaining effective control of parasitism. However, quantitative assessment of such techniques by experimentation can be extremely time-consuming, costly and technically difficult to implement (Barnes et al., 1995). Assessing resistance over a short time-scale can prove problematic, with no techniques yet fully validated for cattle (Besier, 2012; Sutherland and Bullen, 2014). For these reasons, a mathematical model provides an attractive alternative for gaining valuable insights into the use of TST strategies.

To provide useful insight into TST strategies, a mathematical model must adequately address the individual variation within a herd. Previous models accounting for the full lifecycle of gastrointestinal parasitism of cattle have all been demographically deterministic in nature and therefore unable to account for control strategies applied on an individual animal basis (Smith et al., 1987b; Ward, 2006a; Verschave, 2015). Evaluation of TST requires the assessment of the consequences on both treated and non-treated animals, rather than dealing with the average individual animal. The work described in this thesis follows the building of a suitable stochastic population-based model from initial deterministic concepts.

The first aim of the thesis was to develop a model to account for host-parasite interactions and epidemiological factors in order to simulate natural infections of *O. ostertagi* on pasture, focussing on the single most economically significant helminth in temperate beef and dairy cattle systems. The model specification was based on mechanistic concepts of host-parasite interaction, combined with empirical relationships that had previously been developed to

describe parasitism in other species (such as the relationship between immune response and feed intake). In order to place confidence in model outputs the thesis aimed to execute a solid evaluation of the model performance by means of a sensitivity analysis and comparison to experimental studies. Ultimately, the aim was to use model simulations to compare the effects of current control practices and various TST strategies on animal performance and the build-up of anthelmintic resistance, and to assess whether these findings are strongly influenced by external factors, such as initial pasture contamination (IL_0) or calf stocking rates.

The aim of the current chapter is first to identify and justify key assumptions and simplifications associated with the development of the model in terms of defining the key parameters, most importantly those of the host immune response, parasitic effects on the host and epidemiological parameters (as described in Chapters 2 & 3). The second aim of the chapter is to discuss the reliability of the model, including the approach to assigning key parameters and validating the model. Following this, the third aim is to discuss the application of the model, primarily in terms of what would be the recommended TST strategy and under what conditions this would apply (based on Chapters 4 & 5).

6.2 Model development and associated concerns

6.2.1 *Host immune response*

Immune response is an essential aspect of the model. Although various components of the response (such as T-cells, cytokines) have been identified, there are few quantitative measures of the magnitude, time-scale, and variability of responses that could inform the model. This lack of quantitative understanding of the elicited immune response to *O. ostertagi* in calves presented challenges for model parameterisation (Li et al., 2010). In this thesis, the parasitic key life-history traits, classified as parasite establishment, mortality and fecundity, were parameterised through analysis of published literature (Chapter 2). To minimise confounding variables, experimental data were restricted to studies that reported the relevant measures of parasitic load following experimental infections of known infective larval doses. Experiments in which larvae were administered as daily or weekly trickle infections were preferred over single infections (one large dose). It is accepted that trickle infections offer a rough correspondence to natural infections, whereas single infections prove rather more challenging with a sudden burst of larvae eliciting a rapid immune

response (Mihi et al., 2014), a behaviour that was imitated by the model (Appendix B). All of the selected studies involved the administration of mono-infections of *O. ostertagi*; it is hypothesised that in practice, concurrent infections may result in species interactions (Christensen et al., 1987; Poulin, 2001). For example, reports that FEC in concurrent infections of *O. ostertagi* and *Cooperia* exceed the additive effect of mono-specific infections of the respective species suggest there is some impact on the host (Hilderson et al., 1995).

To parameterise the relevant immune parameters (Chapter 2), measurements of worm burdens (WB) and the eggs per female worm are the most valuable form of data. However, the high costs and animal welfare considerations associated with such experiments has resulted in very few reports of such measures, each with few replicates (as low as a single calf for each time point) (Michel, 1969b, 1970; Michel and Sinclair, 1969). As such, a limitation to the parameterisation of immune characteristics was the lack of detailed data. Consequently, the small number of observations on which parameters associated with the immune response were based introduces substantial uncertainty to the model. A sensitivity analysis was conducted to determine the relative effects of altering key parameters, and the influence this may have on model outputs (Section 2.3.2). It was observed that in general the model was most sensitive to development rates of the immune response, and less so to the minimum and maximum values of immunological traits. Provided a sound validation of the model is conducted for predicted parasitological burdens, and general patterns are similar to experimental observations, this is not a major concern.

The interaction between immune response and WB is a complex one. Parasite establishment and mortality are distinct processes but their contributions to the net WB are indistinguishable, given the available data, and were initially parameterised as a combined measure of surviving adult parasites. However, it was necessary to separate the effects of the two to accurately capture WB dynamics. In the absence of more detailed data it was sometimes necessary to draw assumptions based on the closely related parasite species of lambs, *Teladorsagia circumcincta* (formerly *Ostertagia circumcincta*), as was the case for the parasite mortality. The two species share many key characteristics with similar physical features and life cycles (Soulsby, 1965, Taylor et al., 2015). DNA sequencing suggests the phylogenies of the two diverged only recently resulting in these many shared characteristics (Zarlenga et al., 1998). This includes many similarities in the elicited immune response in terms of cytokine profiles in the respective hosts; following infections, similar transcript

levels of interleukins IL-4 and IL-5 have been observed (Claerebout et al., 2005; Craig et al., 2014). For the purpose of comparison it was assumed the two species induce similar immune mechanisms. Worm mortality rate was therefore assumed to follow a sigmoidal curve, consistent with previous models developed for various different parasite species, including *O. ostertagi* (Grenfell et al., 1987b; Verschave et al., 2014). A sensitivity analysis revealed the model was not very sensitive to the minimum or maximum values of parasite mortality, but rather to the rate of development of immunity in mortality and, more conspicuously, rate of development of immunity in the combined effects of establishment and mortality (figure 2.4). It is widely accepted that worm expulsion occurs prior to effects on worm establishment (Vercruysse and Claerebout, 1997); this fitted with model parameterisation for which the best fit was observed when the rate parameter associated with the acquisition of immunity for mortality occurred prior to that for establishment (Chapter 2). It should be noted that the model became more sensitive to parameters as the infection dose increased; natural infections are likely to involve smaller intakes of larvae, meaning the model may be less sensitive to changes in the parameter values.

Density dependent effects have been proposed as a non-immune alternative mechanism that could reduce WB. In this model, no consideration of density-dependence was made for parasite establishment or mortality. It could be questioned as to whether this is appropriate or not; studies both substantiate (Ross, 1963; Anderson and Michel, 1977; Verschave et al., 2014) and refute these claims (Michel, 1970; Barger, 1987). Theoretically, when large burdens are experienced, limited resource availability may result in a larger reduction in surviving worms. However, validation of simulated WBs did not show a consistent over-prediction of large WBs (section 2.4.3), suggesting it was appropriate to omit density-dependence from the model. It is difficult to separate the effects of immunity and density-dependence, meaning such interactions may be accounted for within the immune parameterisation. Additionally, many of the studies corroborating this claim were based on experimental infections in which particularly large WBs can be achieved, on a scale that may not be encountered within naturally occurring infections, justifying this omission given the purpose of the developed model.

Parasite fecundity, parameterised as the number of eggs per female worm, is another distinct aspect of infection which may be affected by immune response. Although numerous other studies have based their predictions of *O. ostertagi* fecundity on FEC this adds a

considerable number of confounding variables, such as variation in daily faecal excretion, aggregated egg distributions within faecal pats and often unusually high infection doses. These factors increase uncertainty and may explain why there is no known correlation between eggs *in utero* and eggs excreted daily (Verschave et al., 2014). A sensitivity analysis (figure 2.4) revealed the peak egg count to be most sensitive to maximum fecundity, implying better estimates of such a parameter would enhance model precision. However, the model does not consistently under- or over-predict FEC, when compared with experimental data. Current parameters could therefore be considered sufficient in representing parasite fecundity.

6.2.2 ***Effects of parasitism***

The sub-clinical effects of *O. ostertagi* manifest as reductions in bodyweight gain attributable to 3 key components: reduced feed intake, protein loss and partitioning of resources to immunity. Although a widely accepted phenomenon, very few studies exist on the exact nature of reduced feed intake resulting from parasitism, particularly in cattle, due to experimental cost and difficulty. Similarities were observed across gastrointestinal parasites of ruminants, e.g. *Trichostrongylus colubriformis,* suggesting a similar mechanism for the reduction in food intake may be in place (Sandberg et al., 2006). There are two key causal hypotheses for this: as a direct result of WB and associated pathophysiological and biochemical changes, or associated with anorexinogenic cytokines produced in the development of an immune response. The former theory links worm damage to parietal cells in the abomasum to reduced acid secretion, leading to an abnormally high pH, which in turn stimulates production and release of gastrin into the bloodstream. Further links to appetite related peptide hormones have been proposed, in particular increased leptin and decreased ghrelin resulting in a down-regulation of Neuropeptide-Y (NPY), a potent stimulator of feed intake (Fox, et al., 1989a; 1989b; 2002). Interestingly Ward (2006a) summarised published studies on the effect of *O. ostertagi* and *Cooperia* infestations on feed intake of cattle and of *T. circumcincta* on feed intake of sheep revealed feed intake reductions to be proportional to the larval intake for sub-clinical infection ranges. Post-mortem WBs were found to show a weaker correlation (Ward, 2006a). This is in contrast to Sandberg et al. (2006) who suggested that over a range of larval intakes, the reduction in food intake is relatively constant (around 20%). Larval intake essentially defined the levels of antigenic exposure and abomasal pathology experienced by a calf, and therefore the level of immunity acquired,

perhaps implying the link between immune-related cytokines and feed intake to be stronger. Ward et al. (2006a) was unable to account for larval intake within their model due to a logical loop between feed intake and larval intake (each dependent on the other), and consequently the reduction was considered as a function of WB. In the model developed within this thesis it was also possible to model reduced feed intake as a function of worm damage using the equation of Vagenas et al. (2007a). The equation relates reduced feed intake directly to WB, although worm mass may prove more appropriate predictor for damage.

Although technically possible to implement, direct linkage between feed intake and WB was not incorporated into the model. The hypothesis that anorexia is a direct function of WB or worm mass did not always fit with observations in the field. For example, Forbes et al. (2009) found infection caused significantly elevated gastrin levels, reduced leptin levels and large egg outputs, but no significant change in feed intake from a control group of healthy calves. Further studies showed a lack of correlation between large WBs and reduced feed intake (Herlich, 1980). Studies on *T. circumcincta* parasitized sheep have found no significant association between feed intake and leptin and gastrin blood levels (Fox et al., 2006; Zaralis et al., 2008, 2011), suggesting this to be merely a correlational effect. Judging from the literature it is more likely that anorexia is observed as a result of certain cytokines which reduce appetite (Langhans, 2000, 2007), for example immune regulators such as IL-1 (Wisse et al., 2004) or TNF-α (Elsasser et al., 1998; Worthington et al., 2013). As a result, the extent of anorexia was instead modelled as a function of the immune development, as a proxy for these cytokine levels.

Again parameterisation of the intake reduction was largely restricted by data availability, with few studies reporting bodyweights and even fewer reporting feed intake in the presence of experimental trickle infections. One of very few studies to look at experimental challenges and the effects on feed intake was conducted by Fox et al. (1989b); however large numbers of larvae were administered to ensure that a reduction in feed intake was observed. This made parameterisation for lower infections difficult. The extent of the reduction was parameterised according to these data, but also from bodyweight data obtained for different weekly infections (Szyszka and Kyriazakis, 2013). When considering bodyweights the proportions of losses attributed to feed intake, to protein loss or as a consequence of nutrient partitioning to immunity/growth were difficult to assign. By

altering the nutritional partitioning rule within the model to favour immunity over growth or vice versa, only small differences were expected in parasitological outputs and on performance (Doeschl-Wilson et al., 2008). Therefore the rate of protein loss and rate of reduction in feed intake were parameterised from measurements of bodyweight to best mirror experimental findings. Although this was possible due to the inclusion of different infection levels, more precise data would increase model confidence considerably. The sensitivity analysis (figure 2.4) revealed that the reduction was most sensitive to the rate of immune development in the combined establishment and mortality of adult worms; this may explain the high correlations observed between WBs and reduced feed intake. The model was far less sensitive to the scaling parameter (relating the rate of reduction in feed intake to the rate of immune acquisition), implying it was critical that the model describe the immune response accurately, whilst errors in the scaling parameter may not have such a large effect.

6.2.3 ***Stochastic components***

In order to account for natural infections on pasture, the model was extended to consider a herd of calves at the phenotypic level, by describing the physical characteristics of each calf (Chapter 3). This was chosen over the genotypic level for simplicity. Each of the defined traits for calf maintenance requirements, growth requirements and immunity were considered to be distributed across the population with a given mean and coefficient of variation (CV). No correlations were assumed between growth and resistance traits; however by altering these assumptions more extreme relationships between parasitism and performance may be observed (Doeschl-Wilson et al., 2008).

Detailed values on the degree of variation in immune parameters were not readily extractable from the literature and therefore were loosely based on previous ruminant models (Laurenson et al., 2012b; Vagenas et al., 2007c). Although a crude assumption, there is evidence that calves show similar immune mechanisms to lambs, which may result in similar CVs, with one study estimating between 2.2 and 23.1% CV for various specific immune mechanisms in cattle (Sellers et al., 2011). Larger variations were assumed in immune acquisition than growth characteristics; growth is subject to obvious selection pressures during breeding, whereas it is difficult to measure and select for immune parameters (Kloosterman et al., 1989). The range of observations of parasitological outputs

of FEC was generally encompassed by the distributions of predicted outputs, although more accurate predictions of immune distributions may improve model performance.

6.2.4 *Epidemiological parameters*

The model aim, to simulate natural infections, necessitated a representation of environmental effects on the free-living stages of the parasitic lifecycle, and on calf nutrition and larval uptake (Chapter 3). This stage of model development posed a challenge to reflect the most important features of the environment while avoiding unnecessary complexity.

Initially the free-living stages were condensed to consider only the relationship between egg output and L_3 larvae. It was assumed that of the eggs excreted to pasture a fixed proportion would develop from egg to L_3 larvae taking no account of transition between intermediate larval stages. Various models have focused explicitly on the free-living stages (Gettinby and Paton, 1981; Grenfell et al., 1986; Chaparro et al., 2011; Rose et al., 2015b), however this was not a focus of this model. Therefore a rather simplified representation was used. Although this may amount to some outputs of the model being over or under-predicted, in particular larval pasture contamination (PC), through for example a lack of consideration for leaching of larvae into the soil, the aim of a modelling exercise is to provide the simplest useful representation of real-life. The model was designed primarily to compare different control strategies, providing the outputs of PC are those that would be recognised as typical, the exact numbers are not important. Simple models have been found to mimic well the pasture surface contamination with no extra benefit gained to model performance from increasing model complexity (Smith et al., 1986). A simple model for free-living stages will suffice, providing the parasitic module is accurate.

Upon considering meteorological effects on the free-living stages a simple interpolation of data was made for temperature (Chapter 3). This was found to mirror the pattern of a mid-summer rise in pasture-contamination well. In field experiments it is impossible to distinguish between the effects of temperature on the proportion of larvae that develop and the time for larval maturation. Consequently only the temperature-dependent larval development time was considered. This is supported by both in laboratory and field experiments (Rose, 1961; Persson, 1974; Pandey, 1974), although the two do not always directly relate, as lab conditions often provide a more favourable environment in other respects (e.g. moisture levels). It was necessary to assume that standard air temperatures

are an accurate representation of the microclimate experienced within the faecal pat. Although this is unlikely to be strictly true (Smith and Wilson, 1980), this was the best approximation due to lack of alternative detailed measurements (Smith et al., 1986). Further, although an important factor in translocation of larvae, moisture levels were assumed to be non-limiting for larval development and transmission in the UK and not considered within the model. This may result in an over-simplification, impacting on patterns of PC. However, the inclusion of such a parameter would complicate the model considerably due to interactions with temperature that must be considered and increasing model complexity may not necessarily aide model functioning.

There is a clear stratification in larvae, with highest concentrations of larvae at the base of the sward, and fewer further up the sward. By assuming an even vertical distribution of larvae on pasture and failing to consider faecal avoidance behaviours it may be that the larval intake is over-estimated for low stocking rates (Gruner and Sauve, 1982). However, there is also a nutritional trade-off associated with faecal avoidance with grass surrounding faecal pats considered to be of higher nutritional quality (Hutchings et al., 2001; Fox et al., 2013). This is less of a concern at high stocking rates with calves forced to eat closer to faecal pats due to decreased grass availability. However, this poses the question as to whether effects on calf performance were attributable to parasitism or a reduction in the grass availability as result of insufficient pasture (Nansen et al. 1988). The model was tested by considering restricted feed intake under limited pasture availability; only trivial differences were observed suggesting the majority of losses were attributable to parasitism. Ultimately, this level of complexity would be expected to contribute little to the model, particularly considering its ultimate purpose.

Grass availability and nutritional quality (ME and MP) were essential parameters to defining larval concentration on pasture and calf larval intake. Grass features were assumed to be dependent on the expected seasonal effects, although enormous variations are evident between farms (AHDB, 2016). Although grass quality often relates to perceived sward length, attempts to model grass quality as a function of sward length did not accurately represent the seasonal patterns in ME and MP content. Overall, the effects perceived by altering grass quality could be considered minimal in the scheme of the model.

6.3 Completed model

The developed model provides the first stochastic model to account for *O. ostertagi* infections in a population of grazing calves. The model accounts for host-parasite interactions, individual variation and parasite epidemiology to provide a description of the complete life cycle. Model parameters were based on scientific literature and theory and parameterised to best reflect the limited data set. A sensitivity analysis revealed the most important parameter to model predictions is the rate of establishment and survival of adult worms. The model provides insights into underlying mechanisms and helps to understand the phenomena of parasite-induced anorexia. Providing the general patterns observed in natural infections are mirrored by model findings the model could be considered fit for purpose. A formal validation revealed this to be the case.

6.3.1 ***Model evaluation***

6.3.1.1 Model sensitivity

Data availability limited the predictions for many important parameter distributions; therefore it was imperative to test how sensitive the model is to small errors and anomalies within these parameters. A sensitivity analysis of the model was performed with the sensitivity indicating the expected changes in model outputs for a given (e.g. 10%) change in model parameter. However, the true uncertainty of most parameters was not known, either in absolute terms (e.g. the CV) or relative to other parameters. Nevertheless, the analysis did give an indication of which parameters were most critical to model predictions, for which better data may increase model accuracy.

6.3.1.2 Model validation

Model validation is an important step in verifying model findings, ensuring the model has some relation to real-life observations. It must further be established whether the underlying mechanisms are represented accurately and that the outputs are not incidental. This can be achieved by validating model components separately, however by using this method alone component interactions may be overlooked and overall model outcomes not substantiated. Thus, evaluation of the current model was performed for underlying parasitological outputs produced under experimental infections (Chapter 2), and also parasitological outputs produced under natural infections (Chapter 3). Models cannot be expected to provide perfect representations of observed data points as in all cases a model

is a simplified version of reality, with many factors unaccounted for, no matter how complex the model. The model produced realistic predictions that mirrored the sparse data available.

Although no consideration was given to varied growth rates between studies for experimental infections (Chapter 2), growth was a more important aspect when considering natural infections (Chapter 3), due to the cumulative effect of interactions with grass intake and availability. Differences in growth rate were accounted for under natural conditions. However, in reality calf bodyweights are sensitive to a number of uncontrolled variables, many of which are complex and not easily accounted for, in particular concurrent infections with *Cooperia*. To account for a mixed infection the most comprehensive method would be to create a model component for predicting the effects of *Cooperia* on the host, and determine species interactions. Although some data exists on artificial *Cooperia* infections, there is very limited data on artificial mixed infections and hence it would be difficult to decipher species interactions for a full range of infection levels. As a consequence a rather simplified method was used in the model to account for such infections, whereby the presence of *Cooperia* was assumed to have the same impact on the rate of protein loss, regardless of infection size. Although this may result in an under- or over-estimation of the impact on calf performance, in relation to the *Cooperia* infection size, the effect is unlikely to be large. Coop et al. (1979) compared 3 different experimental challenge levels of *Cooperia* and observed each to show similar reductions in bodyweight gain, and unimpaired feed intake. Although the effect of *Cooperia* on feed efficiency was small, it may be more significant in mixed infections for which a greater than additive effect may be observed. This is an area for future development; initially a separate component looking solely at calf-*Cooperia* interactions defined by experimental infections would be required. Subsequently the two species model components would be merged and species interactions incorporated and parameterised to the available literature on concurrent infections. Ultimately, the natural infections incorporating both species could be validated against field studies to ensure the model reflects expectancy.

Owing to a lack of experimental studies investigating the effects of varied challenge levels on calf dry matter intake it was not possible to validate performance outputs. Inference was made through measurements of bodyweight gains and losses relative to control animals and vague assertions from studies conducted under natural conditions. Although no formal validation was performed it was observed that the reductions in bodyweight were similar

between predicted and observed values. This was important to model functioning in terms of nutrient partitioning and also dilution of FEC, one of the key outputs validated. A more rigorous validation would entail detailed observations on the growth of healthy and parasitised calves exposed to the same nutritional conditions. Feed composition is often overlooked in experimental designs, with high quality feed able to mask anorectic effect (Mansour et al., 1991; Kyriazakis et al., 1996a; Forbes et al., 2009). Protein availability is hypothesised to significantly affect the duration and rate of recovery of anorexia (Sandberg et al., 2006), with protein supplementation suggested to reduce pathophysiological effects of parasitism (Fox, 1997) and enhance the expression of immunity (Mansour et al., 1991). Measurements of *O.ostertagi* infected calves exposed to low or high protein and energy diets, both within the normal range of husbandry, revealed this to be the case. The high protein and energy diet showed a faster growth rate, reduced by *O. ostertagi* infection, whereas the low protein and energy diet showed a slower growth rate which was not affected by *O. ostertagi* infection (Mansour et al., 1991). Exact feed compositions are not always given in published reports and therefore difficult to account for in a validation.

6.3.2 ***Future model development***

The model was developed with the purpose of comparing various control strategies for *O. ostertagi* under a variety of conditions for first grazing season cattle alone. However, beef steers are more frequently grazed for a second season before slaughter (Phillips, 2010). Adjustments to the model framework would provide further insights into the optimal management of cattle. Although calves are most likely to experience large nematode infections in the first grazing season, the effect of different control strategies on the development of an immune response in the first grazing season may have consequences for subsequent years. For example, strategic treatment is extremely effective at reducing the parasitic burdens experienced by calves, but these animals may be more susceptible in the following grazing season (Claerebout et al., 1999). The model should be extended to account for immunity and growth in the second season. Such an extension would require some representation of the trajectory of immunity in the housing period, consideration for over-winter survival of larvae on pasture and, with greater complexity, a module to account for arrested larvae in hypobiosis.

Besides the three described key life-history traits it is speculated that immunity plays a role in arrested development, otherwise known as hypobiosis, of larvae. Parasitic larvae in the early L_4 stage are also reported to enter hypobiosis in order to avoid sub-optimal environmental conditions (Armour and Duncan, 1987; Fernández et al., 1999). Little is known of the underlying mechanisms and there is a lack of data on which to characterise the effects. The effect of immunity (or environment) on arrested development was not considered, however the majority of damage occurs after development has resumed (Armour and Bruce, 1974). A published meta-analysis concluded that the average proportion of larvae entering hypobiosis is low (0.041) (Verschave et al., 2014). In natural infections this effect may be larger; hypobiosis was significantly increased by the presence of mixed infections involving *Cooperia* sp. (Verschave et al., 2014) and likely to increase as temperatures decline towards the end of the grazing season (Eysker, 1997). However, the effects of hypobiotic larvae on first grazing season cattle could be considered small, only becoming apparent when larval development resumes at the end of winter/early spring (Myers and Taylor, 1989). Once the second grazing season is accounted for the model could be used to look at the long-terms effects of treatments on various factors, such as resistance and performance.

Further, the model is currently based on autumn-born steers. It has been suggested calving season may potentially be a stronger determinant of WBs than factors such as the IL_0, and that variation in calf age at turnout may significantly affect the observed patterns (Eysker, 1986; Höglund et al., 2013b; Taylor et al., 2015). Simple alterations to the population structure in terms of distributions of age and body composition at turnout would allow for predictions to be made for spring-born cattle, or all year round calving. Furthermore, gastrointestinal parasitism is a threat not only to beef cattle, but also to grazing dairy cattle and calving dams. Extension to these groups would require consideration of the reproductive functions, and the additional nutritional requirements associated, along with the consequential periparturient break-down of immunity associated with pregnant and lactating cows (Borgsteede, 1978; Coop and Kyriazakis, 1999). Although these effects are not as distinctive as for ewes they must still be considered. A first step toward this model extension would be to incorporate the extra nutrient requirements, resulting in a compromised immune response.

As mentioned previously, seldom are cattle infected with a single infection, but more frequently mixed infections with two or more parasite species. *Cooperia* can often be more prevalent, particularly in the early stages in the grazing season; although no inter-species interaction have been observed, there were notable consequences on both bodyweight gains and the concentration of eggs in faeces (Kloosterman et al. 1984; Hilderson et al. 1995; Satrija and Nansen, 1993). It is difficult to distinguish between species in faecal samples; large numbers of eggs produced by *Cooperia* worms imply that values of FEC may not be representative of *O. ostertagi* burdens. Other parasitic species such as lungworm (*Dictyocaulus viviparus*) also affect cattle performance, and interactions have been observed. Calf exposure to *O. ostertagi* and *Cooperia* resulted in a significant increase in establishment of lungworm larvae administered subsequently (Kloosterman et al., 1989, 1990). Additionally, bovine liver fluke (*Fasciola hepatica*) is becoming increasingly common and is also known to cause a reduction in performance, with additive effects to *O.ostertagi* (Loyacano et al., 2002). It would therefore be feasible and beneficial to consider these species as separate model components in the future. Previous models have suggested the same basic structure can be used for a multitude of species by simply altering the parameter values (Smith and Grenfell, 1994; Rose et al., 2015b). Species interactions must be considered, with the most notable effects likely to be on bodyweight. However, care must be taken to avoid adding unnecessary levels of model complexity as considering interactions in the presence of many parasite species may prove challenging, although less so due to the differing host sites of the respective parasites.

Although a range of control strategies has been incorporated into the model application, further possibilities exist, in particular aimed at reducing anthelmintic resistance. The model accounts for the use of TST with a single drug, ivermectin. Smith et al. (1987b) have previously shown a benefit to rotational use of anthelmintic drugs, hence it may be of value to account for different drug classes, such as levamisole and benzimidazoles and investigate such strategies. Levamisole is administered via oral drench, injection or pour-on and has no persistent activity. Accompanied by the fact that levamisole activity does not appear to be affected by resistance to benzimidazoles or ivermectin, this makes it an easy drug to model, although less desirable due to poor activity against larval stages of *O. ostertagi* (Williams et al., 1991). However, the inclusion of benzimidazoles and ivermectin may not be as simple, with evidence of an overlap in the mechanism of resistance in which both select on β –

tubulin (Prichard, 2007; Kotze et al., 2014). This would require many assumptions to be made, but may prove a useful tool for exploring the different possible mechanisms for resistance.

An alternate strategy successful implemented for sheep is selective breeding for resistance characteristics, this would be considerably more challenging to conduct in cattle for many practical reasons (Kloosterman et al., 1978). The model is easily adaptable to consider selective breeding for resistance using methods previously applied within a similar model (Vagenas et al., 2007c; Laurenson et al., 2012b). Upon considering the maternal and paternal genotypes it would be necessary to define the heritability of each of the defined key traits. The animal phenotype would ultimately be defined using the mean value, the additive genetic deviation and the environmental deviation. However, the trait for selection for host resistance would need to be investigated more thoroughly as clearly FEC would not provide a good indicator for selection of host resistance due to density-dependent effects (Gasbarre et al., 1990).

Finally, it is within model scope to allow for potential vaccines to be incorporated. Vaccines work on the premise that exposure to antigenic material will help to accelerate the development of an immune response hence prevent the future development of large WBs. A simplified method would be to increase the exposure (*larvaldays*) to heighten development of immunity; however the exact immuno-response stimulated by the form of antigen may be challenging to characterise. Further, vaccination may promote a faster parasite replication rate and consequently more virulent strains (André and Gandon, 2006) making this intervention far more complex than it first appears. If successfully extended, the model could become a valuable tool for developing integrated control combining different treatment mechanisms. The more complex the combination treatment is, the greater the experimental challenge to optimise the combined components, hence modelling may represent an important step towards the best possible outcomes for sustainable and effective treatment in the future.

6.4 Model application- parasitic control

6.4.1 *Stocking Rates*

A number of approaches to controlling gastrointestinal parasitism are aimed at reducing the concentration of larvae on pasture, and logically larval intake per calf. This can be achieved by reducing stocking rates. High stocking rates result in a greater accumulation of larvae on pasture in the latter stages of the grazing season (Henriksen et al. 1976; Nansen et al. 1988). The effect of stocking rates was modelled (Chapter 3) and found to agree with experimental findings with higher stocking rates resulting in larger parasitological burdens and greater reduction in performance as a result (Henriksen et al. 1976; Nansen et al. 1988). The model predictions identify stocking rate as an essential (and controllable) management aspect to consider within any regime of parasitic control, with important consequences for PC and subsequently exposure to infection and emergence of resistance in treated herds.

6.4.2 *Dose and move*

In the 'dose and move' strategy, calves are administered a single dose of anthelmintic and moved to new, cleaner pasture the same day. The timing of the move is generally in unison with the anticipated mid-July rise in PC with the aim of preventing large intakes of larvae (Smith, 2014). Theoretically most overwintered larvae on the clean pasture will have perished by the time of the move. In previous years 'dose and move' strategies have been widely recommended (Jackson, 2013); however the model clearly demonstrates the importance of susceptible genotypes on pasture highlighting the potential detrimental effects of this practice on resistance. Although moving calves to clean pasture initially resulted in smaller parasitic burdens, and improved performance due to a low PC, the impacts are more severe for subsequent years. Upon moving newly treated calves to low PC fields the majority of developing larvae are those excreted by the hosts, hence predominantly resistance genotypes. The lack of *refugia* on clean pasture means rapid accumulation of resistant larvae with negative consequences for subsequent years. The effects on speed of selection for resistance depend on efficacy of drug and size of initial *refugia*. This is consistent with current views and concerns over the rapid development of resistance associated with dose and move strategies for both sheep and cattle populations (van Wyk, 2001; Waller and Thamsborg, 2004; Waghorn et al., 2008, 2009). Hence the model

prediction is in accordance with the current recommendations of the Control of Worms Sustainably (COWS) programme (Taylor et al., 2010) which discourages this strategy.

6.4.3 *Strategic dosing*

Strategic dosing of calves is accepted well-proven methodology for the prevention of gastrointestinal parasitism of set-stocked calves, with the key principle being to administer anthelmintics early in the grazing season to limit worm egg output and hence reduce pasture contamination with infective larvae, thus preventing the acquisition of large infections. An example is in calves that are injected with ivermectin at 3, 8 and 13 weeks post-turnout (Smith, 2014). This strategy was used as a baseline comparison for TST strategies. Although exceptional for reducing PC and preventing the negative effects of parasitism, model simulations revealed a rapid build-up of resistance alleles on pasture (Chapter 4). This was a result of all calves treated in unison with an anthelmintic with persistent activity, resulting in high selection for resistant alleles. This enrichment of resistant alleles, combined with a lack of diluting susceptible genotypes on pasture, increases parasitism in following years due to reduced anthelmintic efficacy.

6.4.4 *Targeted selective treatment (TST)*

Although TST methodologies have been developed for cattle, very few studies have been conducted to quantify their consequences (Greer et al., 2010; McAnulty et al., 2011; Höglund et al., 2013a; O'Shaughnessy et al., 2014a, 2015a; 2015b). Phenotypic traits, used as determinant criteria for treatment selection, have included FEC and pepsinogen (O'Shaughnessy et al., 2014a, 2015a; 2015b) and average daily gains (ADG) (Greer et al., 2010; McAnulty et al., 2011; Höglund et al., 2013a). No studies have looked at the sole use of pepsinogen or FEC, but rather in combination; O'Shaughnessy et al. (2014a; 2015a; 2015b) implemented TST using both pepsinogen and FEC thresholds as a definition for treatment. All three studies defined the thresholds as FEC≥200epg and PP≥2 IU of tyrosine/l (and a third condition for treatment was the presence of lungworm). O'Shaughnessy et al. (2014a, 2015b) found that no treatments were required, a complete elimination of anthelmintic use, with very similar calf performances. However this is likely a result of low levels of gastrointestinal parasitism experienced. A further study by O'Shaughnessy (2015a) suggested TST to be successful with similar FEC and liveweights between control and TST groups and a 50% reduction in anthelmintic use. These studies, conducted using the same

methods, clearly demonstrate the impacts of confounding variables on the number of treatments administered. From the small differences in live weight gains, the authors inferred that ADG may be a poor target for TST.

However, a retrospective study conducted by Höglund et al. (2009) suggested that ADG was in fact the best trait for selection. The authors went on to test this experimentally and found a 92% reduction in treatment application compared to routine monthly treatments, however ADG was compromised slightly whilst FEC were similar to those of untreated groups. Although this study was well controlled, ADG was notably lower than expected for all groups, a result of low nutrient herbage with differences in pasture quality observed between groups as large as 0.6g/kg DM and 36 g/kg DM in ME and CP levels. This will undoubtedly impact on the interactions between growth and immunity. Further studies by Greer et al. (2010) compared groups of dairy calves treated with anthelmintic routinely versus TST; reductions in anthelmintic use of 65% and 84% were observed for two separate farms, with minimal differences in liveweight gains. The experimental design was repeated by McAnulty et al. (2011) who also found reductions in anthelmintic use of 72% and 42%. Again this demonstrates how variable the outcomes may be for the same methods implemented on a different farm. These studies also involved rotational grazing of mixed groups, meaning the effects of different treatment strategies on the build-up of PC could have been masked. The model demonstrates PC to be a key factor in defining treatment success, not only in terms of resistance but also in terms of the estimated bodyweight. Each of the aforementioned studies designed to assess TST varied in many important factors defining infection dynamics and resistance build-up, such as calf genotype (hence growth rate), grazing management (e.g. turnout date, stocking rates), grass quality and availability, initial PC and frequency of assessments for treatment.

From the literature it is unclear as to which strategy is most successful in treating the effects of parasitism whilst preventing the development of resistance. In practice it is extremely challenging to evaluate the relative success, with no validated measures for assessing the development of resistance, particularly over a short time-scale (Besier, 2012; Sutherland and Bullen, 2014). Comparisons are also limited due to large numbers of uncontrolled confounding variables, as previously mentioned, which will have consequences on the underlying infection levels and subsequently affect the perceived success of any one treatment strategy (O'Shaughnessy et al., 2015b). Of the few studies that have been

conducted on cattle there is no direct comparison of different traits for TST; nor is there a comparison to current practices with many of the control groups representing control strategies that would not themselves be recommended in practice (Höglund et al., 2013a). The model was able to make direct comparisons of strategies, hence providing insights into an otherwise impossible comparison for a range of scenarios (Chapters 4 and 5).

All experimental testing of strategies in cattle have investigated the use of threshold values, however none looked at the value of treating a fixed percentage of calves at given time points. Thus model simulations provide the first insights into what may occur. Treatment success was assessed in terms of benefit per R (BPR), the ratio of average benefit in weight gain to change in frequency of resistance alleles R (relative to an untreated population). Although in the immediate short-term treating more calves provided the greatest improvement to host performance, in terms of BPR treating a smaller percentage of calves showed the greatest benefit. The most beneficial scenario is one in which treatment is given to the calves for which the most damaging consequences of parasitism are observed, most frequently indicated by large WBs, whilst simultaneously maintaining *refugia*. This is achieved most effectively by allowing individuals who are less affected, generally displaying smaller WBs or tolerant individuals, who exhibit large FECs due to reduced immunity and density-dependence effects to continue to contribute susceptible alleles to pasture. This was accomplished most effectively by using pepsinogen, or random selection, dependent on IL_0 levels and stocking rates, with no significant difference between the two criteria in most cases. The remaining determinant criteria, ADG and FEC, were less effective due to their detrimental effects on *refugia* incurred through large reductions in susceptible egg outputs.

The parameterisation of the relationship between plasma pepsinogen and WB assumed a high correlation of 0.7 based on a range of 0.638-0.8 observed (Anderson et al., 1966; Allen et al., 1970; Baker and Gershwin, 1993; Dorny et al., 1999). However in some cases it is suggested that the correlation between pepsinogen and WB may be weak (R=0.34) (Höglund, 2010). This uncertainty in values may obscure the identification of heavily infected cattle. However, none of these drawbacks are attached to random selection, suggesting this may in fact be the most beneficial target for selection when treating a fixed percentage of calves.

Although the degree of difference between determinant criteria varied between IL_0 levels and stocking rates, the same general patterns were observed. There was a greater benefit to treatment as IL_0 decreased due to the greater potential for improvement in EBW gain, but there was similar build-up of resistance on pasture. This was a result of interactions between the acquisition of immunity and impacts on host performance. Larger IL_0 presented a larger amount of antigenic material from an early stage, allowing the host to mount a stronger immune response but displaying greater signs of reduced performance. Consequently there was less scope for preventing reductions in EBW gain through anthelmintic treatments. Stocking rate influenced the determinant criteria more significantly. It was hypothesised that as stocking rate increased, the greater potential for improvement in EBW gain would outweigh the increased build-up of resistance alleles. Although this was indeed the case for calves treated according to ADG, this could not be said for the remaining determinant criteria for which treatment yielded the largest benefit at conventional stocking rates. At high stocking rates large PC levels built-up prior to treatment meaning calves quickly became re-infected and improvements in EBW gain were smaller than expected. Consequently these benefits were outweighed by the more rapid build-up of resistance on pasture.

Alternatively, strategies to treat only those individuals who cross a threshold value have been investigated more thoroughly, and model findings generally agree with current observations. Model simulations revealed the combined use of FEC and plasma pepsinogen to be extremely ineffective, primarily due to a lack of correlation between the timing for peak values. The other experimentally investigated TST strategy in cattle has been the treatment of calves according to ADG thresholds. The model supports this method as the best overall strategy, with the greatest BPR values observed under all stocking rates and IL_0 levels. This was largely attributable to the fact that treating calves to ADG thresholds prevented a build-up of PC and reductions in bodyweight gain from an early stage. In contrast, by the time threshold values were reached for parasitological measurements of FEC and pepsinogen, large PC levels had already been attained, resulting in an increased infection pressure. This necessitated large numbers of anthelmintic treatments in unison, negatively impacting on the frequency of R on pasture. These model predictions are in line with findings of Jackson (2013) who found that ADG showed no correlation to parasitological markers of FEC or pepsinogen. These patterns were consistent for all IL_0 levels and stocking rates; as IL_0 decreased or as stocking rate increased there was a greater benefit to treatment

for determinant criteria of ADG and pepsinogen due to the greater potential for improvement in EBW gain. The exception to this was treatments according to threshold triggers of FEC which were less affected, primarily due to density-dependent effects resulting in similar FEC outputs regardless of IL_0 or stocking rates, and consequently similar frequencies of treatment.

The model highlights the importance of selection method on the best determinant criterion for selection; treating a fixed percentage of calves showed the opposite pattern to threshold treatments. This is explained by the frequency and timing of treatment assessments. ADG provided the best determinant criterion for threshold treatments as reductions in EBW gain were detected early enough to prevent later large reductions. However this was a poor determinant criterion when treating a fixed percentage as calves showing the largest losses in EBW gains at the point of assessment were generally entering the recovery phase and therefore showed little benefit from treatment. Pepsinogen was the worst threshold trigger as WBs did not peak until late in the season, at which point thresholds were crossed and large numbers of treatments were required in unison, causing a large increase in resistance. Conversely, pepsinogen was the best determinant criterion for treating fixed percentages as it indicated those individuals with the greatest parasitic burdens. Overall, the strategy providing the greatest benefit was threshold treatments according to ADG. Of course this is not to say threshold ADG provides the best determinant criterion for treatment, although a wide range of theoretical determinant criteria were tested it may be that other markers are yet to be identified. For example, Kenyon and Jackson (2012) suggest an indicator of appetite could prove a sensitive marker, however this may be challenging to measure as indicated by the limited literature on feed intake.

6.4.5 ***Practical implementation of TST strategies***

The developed model aimed to compare, evaluate and explain different control strategies for the control of *O. ostertagi* in calves. Although the model produced clear outputs for the most beneficial TST strategy, considerably more factors must be considered alongside those addressed by the model (i.e. compromised performance and resistance). For these strategies to be adopted, farmers must be convinced of the merits of TST. This may not be straightforward, as has been suggested for sheep, especially because the benefits from reducing the rate of anthelmintic resistance development may not be immediately obvious.

The relative advantage, complexity and compatibility of TST strategies are all important factors taken into consideration by the farming industry (for which varied priorities exist) (Woodgate and Love, 2012). Difficulties in quantifying such factors make it challenging for farmers to visualise the problem and subsequent benefits of TST. Steps towards quantifying these are essential as change is more likely to be adopted when the problem is obvious (Rogers, 1995). Dealing with this challenge may constitute a new field of research that requires collaboration between parasitologists and social scientists.

In practice, the implementation of TST on cattle farms requires further optimisation, cost-benefit analysis, and attention to practical issues related to assessment of individuals for treatment. The most feasible option is treatment according to ADG as measurements are instantaneous with fewer additional diagnostic costs. Although currently weighing scales are expensive, individual weighing can be labour intensive and poses risks of injury to both cattle and humans when poor facilities are offered. The rapid advances in precision farming may change overcome these obstructions (Laca, 2009). In any case, it would be necessary to base a threshold trigger for treatment on the expected growth rate, which can be dependent on the genotype of a particular cattle breed. However, considerations for other influences of growth, such as grass quality, additional disease or parasitism affecting performance, make this challenging. The development of a parameter such as the 'happy factor' developed for TST in sheep (Greer et al., 2009) could prove valuable in determining a universal, practical threshold recommendation. However, threshold treatments require frequent assessments and constitute a cost, making the methodology more complex. Treating a fixed percentage of calves at random at fixed time points may prove more easily applicable and be more readily accepted by farmers. The use of pen-side automated systems may prove essential to treating a fixed percentage of calves according to determinant criteria. This method could otherwise be considered impractical, due to extra cost and labour associated with the whole herd having to be measured to calculate the top "x" percentage, and re-gathering of the individuals requiring treatment, incurring extra stress. There is potential for the percentage of calves selected for treatment to be based on characteristics of an individual herd. For example, dependent on the risk of resistance, farms at a high risk from anthelmintic resistance should treat a lower percentage of calves.

6.5 Concluding remarks

In this thesis I use a mathematical approach to describe the interactions between calves, *O. ostertagi* and parasite epidemiology with the aim of comparing different control strategies, particularly those aiming to slow the development of parasite resistance. The developed model appears to show a reasonable likeness to field observations, and therefore would be appropriate for conducting purposeful comparisons. In essence, this thesis supports the call for changes in current practice for anthelmintic treatment of calves which recommends whole-herd treatment based on herd monitoring of FEC (Taylor, 2010). In all cases TST strategies provided a clear benefit to the sustainable control of gastrointestinal parasitism of calves by treating only individuals who require it and allowing those less affected to contribute susceptible parasite genotypes to pasture. The model is in agreement with others in questioning the value of FECs as a marker of disease (McAnulty and Greer, 2011; Jackson, 2013), with parasitological measures of FEC and pepsinogen reported to bear little correlation to bodyweight gain (McAnulty and Greer, 2011; Jackson, 2013). In concordance with previous suggestions (Höglund et al., 2009; Jackson, 2013) the model supports the recommendation of TST using threshold triggers for ADG, as was the case for all tested scenarios. The value of this targeting approach lies in the treatment of calves experiencing damaging effects of parasitism, whereas tolerant individuals are left untreated and contributing susceptible genotypes to pasture without displaying the negative effects of parasitism, hence maintaining *refugia*. However, before this can be recommended as best practice for on-farm application further considerations must be made. Further work is required to define the threshold for treatment, to account for the dependence of ADG on calf genetics, herd nutrition and additional disease including co-infections with parasitic species such as *Cooperia*, lungworm and liver fluke. The relative advantages, complexity and compatibility of TST strategies are all factors to be considered as they affect the likelihood of change being adopted by farmers. Future modelling work involving a cost-benefit analysis is required, possibly followed by pilot studies for proof of principle. However, the model suggests that even small changes in policy can help to slow the development of anthelmintic resistance dramatically.

Chapter 7: References

AFRC, 1993. Energy and Protein Requirements of Ruminants: An advisory manual prepared by the AFRC Technical Committee on Responses to Nutrients. CAB INTERNATIONAL, Wallingford, UK.

Agriculture and Horticulature Development Board (AHDB), 2013. Technical Article: Season Overview. Agriculture and Horticulture Development Board, UK. http://dairy.ahdb.org.uk/news/technical-article-archive/december-2013/season-overview/#.VZ5LZPIViko [Accessed: May 2016]

Agriculture and Horticulature Development Board (AHDB), 2015. http://beefandlamb.ahdb.org.uk/wp/wp-content/uploads/2015/07/UK-Yearbook-2015-Cattle-200715.pdf [Accessed: October 2016]

Agriculture and Horticulture Development Board (AHDB), 2016a. http://beefandlamb.ahdb.org.uk/wp/wp-content/uploads/2016/07/BRP-Planning-grazing-strategies-manual-8-150716.pdf [Accessed October 2016]

Agriculture and Horticulture Development Board (AHDB), 2016b. https://dairy.ahdb.org.uk/technical-information/grassland-management/forage-for-knowledge/latest-measures/#.WCSgDi2LSHs [Accessed October 2016]

Al Saqur, I., Armour, J., Bairden, K., Dunn, A. M., Jennings, F. W. and Murray, M., 1982. Field study on the epidemiology and pathogenicity of different isolates of bovine *Ostertagia* spp. *Research in Veterinary Science*, 33, 313–318.

Alexandratos, N. and Bruinsma, J., 2012. World agriculture towards 2030/2050: the 2012 revision. ESA Working paper No. 12-03. Rome, FAO http://www.fao.org/docrep/016/ap106e/ap106e.pdf

Allen, W., Sweasey, D., Berret, S., Nancy Hebert, C. and Patterson, D., 1970. Clinical pathology of ostertagiasis in calves during prolonged experimental infection. *Journal of Comparative Pathology*, 80, 441–454.

Almería, S., Llorente, M. M. and Uriarte, J., 1996. Monthly fluctuations of worm burdens and hypobiosis of gastrointestinal nematodes of calves in extensive management systems in the Pyrenees (Spain). *Veterinary Parasitology*, 67, 225–236.

Anderson, N., Armour, J., Jarrett, W. F. H., Jennings, F. W., Ritchie, J. S. D. and Urquhart, G. M., 1967. Experimental Infections of Ostertagia ostertagi in Calves: Results of Two Regimens of Multiple Innoculations. *American Journal of Veterinary Research*, 28, 1073–1077.

Anderson, N., Armour, J., Rosalind, M. E., Jarrett, W. F. H., Jennings, F.W., Ritchie, J. S. D. and Urquhart, G.M., 1966. Experimental *Ostertagia ostertagi* infections in calves: results of single infections with five graderd dose levels of larvae. *American Journal of Veterinary Research*, 27, 1259–1265.

Anderson, N. and Lord, V., 1979. Anthelmintic efficiency of oxfendazole fenbendazole and levamisole against naturally acquired infections of *Ostertagia ostertagi* and *T. axei* in cattle. *Australian Veterinary Journal*, 55, 158-162.

Anderson, R. C., 2000. Order Strongylida (the Burrsate Nematodes). In *Nematode Parasites of Vertebrates: Their Development and Transmission*, 2nd Edn. CABI Pub., Wallingford, Oxon, UK; New York, NY, pp. 96–98.

Anderson, R. M. and Michel, J. F., 1977. Density-dependent survival in populations of *Ostertagia ostertagi*. *International Journal of Parasitology*, 7, 321–329.

André, J.B. and Gandon, S., 2006. Vaccination, within-host dynamics, and virulence evolution. *Evolution*, 60, 13–23.

Areskog, M., Ljungström, B. and Höglund, J., 2013a. Limited efficacy of pour-on anthelmintic treatment of cattle under Swedish field conditions. *International Journal for Parasitology: Drugs and Drug Resistance*, 3, 129–134.

Areskog, M., Sollenberg, S., Engström, A., von Samson-Himmelstjerna, G. and Höglund, J. 2013b. A controlled study on gastrointestinal nematodes from two Swedish cattle farms showing field evidence of ivermectin resistance. *Parasites and Vectors*, 7, 13.

Armour, J., Bairden, K., Batty, A. F., Davidson, C.C. and Ross, D. B., 1985. Persistent anthelmintic activity of ivermectin in cattle. *Veterinary Record*, 116, 151–153.

Armour, J., Bairden, K., Duncan, J. L., Jennings, F.W. and Parkins, J. J., 1979. Observations on ostertagiasis in young cattle over two grazing seasons with special reference to plasma pepsinogen levels. *Veterinary Record*, 105, 500–503.

Armour, J. and Bruce, R. G., 1974. Inhibited development in *Ostertagia ostertagi* infections-a diapause phenomenon in a nematode. *Parasitology*, 69, 161–74.

Armour, J. and Duncan, M., 1987. Arrested larval development in cattle nematodes. *Parasitology Today (Personal ed.)*, 3, 171–6.

Bairden, K., Armour, J. and Duncan, J. L., 1995. A 4-year study on the effectiveness of alternate grazing of cattle and sheep in the control of bovine parasitic gastro-enteritis. *Veterinary Parasitology*, 60, 119–132.

Baker, D. G. and Gershwin, L. J., 1993. Inverse relationship between IgE and worm burdens in cattle infected with *Ostertagia ostertagi*. *Veterinary Parasitology*, 47, 87–97.

Barger, I. A., 1987. Population regulation in trichostrongylids of ruminants. *International Journal of Parasitology*, 17, 531–540.

Barnes, E. H. and Dobson, R. J., 1990. Population dynamics of *Trichostrongylus colubrimformis* in sheep: computer model to simulate grazing systems and the evolution of anthelmintic resistance. *International Journal of Parasitology,* 20, 823–831.

Barnes, E. H., Dobson, R. J. and Barger, I. A., 1995. Worm control and anthelmintic resistance: adventures with a model. *Parasitology Today*, 11, 56–63.

Barton, C. H. J., Dale, E. F., Dixon, C. and Coles, G. C., 2006. Short Communications Survey of parasite control on beef farms in south-west England. *Veterinary Parasitology*, 159, 682–684.

Bell, S. L., Thomas, R. J. and Ferber, M. T., 1990. Appetite, digestive efficiency, feed utilization and carcass evaluation of housed calves naturally infected with gastrointestinal nematodes. *Veterinary Parasitology*, 34, 323–333.

Bellosa, M. L., Nydam, D. V., Liotta, J. L., Zambriski, J. A., Linden, T. C. and Bowman, D. D., 2011. A comparison of fecal percent dry matter and number of *Cryptosporidium parvum* oocysts shed to observational fecal consistency scoring in dairy calves. *Journal of Parasitology*, 97, 349–351.

Berghen, P., Hilderson, H., Vercruysse, J. and Dorny, P., 1993. Evaluation of pepsinogen, gastrin and antibody response in diagnosing ostertagiasis. *Veterinary Parasitology*, 46, 175–195.

Besier, R. B., 2012. Refugia-based strategies for sustainable worm control: Factors affecting the acceptability to sheep and goat owners. *Veterinary Parasitology,* 186, 2–9.

Bishop, S. C. and Stear, M. J., 1997. Modelling responses to selection for resistance to gastro-intestinal parasites in sheep. *Animal Science*, 64, 469–478.

Boag, B., Topham, P. B. and Webster, R., 1989. Spatial distribution on pasture of infective larvae of the gastro-intestinal nematode parasites of sheep. *International Journal for Parasitology*, 19, 681–685.

Borgsteede, F. H. M., 1978. Observations on the post-parturient rise of nematode egg-output in cattle. *Veterinary Parasitology*, 4, 385-391.

Borgsteede, F. H. and Hendriks, J., 1979. Experimental infections with *Cooperia oncophora* (Railliet, 1918) in calves. Results of single infections with two graded dose levels of larvae. *Parasitology*, 78, 331–342.

Borgsteede, F. H. and Hendriks, J., 1986. The residual effect of treatment with ivermectin after experimental reinfection with nematodes in calves. *Veterinary Quarterly*, 8, 98–104.

Bosco, A., Rinaldi, L., Maurelli, M. P., Musella, V., Coles, G. C. and Cringoli, G. (2014). The comparison of FLOTAC, FECPAK and McMaster techniques for nematode egg counts in cattle. *Acta Parasitologica*, 59, 625–628.

Bown, M., Poppi, D. and Sykes, A., 1991. The effect of post-ruminal infusion of protein or energy on the pathophysiology of *Trichostrongylus colubriformis* infection and body composition in lambs. *Australian Journal of Agricultural Research*, 42, 253–267.

Busin, V., Kenyon, F., Laing, N., Denwood, M. J., Mcbean, D., Sargison, N. D. and Ellis, K., 2013. Addressing sustainable sheep farming: Application of a targeted selective treatment approach for anthelmintic use on a commercial farm. *Small Ruminant Research*, 110, 100–103.

Cammack, K. M., Leymaster, K. A, Jenkins, T. G., Nielsen, M. K., 2005. Estimates of genetic parameters for feed intake, feeding behavior, and daily gain in composite ram lambs. *Journal of Animal Science*, 83, 777–785.

Campolongo, F., Saltelli, A. and Cariboni, J., 2011. From screening to quantitative sensitivity analysis. A unified approach. *Computer Physics Commununications*, 182, 978–988.

Canul-Ku, H. L., Rodríguez-Vivas, R. I., Torres-Acosta, J. F. J., Aguilar-Caballero, A. J., Pérez-Cogollo, L. C. and Ojeda-Chi, M. M., 2012. Prevalence of cattle herds with ivermectin resistant nematodes in the hot sub-humid tropics of Mexico. *Veterinary Parasitology*, 183, 292–298.

Cattadori, I. M., Boag, B., Bjørnstad, O. N., Cornell, S. J., and Hudson, P. J., 2005. Peak shift and epidemiology in a seasonal host−nematode system. *Proceeding of the Royal Society B*, 272, 1163-1169.

Chaparro, M. A. E. and Canziani, G. A., 2010. A discrete model for estimating the development time from egg to infecting larva of *Ostertagia ostertagi* parametrized using a fuzzy rule-based system. *Ecological Modelling*, 221, 2582–2589.

Chaparro, M. A. E., Canziani, G. A. and Fiel, C. A., 2013. Parasitic stage of *Ostertagia ostertagi*: A mathematical model for the livestock production region of Argentina. *Ecological Modelling*, 265, 56–63.

Chaparro, M. A. E., Canziani, G. A., Saumell, C. A. and Fiel, C. A., 2011. Estimation of pasture infectivity according to weather conditions through a fuzzy parametrized model for the free-living stage of *Ostertagia ostertagi*. *Ecological Modelling*, 222, 1820–1832.

Charlier, J., Demeler, J., Höglund, J., von Samson-Himmelstjerna, G., Dorny, P. and Vercruysse, J., 2010. *Ostertagia ostertagi* in first-season grazing cattle in Belgium, Germany and Sweden: general levels of infection and related management practices. *Veterinary Parasitology*, 171, 91–98.

Charlier, J., Dorny, P., Levecke, B., Demeler, J., von Samson-Himmelstjerna, G., Höglund, J. and Vercruysse, J., 2011. Serum pepsinogen levels to monitor gastrointestinal nematode infections in cattle revisited. *Research in Veterinary Science*, 90, 451–456.

Charlier, J., Morgan, E. R., Rinaldi, L., Dijk, J. Van, Demeler, J., Höglund, J., Hertzberg, H., Ranst, B. Van, Hendrickx, G., Vercruysse, J. and Kenyon, F., 2014. Review Practices to optimise gastrointestinal nematode control on sheep , goat and cattle farms in Europe using targeted (selective) treatments. *Veterinary Record*, 175, 250–255.

Christensen, N., Nansen, P., Fagbemi, B. O. and Monrad, J., 1987. Heterologous antagonistic and synergistic interactions between helminths and between helminths and protozoans in concurrent experimental infection of mammalian hosts. *Parasitology Research*, 73, 387–410.

Chylinski, C., Cortet, J., Neveu, C. and Cabaret, J., 2015. Exploring the limitations of pathophysiological indicators used for targeted selective treatment in sheep experimentally infected with *Haemonchus contortus*. *Veterinary Parasitology,* 207, 85–93.

Claerebout, E., Dorny, P., Agneessens, J., Demeulenaere, D. and Vercruysse, J., 1999. The effect of first season chemoprophylaxis in calves on second season pasture contamination and acquired resistance and resilience to gastrointestinal nematodes. *Veterinary Parasitology*, 80, 289–301.

Claerebout, E., Hilderson, H., Meeus, P., De Marez, T., Behnke, J., Huntley, J. and Vercruysse, J., 1996. The effect of truncated infections with *Ostertagia ostertagi* on the development of acquired resistance in calves. *Veterinary Parasitology,* 66, 225–239.

Claerebout, E., Vercauteren, I., Geldhof, P., Olbrechts, A., Zarlenga, D. S., Goddeeris, B. M. and Vercruysse, J., 2005. Cytokine responses in immunized and non-immunized calves after *Ostertagia ostertagi* infection. *Parasite Immunology*, 27, 325–331.

Claerebout, E. and Vercruysse, J., 2000. The immune response and the evaluation of acquired immunity against gastrointestinal nematodes in cattle: a review. *Parasitology*, 120, 25–42.

Cockroft, P., 2015. *Bovine Medicine*, 3rd Edn. John Wiley & Sons, Chichester, UK

Coffey, M. P., Emmans, G. C., Brotherstone, S., 2001. Genetic evaluation of dairy bulls for energy balance traits using random regression. *Animal Science*, 73, 29–40.

Colditz, I. G., 2002. Effects of the immune system on metabolism: Implications for production and disease resistance in livestock. *Livestock Production Science*, 75, 257–268.

Coles, G. C., Bauer, C., Borgsteede, F. H., Geerts, S., Klei, T. R., Taylor, M. A.,Waller, P. J., 1992. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Veterinary Parasitology*, 44, 35–44.

Coles, G. C., Stafford, K. A., MacKay, P. H., 1998. Ivermectin-resistant Cooperia species from calves on a farm in Somerset. *Veterinary Record*, 142, 255–256.

Coop, R. L., Sykes, A.R. and Angus, K. W., 1979. The pathogenicity of daily intakes of *Cooperia oncophora* larvae in growing calves. *Veterinary Parasitology*, 5, 261–269.

Coop, R. L. and Kyriazakis, I., 1999. Nutrition-parasite interaction. *Veterinary Parasitology*, 84, 187–204.

Coop, R. L. and Kyriazakis, I., 2001. Influence of host nutrition on the development and consequences of nematode parasitism in ruminants. *Trends in parasitology*, 17, 325–330.

Coop, R. L., Sykes, A. R. and Angus, K. W., 1979. The pathogenicity of daily intakes of *Cooperia oncophora* larvae in growing calves. *Veterinary Parasitology*, 5, 261–269.

Corwin, R. M., 1997. Economics of gastrointestinal parasitism of cattle. *Veterinary Parasitology*, 72, 451-460.

Cotter, J. L., van Burgel, A. and Besier, R. B., 2015. Anthelmintic resistance in nematodes of beef cattle in south-west Western Australia. *Veterinary Parasitology* 207, 276–284.

Craig, N. M., Smith, D. W., Pate, J. A, Morrison, I. W. and Knight, P. A., 2014. Local cytokine transcription in naïve and previously infected sheep and lambs following challenge with *Teladorsagia circumcincta*. *BMC Veterinary Research*, 10, 87.

Cringoli, G., Rinaldi, L., Maurelli, M. P. and Utzinger, J., 2010. FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. *Nature Protocols*, 5, 503–515.

Dale, A., Mayne, S., Ferris, C., Hopps, A., Verner, M., Mulholland, M., McCluggage, I. and Matthews, D., 2012. Measurement of physical and financial performance across 10 farms over three years to identify the main drivers of profitability on Northern Ireland dairy farms. Final report of AgriSearch project D-28-06. Agri-Food and Biosciences Institute, Northern Ireland.

De Maere, V., Vercauteren, I., Gevaert, K., Vercruysse, J. and Claerebout, E., 2005. An aspartyl protease inhibitor of *Ostertagia ostertagi*: molecular cloning, analysis of stage and tissue specific expression and vaccine trial. *Molecular and Biochemical Parasitology*, 141, 81–88.

Demeler, J., Kuttler, U. and von Samson-Himmelstjerna, G., 2010. Adaptation and evaluation of three different in vitro tests for the detection of resistance to anthelmintics in gastrointestinal nematodes of cattle. *Veterinary Parasitology,* 170, 61–70.

Demeler, J., Van Zeveren, A. M. J., Kleinschmidt, N., Vercruysse, J., Höglund, J., Koopmann, R., Cabaret, J., Claerebout, E., Areskog, M. and von Samson-Himmelstjerna, G., 2009. Monitoring the efficacy of ivermectin and albendazole against gastrointestinal nematodes of cattle in Northern Europe. *Veterinary Parasitology*, 160, 109–115.

Dimander, S. O., Höglund, J., Uggla, A., Spörndly, E. and Waller, P. J., 2003. Evaluation of gastro-intestinal nematode parasite control strategies for first-season grazing cattle in Sweden. *Veterinary Parasitology*, 111, 193–209.

Doeschl-Wilson, A., Vagenas, D., Kyriazakis, I. and Bishop, S., 2008. Exploring the assumptions underlying genetic variation in host nematode resistance. *Genetic Selection*, 40, 241–264.

Dorny, P., Shaw, D. J. and Vercruysse, J., 1999. The determination at housing of exposure to gastrointestinal nematode infections in first-grazing season calves. *Veterinary Parasitology*, 80, 325–340.

Dorny, P., Vercruysse, J., Hilderson, H., Berghen, P., Van Ommeslaeghe, K. and Kloosterman, A., 1988. Field evaluation of an experimental albendazole pulse release bolus in the control of parasitic gastroenteritis in first-season grazing calves. *Veterinary Research Communiations*, 12, 335–342.

Eddi, C., Muniz, R. A., Caracostantogolo, J., Errecalde, J. O., Rew, R. S., Michener, S. L. and McKenzie, M. E., 1997. Comparative persistent efficacy of doramectin, ivermectin and fenbendazole against natural nematode infections in cattle. *Veterinary Parasitology,* 72, 33–41.

Edmonds, M. D., Johnson, E. G. and Edmonds, J. D., 2010. Anthelmintic resistance of *Ostertagia ostertagi* and *Cooperia oncophora* to macrocyclic lactones in cattle from the western United States. *Veterinary Parasitology*, 170, 224–229.

El-abdellati, A., Charlier, J., Geldhof, P., Levecke, B. and Demeler, J., 2010. The use of a simplified faecal egg count reduction test for assessing anthelmintic efficacy on Belgian and German cattle farms. *Veterinary Parasitology,* 169, 352–357.

Elsasser, T. H., Sartin, J. L., McMahon, C., Romo, G., Fayer, R., Kahl, S. and Blagburn, B., 1998. Changes in somatotropic axis response and body composition during growth hormone administration in progressive cachetic parasitism. *Domestic Animal Endocrinology,* 15, 239–255.

Else, K. J. and Finkelman, F. D., 1998. Intestinal nematode parasites, cytokines and effector mechanisms. *International Journal for Parasitology*, 28, 1145–1158.

Emmanouil, G., Galanis, G. and Kallos, G., 2006. Statistical methods for the prediction of night-time cooling and minimum temperature. *Meteorological Applications*, 13, 169-178.

Emmans, G. C., 1994. Effective energy – a concept of energy-utilization applied across species. *British Journal of Nutrition*, 71, 801-821.

Emmans, G. C., 1997. A method to predict the food intake of domestic animals from birth to maturity as a function of time. *Journal of Theoretical Biology*, 186, 189–200.

Emmans, G. C. and Fisher, C., 1986. Problems of nutritional theory, in: *Nutritional Requirements and Nutritional Theory*. Butterworths, London. pp. 9-57.

Emmans, G. C. and Kyriazakis, I., 1997. Models of pig growth: problems and proposed solutions. *Livestock Production Science*, 51, 119–129.

Emmans G. C. and Kyriazakis, I., 1999. Growth and body composition, in: *A Quantitative Biology of the Pig*. CAB International, Wallingford, UK. pp. 181–197.

Emmans, G. C. and Kyriazakis, I., 2001. Consequences of genetic change in farm animals on food intake and feeding behaviour. *Proceedings of the Nutritional Society*, 60, 115–125.

English Beef and Lamb Executive (EBLEX), 2005. http://www.eblex.org.uk/wp/wp-content/uploads/2013/06/beefactionforprofit1betterreturnsfromcontinentalcrosssteers.pdf [Accessed: June 2015]

Eysker, M., 1997, Some aspects of inhibited development of trichostrongylids in ruminants. *Veterinary Parasitol*ogy, 72, 265-283

Eysker, M., 1986. The prophylactic effect of ivermectin treatment of calves, three weeks after turnout, on gastro-intestinal helminthiasis. *Veterinary Parasitology*, 22, 95–103

Eysker, M. and Ploeger, H. W., 2000. Value of present diagnostic methods for gastrointestinal nematode infections in ruminants. *Parasitology*, 120, 109–119.

Eysker, M., van der Aar, W. M., Boersema, J. H., Dop, P. Y. and Kooyman, F. N., 1998. The efficacy of Michel's dose and move system on gastrointestinal nematode infections in diary calves. *Veterinary Parasitology*, 75, 99-114.

Fahrenkrog, J., 2013. *Optimierung der Parasitenbekämpfung bei Weidehaltung von Rindern INAUGURAL - DISSERTATION zur Erlangung des Grades eines Doktors*.

Fernández, A. S., Fiel, C. A. and Steffan, P. E., 1999. Study on the inductive factors of hypobiosis of *Ostertagia ostertagi* in cattle. *Veterinary Parasitology*, 81, 295–307.

Ferreira, G. B., MacNeil, M. D. and Van Vleck, L. D., 1999. Variance components and breeding values for growth traits from different statistical models. *Journal of Animal Science*, 77, 2641–2650.

Fiel, C. A., 2009. Multiple anthelmintic resistance in grazing cattle of Argentina: revertion evidences throughout the use of a three year rational control programme. In *Proceedings of the 22nd International Conference of the World Association for the Advancement of Veterinary Parasitology.*, pp. 20, 68.

Fisher, M. and Jacobs, D., 1995. Evaluation of doramectin in a programme for season-long control of parasitic gastroenteritis in calves. *The Veterinary Record*, 137, 281–284.

Flota-Bañuelos, C., Martínez, I., López-Collado, J., Vargas Mendoza, M., González Hernández, H. and Fajersson, P., 2013. Spatio-temporal pattern of larvae and eggs of gastrointestinal nematodes in cattle pastures in Veracruz, Mexico. *Revista De Biología Tropical,* 61, 1747-1758.

Forbes, A. B., Huckle, C. A., Gibb, M. J., Rook, A. J. and Nuthall, R., 2000. Evaluation of the effects of nematode parasitism on grazing behaviour, herbage intake and growth in young grazing cattle. *Veterinary Parasitology,* 90, 111–118.

Forbes, A. B., Warren, M., Upjohn, M., Jackson, B., Jones, J., Charlier, J. and Fox, M. T., 2009. Associations between blood gastrin, ghrelin, leptin, pepsinogen and *Ostertagia ostertagi* antibody concentrations and voluntary feed intake in calves exposed to a trickle infection with *O. ostertagi*. *Veterinary Parasitology*, 162, 295–305.

Fox, M. T., 1993. Pathophysiology of infection with *Ostertagia ostertagi* in cattle. *Veterinary Parasitology*, 46, 143–58.

Fox, M. T., 1997. Pathophysiology of infection with gastrointestinal nematodes in domestic ruminants: recent developments. *Veterinary Parasitology*, 72, 285-308.

Fox, M. T., Gerrelli, D., and Pitt, S. R., 1989a. *Ostertagia ostertagi* infection in the calf: effects of a trickle challenge on the hormonal control of digestive and metabolic function. *Research in Veterinary Science,* 47, 299–304.

Fox, M. T., Gerrelli, D., Pitt, S. R., Jacobs, D. E., Gill, M., Gale, D. L., 1989b. *Ostertagia ostertagi* infection in the calf: effects of a trickle challenge on appetite, digestibility, rate of passage of digesta and liveweight gain. *Research in Veterinary Science,* 47, 294–298.

Fox, M. T., Gerrelli, D., Pitt, S. R., Jacobs, D. E., Hart, I. C. and Simmonds, A. D., 1987. Endocrine effects of a single infection with *Ostertagia ostertagi* in the calf. *International Journal of Parasitology,* 17, 1181–1185.

Fox, M. T., Reynolds, G. W., Scott, I., Simcock, D. C. and Simpson, H. V., 2006. Vagal and splanchnic afferent nerves are not essential for anorexia associated with abomasal parasitism in sheep. *Veterinary Parasitology*, 135, 287–295.

Fox, M. T., Uche, U. E., Vaillant, C., Ganabadi, S. and Calam, J., 2002. Effects of *Ostertagia ostertagi* and omeprazole treatment on feed intake and gastrin-related responses in the calf. *Veterinary Parasitology*, 105, 285–301.

Fox, N. J., Marion, G., Davidson, R. S., White, P. C. L. and Hutchings, M. R., 2013. Modelling Parasite Transmission in a Grazing System: The Importance of Host Behaviour and Immunity. *PLoS ONE 8*, e77996.

Frisch, J. E., 1981. Changes occurring in cattle as a consequence of selection for growth rate in a stressful environment. *The Journal of Agricultural Science*, 96, 23-38.

Gaba, S., Cabaret, J., Sauvé, C., Cortet, J. and Silvestre, A., 2010. Experimental and modeling approaches to evaluate different aspects of the efficacy of Targeted Selective Treatment of anthelmintics against sheep parasite nematodes. *Veterinary Parasitology*, 171, 254–262.

Gallidis, E., Papadopoulos, E., Ptochos, S. and Arsenos, G., 2009. The use of targeted selective treatments against gastrointestinal nematodes in milking sheep and goats in Greece based on parasitological and performance criteria. *Veterinary Parasitology*, 164, 53–58.

Gasbarre, L. C., Leighton, E. A. and Davies, C. J., 1990. Genetic control of immunity to gastrointestinal nematodes of cattle. *Veterinary Parasitology*, 37, 257–272.

Gasbarre, L. C., Smith, L. L., Hoberg, E. and Pilitt, P. A., 2009. Further characterization of a cattle nematode population with demonstrated resistance to current anthelmintics. *Veterinary Parasitology*, 166, 275–280.

Geerts, S., Brandt, J., Kumar, V. and Biesemans, L., 1987. Suspected resistance of *Ostertagia ostertagi* in cattle to levamisole. *Veterinary Parasitology*, 23, 77–82.

Geldhof, P., Claerebout, E., Knox, D., Vercauteren, I., Looszova and A., Vercruysse, J., 2002. Vaccination of calves against *Ostertagia ostertagi* with cysteine proteinase enriched protein fractions. *Parasite Immunology*, 24, 263–270.

Gernand, E., Rehbein, P., von Borstel, U. U. and König, S., 2012. Incidences of and genetic parameters for mastitis, claw disorders, and common health traits recorded in dairy cattle contract herds. *Journal of Dairy Science*, 95, 2144–2156.

Gettinby, G., Bairden, K., Armour, J. and Benitez-usher, C., 1979. A prediction model for bovine ostertagiasis. *The Veterinary Record: Journal of the British Veterinary Association*, 105, 57–59.

Gettinby, G. and Paton, G., 1981. The role of temperature and other factors in predicting the pattern of bovine ostertagia spp. infective larvae on pasture. *Journal of Thermal Biology*, 6, 395–402.

Geurden, T., Chartier, C., Fanke, J., di Regalbono, A. F., Traversa, D., von Samson-Himmelstjerna, G., Demeler, J., Vanimisetti, H. B., Bartram, D. J. and Denwood, M. J., 2015. Anthelmintic resistance to ivermectin and moxidectin in gastrointestinal nematodes of cattle in Europe. *International Journal for Parasitology: Drugs and Drug Resistance*, 5, 163–171.

Gilleard, J. S., 2006. Understanding anthelmintic resistance: The need for genomics and genetics. *International Journal of Parasitology,* 36, 1227–1239.

Gilleard, J. S. and Beech, R. N., 2007. Population genetics of anthelmintic resistance in parasitic nematodes. *Parasitology*, 134, 1133–1147.

Godber, O. F., Phythian, C. J., Bosco, A., Ianniello, D., Coles, G., Rinaldi, L. and Cringoli, G., 2015. A comparison of the FECPAK and Mini-FLOTAC faecal egg counting techniques. *Veterinary Parasitology*, 207, 342–345.

Goddard, M. E. and Hayes, B. J., 2009. Mapping genes for complex traits in domestic animals and their use in breeding programmes. *Nature Reviews Genetics*, 10, 381–391.

González-Hernández, A., Van Coppernolle, S., Borloo, J., Van Meulder, F., Paerewijck, O., Peelaers, I., Leclercq, G., Claerebout, E. and Geldhof, P., 2016. Host protective ASP-based vaccine against the parasitic nematode *Ostertagia ostertagi* triggers NK cell activation and mixed IgG1-IgG2 response. *Scientific Reports*, 6, 29496.

Greer, A. W., Huntley, J. F., Mackellar, A., Mcanulty, R. W., Jay, N. P., Green, R. S., Stankiewicz, M. and Sykes, A. R., 2008. The effect of corticosteroid treatment on local immune responses, intake and performance in lambs infected with *Teladorsagia circumcincta*. *International Journal for Parasitology*, 38, 1717–1728.

Greer, A. W., Kenyon, F., Bartley, D. J., Jackson, E. B., Gordon, Y., Donnan, a. a., McBean, D. W. and Jackson, F., 2009. Development and field evaluation of a decision support model for anthelmintic treatments as part of a targeted selective treatment (TST) regime in lambs. *Veterinary Parasitology*, 164, 12–20.

Greer, A. W., Mcanulty, R. W. and Gibbs, S. J., 2010. Performance-based targeted selective anthelmintic treatment regime for grazing dairy calves. *Proceedings of the 4th Australasian Dairy Science Symposium*, 385–389.

Greer, A. W., Stankiewicz, M., Jay, N. P., McAnulty, R. W. and Sykes, A. R., 2005. The effect of concurrent corticosteroid induced immuno - suppression and infection with the intestinal parasite Trichostrongylus colubriformis on food intake and utilization in both immunologically naïve and competent sheep. *Animal Science*, 80, 89–99.

Grenfell, B. T., Smith, G. and Anderson, R. M., 1986. Maximum-likelihood estimates of the mortality and migration rates of the infective larvae of *Ostertagia ostertagi* and *Cooperia oncophora*. *Parasitology*, 92, 643–652.

Grenfell, B. T., Smith, G. and Anderson, R. M., 1987a. The regulation of *Ostertagia ostertagi* populations in calves: the effect of past and current experience of infection on proportional establishment and parasite survival. *Parasitology*, 95, 363–372.

Grenfell, B. T., Smith, G. and Anderson, R. M., 1987b. A mathematical model of the population biology of *Ostertagia osertagi* in calves and yearlings. *Parasitology*, 95, 389–406.

Grenfell, B. T., Wilson, K., Isham, V. S., Boyd, H. E. G. and Dietz, K., 1995. Modelling patterns of parasite aggregation in natural populations: trichostrongylid nematode-ruminant interactions as a case study. *Parasitology*, 111, 135–151.

Grønvold, J., 1979. On the possible role of earthworms in the transmission of *Ostertagia ostertagi* third-stage larvae from feces to soil. *Journal of Parasitology*, 65, 831- 832.

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

Gruner, L. and Sauve, C., 1982. The distribution of trichostrongyle infective larvae on pasture and grazing behaviour in calves. *Veterinary Parasitology*, 11, 203–213.

Gruner, L., Mauleon, H. and Sauve, C., 1982. Migrations of trichostrongyle infective larvae: experiments with ovine parasites in soil. *Annals of Veterinary Research*, 13, 51–59.

Hansen, J. W., Zajac, A. M., Eversole, D. E. and Gerken, H. J., 1989. The effect of stocking rate and parasite control on the performance of replacement beef heifers on pasture. *Veterinary Parasitology*, 34, 103–115.

Hayhurst, C., Bradley, A., Forbes, A. B., Hunter, K. and Royal, M. D., 2010. Short communication: Genetic and nongenetic factors influencing *Ostertagia ostertagi* antibodies in UK Holstein-Friesian cattle. *Journal of Dairy Science*, 93, 2239–2243.

Hays, W. V. and Preston, R. L., 2012. Nutrition and feeding management to alter carcass composistion of pigs and cattle. In *Low-fat meats: design strategies and human implications* (ed. Hafs, H. D. and Zimbelman, R. G.). Academic Press, London, UK. pp. 13-33

Heizer, E., Zarlenga, D. S., Rosa, B., Gao, X., Gasser, R. B., De Graef, J., Geldhof, P. and Mitreva, M., 2013. Transcriptome analyses reveal protein and domain families that delineate stage-related development in the economically important parasitic nematodes, *Ostertagia ostertagi* and *Cooperia oncophora*. *BMC genomics*, 14, 118–132.

Henriksen, S., Jørgensen, R. and Nansen, P., 1976. Ostertagiasis in calves. I. The effect of control measures on infection levels and body weight gains during the grazing season in Denmark. *Veterinary Parasitology*, 2, 259–272.

Herd, R., 1988. Control strategies for ostertagiasis. *Veterinary Parasitology*, 27, 111–123.

Herlich, H., 1980. *Ostertagia ostertagi* infection and reinfection in cattle of different ages. *American Journal of Veterinary Research*, 41, 259–261.

Herlich, H., Gasbarre, L. C. and Douvres, F., 1984. Infectivity and pathogenicity of three isolates of *Ostertagia ostertagi* in cattle. *Veterinary Parasitology,* 16, 253–260.

Hertzberg, H., Schnieder, T., Lopmeier, F. J. and Stoye, M., 1992. The influence of weather and egg contamination on the development of third-stage larvae of *Cooperia oncophora* on pasture. *International Journal for Parasitology*, 22, 719–730.

Hilderson, H., Berghen, P., Vercruysse, J., Dorny, P. and Braem, L., 1989. Diagnostic value of pepsinogen for clinical ostertagiosis. *Veterinary Record*, 125, 376–377.

Hilderson, H., Vercruysse, J., Claerebout, E., De Graaf, D. C., Fransen, J. and Berghen, F. P., 1995. Interactions between *Ostertagia ostertagi* and *Cooperia oncophora* in calves. *Veterinary Parasitology*, 56, 107–119.

Hilderson, H., Vercruysse, J., de Graaf, D. C., Bastiaensen, P., Fransen, J., Berghen, P., 1993. The presence of an early L4 larvae population in relation to the immune response of calves against *Ostertagia ostertagi*. *Veterinary Parasitology,* 47, 255–266.

Hilderson, H., Vercruysse, J., Dorny, P., Berghen, P. and Kloosterman, A., 1990. Subclinical parasitism in calves: Biochemical and serological evaluation of preventive treatment against gastroenteritis and husk. *Preventative Veterinary Medicine*, 8, 283–290.

Höglund, J., 2010. Parasite surveillance and novel use of anthelmintics in cattle. *Acta Veterinaria Scandinavica*, 52, 2-5.

Höglund, J., Dahlström, F., Sollenberg, S. and Hessle, A., 2013a. Weight gain-based targeted selective treatments (TST) of gastrointestinal nematodes in first-season grazing cattle. *Veterinary Parasitology*, 196, 358–365.

Höglund, J., Hessle, A. and Dahlström, F., 2013b. Calving season is a stronger determinant of worm burdens in pasture-based beef production than the level of residual larval contamination at turnout. *The Veterinary Record*, 172, 472-477.

Höglund, J., Morrison, D. A., Charlier, J., Dimander, S. O., Larsson, A., 2009. Assessing the feasibility of targeted selective treatments for gastrointestinal nematodes in first-season grazing cattle based on mid-season daily weight gains. *Veterinary Parasitology,* 164, 80–88.

Hollanders, W., Berghen, P., Dorny, P., Hilderson, H., Vercruysse, J. and Ryan, W. G., 1992. Prevention of parasitic gastroenteritis and parasitic bronchitis in first and second season grazing cattle. *The Veterinary Record*, 130, 355–356.

Holmes, P.H., 1993. Interactions between parasites and animal nutrition: the veterinary consequences. *Proceedings of the Nutrition Society*, 52, 113–120.

Holter, P., 1979. Effect of Dung-Beetles (*Aphodius* spp.) and earthworms on the disappearance of cattle dung. *Oikos*, 32, 393–402.

Hosking, B. C., Watson, T. G. and Leathwick, D. M., 1996. Multigeneric resistance to oxfendazole by nematodes in cattle. *Veterinary Record*, 138, 67–68.

Houdijk, J. M., Jessop, N. S. and Kyriazakis, I., 2001. Nutrient partitioning between reproductive and immune functions in animals. *Proceedings of the Nutritional Society*, 60, 515-525.

Hutchings, M. R., Gordon, I. J., Kyriazakis, I. and Jackson, F., 2001. Sheep avoidance of faeces-contaminated patches leads to a trade-off between intake rate of forage and parasitism in subsequent foraging decisions. *Animal Behaviour*, 62, 955–964.

Hutchings, M. R., Knowler, K. J., McAnulty, R. and McEwan, J. C., 2007. Genetically resistant sheep avoid parasites to a greater extent than do susceptible sheep. *Proceedings of the Royal Society B: Biological Science*, 274, 1839-1844.

Jabbar, A., Iqbal, Z., Kerboeuf, D., Muhammad, G., Khan, M. N., Afaq, M., 2006. Anthelmintic resistance: the state of play revisited. *Life Sciences*, 79, 2413–2431.

Jackson, A., 2013. Parasitic gastroenteritis in calves during their first season at grass: The potential for a performance-based targeted selective anthelmintic treatment programme. Thesis (PhD). Glasgow Univesity, Glasgow, UK

Jacobs, D., Foster, J. and Gowling, G., 1989. Comparative study of early-season prophylaxis using ivermectin with lungworm vaccination in the control of parasitic bronchitis and gastroenteritis in cattle. *Veterinary Parasitology*, 34, 45–56.

Jalali, A. R., Weisbjerg, M. R., Nadeau, E., Randby, A. T., Rustas, B., Eknæs, M. and Nørgaard, P., 2015. Effects of forage type , animal characteristics and feed intake on faecal particle size in goat , sheep , llama and cattle. *Animal Feed Science and Technology*, 208, 53–65.

Jennings, F. W., Armour, J., Lawson, D. D. and Roberts, R., 1966. Experimental *Ostertagia ostertagi* infections in calves: studies with abomasal cannulas. *American Journal of Veterinary Science*, 27, 1249–1257.

Kahn, L., 2003. Regulation of the resistance and resilience of periparturient ewes to infection with gastrointestinal nematode parasites by dietary supplementation. *Animal Production Science*, 43, 1477–1485.

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

Kao, R. R., Leathwick, D.M., Roberts, M. G., Sutherland, I. A, 2000. Nematode parasites of sheep: a survey of epidemiological parameters and their application in a simple model. *Parasitology*, 121, 85–103.

Kaplan, R.M., 2004. Drug resistance in nematodes of veterinary importance: A status report. *Trends in Parasitology*, 20, 477–481.

Kenyon, F. and Jackson, F., 2012. Targeted flock/herd and individual ruminant treatment approaches. *Veterinary Parasitology*, 186, 10–17.

Kenyon, F., Greer, A. W., Coles, G. C., Cringoli, G., Papadopoulos, E., Cabaret, J., Berrag, B., Varady, M., Van Wyk, J. A., Thomas, E., Vercruysse, J. and Jackson, F., 2009. The role of targeted selective treatments in the development of refugia-based approaches to the control of gastrointestinal nematodes of small ruminants. *Veterinary Parasitology,* 164, 3–11.

Kenyon, F., McBean, D., Greer, A. W., Burgess, C. G. S., Morrison, A. A., Bartley, D. J., Bartley, Y., Devin, L., Nath, M. and Jackson, F., 2013. A comparative study of the effects of four treatment regimes on ivermectin efficacy, body weight and pasture contamination in lambs naturally infected with gastrointestinal nematodes in Scotland. *International Journal of Parasitology*: *Drugs Drug Resist*ance, 3, 77–84.

Klesius, P. H., 1988. Immunity to *Ostertagia ostertagi*. *Veterinary Parasitology*, 27, 159–167.

Kloosterman, A., Parmentier, H. K. and Ploeger, H. W., 1992. Breeding cattle and sheep for resistance to gastrointestinal nematodes. *Parasitology Today (Personal ed.)*, 8, 330–335.

Kloosterman, A., Albers, G. A. A. and Van Den Brink, R., 1978. Genetic variation among calves in resistance to nematode parasites. *Veterinary Parasitology*, 4, 353–368.

Kloosterman, A., Albers, G. A. A. and Van Den Brink, R., 1984. Negative interactions between *Ostertagia ostertagi* and *Cooperia oncophora* in calves. *Veterinary Parasitology*, 15, 135–150.

Kloosterman, A., Ploeger, H. W. and Frankena, K., 1990. Increased establishment of lungworms after exposure to a combined infection of *Ostertagia ostertagi* and *Cooperia oncophora*. *Veterinary Parasitology,* 36, 117–122.

Knap, P. W. and Schrama, J. W., 1996. Simulation of growth in pigs: approximation of protein turn-over parameters. *Animal Science*, 63, 533-547.

Kotze, A. C., Hunt, P. W., Skuce, P., von Samson-Himmelstjerna, G., Martin, R. J., Sager, H., Krücken, J., Hodgkinson, J., Lespine, A., Jex, A. R., Gilleard, J. S., Beech, R. N., Wolstenholme, A. J., Demeler, J., Robertson, A. P., Charvet, C. L., Neveu, C., Kaminsky, R., Rufener, L., Alberich, M., Menez, C. and Prichard, R. K., 2014. Recent advances in candidate-gene and whole-genome approaches to the discovery of anthelmintic resistance markers and the description of drug/receptor interactions. *International Journal of Parasitology*: *Drugs Drug Resist*ance, 4, 164-184.

Kyriazakis, I., 2010. Is anorexia during infection in animals affected by food composition? *Animal Feed Sciene and Technology*, 156, 1–9.

Kyriazakis, I., 2011. Opportunities to improve nutrient efficiency in pigs and poultry through breeding. *Animal*, 5, 821–832.

Kyriazakis, I., 2014. Pathogen-induced anorexia: a herbivore strategy or an unavoidable consequence of infection? *Animal Production Science*, 54, 1190–1197.

Kyriazakis, I., Anderson, D. H., Coop, R. L. and Jackson, F., 1996a. The pathophysiology and development of immunity during long-term subclinical infection with *Trichostrongylus colubriformis* of sheep receiving different nutritional treatments. *Veterinary Parasitology,* 65, 41–54.

Kyriazakis, I., Anderson, D. H., Oldham, J. D., Coop, R. L. and Jackson, F., 1996b. Long-term subclinical infection with *Trichostrongylus colubriformis*: effects on food intake, diet selection and performance of growing lambs. *Veterinary Parasitology*, 61, 297–313.

Kyriazakis, I. and Emmans, C., 1995. The voluntary feed intake of pigs given feed based on wheat bran, dried citrus pulp and grass meal , in relation to measurements of feed bulk. *The British Journal of Nutrition*, 73, 191–207.

Kyriazakis, I. and Houdijk, J., 2007. Food intake and performance of pigs during health, disease and recovery, in *Paradigms in Pig Science* (ed. Wiseman, J., Varley, J., McOrist, M., Kemp, B.). Nottingham University Press, Nottingham, UK., pp. 493–513.

Kyriazakis, I., Tolkamp, B. and Hutchings, M., 1998. Towards a functional explanation for the occurrence of anorexia during parasitic infections. *Animal Behaviour*, 56, 265–274.

Laca, E. A., 2009. Precision livestock production: tools and concepts. Brazilian Journal of Animal Science, 38, 123–132.

Langhans, W., 2000. Anorexia of infection: current prospects. *Nutrition*, 16, 996–1005.

Langhans, W., 2007. Signals generating anorexia during acute illness. *The Proceedings of the Nutrition Society*, 66, 321–30.

Larsson, A., Dimander, S. O., Rydzik, A., Uggla, A., Waller, P. J., Höglund, J., 2007. A 3-year field evaluation of pasture rotation and supplementary feeding to control parasite infection in first-season grazing cattle-Dynamics of pasture infectivity. *Veterinary Parasitology,* 145, 129–137.

Laurenson, Y. C. S. M., Bishop, S. C. and Kyriazakis, I., 2011. In silico exploration of the mechanisms that underlie parasite-induced anorexia in sheep. *The British Journal of Nutrition*, 106, 1023–1039.

Laurenson, Y. C. S. M., Bishop, S. C., Forbes, A. B., Kyriazakis, I., 2013a. Modelling the short- and long-term impacts of drenching frequency and targeted selective treatment on the performance of grazing lambs and the emergence of anthelmintic resistance. *Parasitology*, 140, 780-791.

Laurenson, Y. C. S. M., Kahn, L. P., Bishop, S. C., Kyriazakis, I., 2016. Which is the best phenotypic trait for use in a targeted selective treatment strategy for growing lambs in temperate climates? *Veterinary Parasitology,* 226, 174–188.

Laurenson, Y. C. S. M., Kyriazakis, I., Bishop, S. C., 2012b. In silico exploration of the impact of pasture larvae contamination and anthelmintic treatment on genetic parameter estimates for parasite resistance in grazing sheep. *Journal of Animal Science*, 90, 2167-2180.

Laurenson, Y. C. S. M., Kyriazakis, I., Bishop, S. C., 2013b. Can we use genetic and genomic approaches to identify candidate animals for targeted selective treatment. *Veterinary Parasitology,* 197, 379–383.

Laurenson, Y.C.S.M., Kyriazakis, I., Forbes, A. B., Bishop, S.C., 2012a. Exploration of the epidemiological consequences of resistance to gastro-intestinal parasitism and grazing management of sheep through a mathematical model. *Veterinary Parasitology,* 189, 238–249.

Leathwick, D. M., 2013. Managing anthelmintic resistance – Parasite fitness, drug use strategy and the potential for reversion towards susceptibility. *Veterinary Parasitology,* 198, 145–153.

Leathwick, D. M. and Miller, C. M., 2013. Efficacy of oral, injectable and pour-on formulations of moxidectin against gastrointestinal nematodes in cattle in New Zealand. *Veterinary Parasitology*, 191, 293–300.

Leathwick, D. M., Vlassofft, A., Barlow, N. D., 1995. A Model for Nematodiasis in New Zealand Lambs : the Effect of Drenching Regime and Grazing Management on the Development of Anthelmintic Resistance. *International Journal of Parasitology*, 25, 1479–1490.

Leathwick, D. M., Waghorn, T. S., Miller, C. M., Atkinson, D. S., Haack, N. A. and Oliver, A. M. (2006). Selective and on-demand drenching of lambs: Impact on parasite populations and performance of lambs. *New Zealand Veterinary Journal*, 54, 305–331.

Leighton, E. A., Murrell, K. D. and Gasbarre, L. C., 1989. Evidence for genetic control of nematode egg-shedding rates in calves. *The Journal of Parasitology*, 75, 498–504.

Lewis R. M., Macfarlane J. M., Simm G. and Emmans, G. C., 2004. Effects of food quality on growth and carcass composition in lambs of two breeds and their cross. *Animal Science*, 78, 355–367.

Li, R. W., Hou, Y., Li, C., Gasbarre, L. C., 2010. Localized complement activation in the development of protective immunity against *Ostertagia ostertagi* infections in cattle. *Veterinary Parasitology*, 174, 247–256.

Lifschitz, A., Virkel, G., Sallovitz, J., Sutra, J. F., Galtier, P., Alvinerie, M. and Lanusse, C., 2000. Comparative distribution of ivermectin and doramectin to parasite location tissues in cattle. *Veterinary Parasitology*, 87, 327–338.

Louie, K., Vlassoff, A. and Mackay, A., 2005. Nematode parasites of sheep: extension of a simple model to include host variability. *Parasitology*, 130, 437-446.

Louw, B. P., 1988. The influence of loss and gain of body mass on ovarian activity in beef cows. *South African Journal of Animal Science*, 18, 1–7.

Loyacano, A. F., Williams, J. C., Gurie, J. and DeRosa, A. A., 2002. Effect of gastrointestinal nematode and liver fluke infections on weight gain and reproductive performance of beef heifers. *Veterinary Parasitology*, 107, 227–234.

Mackinnon, M. J., Hetzel, D. J. S., Corbet, N. J., Bryan, R. P. and Dixon, R., 1990. Correlated responses to selection for cow fertility in a tropical beef herd. *Animal Production*, 50, 417–424.

Mackinnon, M., Meyer, K. and Hetzel, D. J., 1991. Genetic variation and covariation for growth, parasite resistance and heat tolerance in tropical cattle. *Livestock Production Science*, 27, 105–122.

Mansour, M. M., Dixon, J. B., Rowan, T. G. and Carter, S. D., 1992. Modulation of calf immune responses by *Ostertagia ostertagi*: the effect of diet during trickle infection. *Veterinary Immunology and Immunopathology*, 33, 261–269.

Mansour, M. M., Rowan, T. G., Dixon, J. B. and Carter, S. D., 1991. Immune modulation by *Ostertagia ostertagi* and the effects of diet. *Veterinary Parasitology*, 39, 321–332.

Martínez-Valladares, M., Geurden, T., Bartram, D. J., Martínez-Pérez, J. M., Robles-Pérez, D., Bohórquez, A., Florez, E., Meana, A. and Rojo-Vázquez, F. A., 2015. Resistance of gastrointestinal nematodes to the most commonly used anthelmintics in sheep, cattle and horses in Spain. *Veterinary Parasitology*, 211, 228–233.

MATLAB and statistics toolbox release 2012, The Math Works, Inc., Natick, Massachusetts, United States.

MATLAB and statistics toolbox release 2015b, The Math Works, Inc., Natick, Massachusetts, United States.

Mayer, D. G. and Butler, D. G., 1993. Statistical validation. *Ecological Modelling*, 68, 21–32.

McHugh, N., Evans, R. D., Amer, P. R., Fahey, A. G. and Berry, D. P., 2011. Genetic parameters for cattle price and body weight from routinely collected data at livestock auctions and commercial farms. *Journal of Animal Science*, 89, 29–39.

McAnulty, R. W., Gibbs, S. J. and Greer, A. W., 2011. BRIEF COMMUNICATION: Liveweight gain of grazing dairy calves in their first season subjected to a targeted selective anthelmintic treatment (TST) regime. *Proceedings of the New Zealand Society of Animal Production*, 71, 301–303.

McAnulty, R. W. and Greer, A. W., 2011. Developing and refining a performance-based targeted selective anthelmintic treatment regime for grazing dairy calves. *Proceedings of the 21st International Conference of the WAAVP*. Buenos Aires August 2011 pp. 259.

McKenna, P. B., 1991. Resistance to benzimidazole anthelmintics in cattle in New Zealand. *New Zealand Veterinary Journal*, 39, 154–155.

Medley G.F., 2002. The epidemiological consequences of optimisation of the individual host immune response. *Parasitology ,*125, S61–S70.

Michel, J. F., 1968. The Control of Stomach-Worm Infection in Young Cattle. *Grass and Forage Science*, 23, 165–173.

Michel, J. F., 1969a. The regulation of egg output by Ostertagia ostertagi in calves infected once only. *Parasitology*, 59, 767–774.

Michel, J. F. 1969b. Some observations on the worm burdens of calves infected daily with *Ostertagia ostertagi*. *Parasitology*, 59, 575–595.

Michel, J. F., 1970. The regulation of populations of *Ostertagia ostertagi* in calves. *Parasitology*, 61, 435–447.

Michel, J. F., 1985. Strategies for the use of anthelmintics in livestock and their implications for the development of drug resistance. *Parasitology*, 90, 621–628.

Michel, J. F. and Lancaster, M. B., 1970. Experiments on the control of parasitic gastroenteritis in calves. *Journal of Helminthology*, 44, 107–140.

Michel, J. F., Lancaster, M. B., Hong, C., 1978. The length of *Ostertagia ostertagi* in populations of uniform age. *International Journal of Parasitology*, 8, 437–441.

Michel, J. F. and Sinclair, I. J., 1969. The effect of cortisone on the worm burdens of calves infected daily with *Ostertagia ostertagi*. *Parasitology*, 59, 691–708.

Mihi, B., van Meulder, F., Vancoppernolle, S., Rinaldi, M., Chiers, K., van den Broeck, W., Goddeeris, B. M., Vercruysse, J., Claerebout, E. and Geldhof, P., 2014. Analysis of the mucosal immune responses induced by single and trickle infections with the bovine abomasal nematode *Ostertagia ostertagi*. *Parasite Immunology*, 36, 150–156.

Morgan, E., Charlier, J., Hendrickx, G., Biggeri, A., Catalan, D., von Samson-Himmelstjerna, G., Demeler, J., Müller, E., van Dijk, J., Kenyon, F., Skuce, P., Höglund, J., O'Kiely, P., van Ranst, B., de Waal, T., Rinaldi, L., Cringoli, G., Hertzberg, H., Torgerson, P., Wolstenholme, A. and Vercruysse, J., 2013. Global Change and Helminth Infections in Grazing Ruminants in Europe: Impacts, Trends and Sustainable Solutions. *Agriculture*, 3, 484–502.

Murray, M., Jennings, F. and Armour, J., 1970. Bovine ostertagiasis: structure, function and mode of differentiation of the bovine gastric mucosa and kinetics of the worm loss. *Research in Veterinary Science*, 11, 417–427.

Myers, G. H. and Taylor, R. F., 1989. Ostertagiasis in cattle. *Journal of Veterinary Diagnostic Investigation*, 1, 195–200.

Nansen, P., Foldager, J., Hansen, J. W., Henriksen, S. A and Jørgensen, R. J., 1988. Grazing Pressure and Acquisition of *Ostertagia ostertagi* in Calves. *Veterinary Parasitology*, 27, 325–335.

Nansen, P., Grønvold, J., Jørgensen, R. J., Henriksen, S. A., Foldager, J. and Sejrsen, K., 1989. Outbreaks of early-season trichostrongylosis in calves in Denmark. *Veterinary Parasitology*. 32, 199–211.

National Office of Animal Health (NOAH) http://www.noahcompendium.co.uk/merial_animal_health_ltd/ivomec_classic_injection_for_cattle_and_sheep/-66181.html (Accessed: November 2015).

NRC., 2001. *Nutrient Requirements of Dairy Cattle*. National Academies Press, pp. 13–28.

O'Shaughnessy, J., Earley, B., Mee, J. F., Doherty, M. L., Crosson, P., Barrett, D., de Waal, T., 2015a. Nematode control in suckler beef cattle over their first two grazing seasons using a targeted selective treatment approach. *Irish Veterinary Journal*, 68, 13.

O'Shaughnessy, J., Earley, B., Mee, J. F., Doherty, M. L., Crosson, P., Barrett, D., de Waal, T., 2015b. Controlling nematodes in dairy calves using targeted selective treatments. *Veterinary Parasitology*, 209, 221–228.

O'Shaughnessy, J., Earley, B., Mee, J. F., Doherty, M. L., Crosson, P., Barrett, D., Macrelli, M., de Waal, T., 2014a. Nematode control in spring-born suckler beef calves using targeted selective anthelmintic treatments. *Veterinary Parasitology,* 205, 150–157.

O'Shaughnessy, J., Earley, B., Mee, J.F., Doherty, M. L., Crosson, P., Barrett, D., Prendiville, R., Macrelli, M., de Waal, T., 2014b. Detection of anthelmintic resistance on two Irish beef research farms. *Veterinary Record*, 175, 120–121.

Pandey, V. S., 1972. Effect of temperature on survival of the free-living stages of *Ostertagia ostertagi*. *The Journal of Parasitology*, 58, 1042–1046.

Pandey, V. S., 1974. Ecological observations on the free-living stages of *Ostertagia ostertagi*. *Annales de Recherches Vétérinaires*, 5, 261–279.

Papadopoulos, E., Gallidis, E., Ptochos, S., 2012. Anthelmintic resistance in sheep in Europe: A selected review. *Veterinary Parasitology,* 189, 85–88.

Parkins, J. J. and Holmes, P. H., 1989. Effects of gastrointestinal helminth parasites on ruminant nutrition. *Nutrition Research Reviews*, 2, 227–246.

Parkins, J. J., Taylor, L. M., Holmes, P. H., Bairden, K., Salman, S. K. and Armour, J., 1990. Pathophysiological and parasitological studies on a concurrent infection of *Ostertagia ostertagi* and *Cooperia oncophora* in calves. *Research in Veterinary Science*, 48, 201-208.

Persson, L., 1974. Studies on the survival of eggs and infective larvae of *Ostertagia ostertagi* and *Cooperia oncophora* in herbage. *Zentralblatl für Veterinärmedizin*, 21B, 787-798

Phillips, C. J. C., 2010. Principles of Cattle Production, 2nd Edn. CAB INTERNATIONAL, Cambridge, UK, pp. 154–187.

Ploeger, H. W. and Kloosterman, A., 1993. Gastrointestinal nematode infections and weight gain in dairy replacement stock: first-year calves. *Veterinary Parasitology,* 46, 223–241.

Ploeger, H. W., Kloosterman, A., Borgsteede, F. H. and Eysker, M., 1990. Effect of naturally occurring nematode infections in the first and second grazing season on the growth performance of second-year cattle. *Veterinary Parasitology*, 36, 57–70.

Ploeger, H. W., Kloosterman, A and Rietveld, F. W., 1995. Acquired immunity against *Cooperia* spp. and *Ostertagia* spp. in calves: effect of level of exposure and timing of the midsummer increase. *Veterinary Parasitology,* 58, 61–74.

Poulin, R., 2001. Interactions between species and the structure of helminth communities. *Parasitology*, 122, S3–S11.

Prada Jiménez de Cisneros, J., Stear, M. J., Mair, C., Singleton, D., Stefan, T., Stear, A., Marion, G. and Matthews, L., 2014. An explicit immunogenetic model of gastrointestinal nematode infection in sheep. *Journal of the Royal Society Interface*, 11, 1–10.

Prakash, M., 2009. Genetics, Inbreeding and Crossbreeding *in Introduction to Veterinary Genetics*, 1st Edn. Discovery Publishing House, Pvt. Ltd., New Delhi, pp. 36–74.

Prichard, R. K., Steel, J. W. and Hennessy, D. R., 1981. Fenbendazole and thiabendazole in cattle: partition of gastrointestinal absorption and pharmacokinetic behaviour. *Journal of Veterinary Pharmacology and Therapeutics*, 4, 295-304.

Prichard, R. K., 2007. Ivermectin resistance and overview of the Consortium for Anthelmintic Resistance SNPs. *Expert Opinion on Drug Discovery*, 2, S41–S52.

Ramos, F., Portella, L. P., Rodrigues, F. de S., Reginato, C. Z., Pötter, L., Cezar, A. S., Sangioni, L. A. and Vogel, F. S. F., 2016. Anthelmintic resistance in gastrointestinal nematodes of beef cattle in the state of Rio Grande do Sul, Brazil. *International Journal for Parasitology: Drugs and Drug Resistance*, 6, 93–101.

Ranjan, S., Trudeau, C., Prichard, R. K., Daigneault, J. and Rew, R. S., 1997. Nematode reinfection following treatment of cattle with doramectin and ivermectin. *Veterinary Parasitology,* 72, 25–31.

Rehbein, S., Baggott, D. G., Johnson, E. G., Kunkle, B. N., Yazwinski, T. A., Yoon, S., Cramer, L. G. and Soll, M. D., 2013. Nematode burdens of pastured cattle treated once at turnout with eprinomectin extended-release injection. *Veterinary Parasitology*, 192, 321–331.

Rendell, D. K., 2010. Anthelmintic resistance in cattle nematodes on 13 south-west Victorian properties. *Australian Veterinary Journal*, 88, 504–509.

Renshaw, E., 1991. *Modelling Biological Populations in Space and Time*, 1st Edn. Cambridge University Press, Cambridge, UK.

Ritchie, J. S. D., Anderson, N., Armour, J., Jarrett, W. F. H., Jennings, F. W. and Urquhart, G. M., 1966. Experimental *Ostertagia ostertagi* Infections in Calves: Parasitology and Pathogenesis of a Single Infection. *American Journal of Veterinary Science*, 27, 659–667.

Rogers, E. M., 1995. Diffusion of Innovations. The Free Press, New York, USA.

Rose, H., Rinaldi, L., Bosco, A., Mavrot, F., de Waal, T., Skuce, P., Charlier, J., Torgerson, P. R., Hertzberg, H., Hendrickx, G., Vercruysse, J. and Morgan, E. R., 2015a. Widespread anthelmintic resistance in European farmed ruminants: a systematic review. *Veterinary Record*, 176, 546.

Rose, H., Wang, T., Dijk, J. Van and Morgan, E. R., 2015b. GLOWORM-FL : A simulation model of the effects of climate and climate change on the free-living stages of gastro-intestinal nematode parasites of ruminants. *Ecological Modelling*, 297, 232–245.

Rose, J. H., 1961. Some observations on the free-living stages of *Ostertagia ostertagi*, a stomach worm of cattle. *Parasitology*, 51, 295–307.

Rose, J. H., 1969. The development of the parasitic stages of *Ostertagia ostertagi*. *Journal of Helminthology*, 43, 173–184.

Ross, J. G., 1963. Experimental infections of calves with the nematode parasite *Ostertagia ostertagi*. *Veterinary Record*, 75, 129–132.

Rykiel, E. J., 1996. Testing ecological models: The meaning of validation. *Ecological Modelling*, 90, 229–244.

Saltelli, A., Annoni, P., Azzini, I., Campolongo, F., Ratto, M. and Tarantola, S., 2010. Variance based sensitivity analysis of model output. Design and estimator for the total sensitivity index. *Computer Physics Commununications*, 181, 259–270.

Sandberg, F. B., Emmans, G. C., Kyriazakis, I., 2006. A model for predicting feed intake of growing animals during exposure to pathogens. *Journal of Animal Science*, 84, 1552–1566.

Sarkũnas, M., Malakauskas, A., Nansen, P., Hansen, J. W. and Paulikas, V., 1999. Effect of strategic treatments with ivermectin on parasitism of set-stocked calves exposed to natural trichostrongyle infection in Lithunia. *Acta Veterinaria Scandinavica*, 40, 163-171.

Satrija, F. and Nansen, P., 1993. Experimental concurrent infections with *Ostertagia ostertagi* and *Cooperia oncophora* in the calf. *Research in Veterinary Science*, 55, 92-97.

Satrija, F., Nansen, P. and Jørgensen, R., 1996. The effects of first-season strategic and tactical ivermectin treatments on trichostrongylosis in the first-and second-season grazing. *Veterinary Parasitology*, 64, 219–237.

Sellers, M. D., Hulbert, L. E., Cobb, C. J. and Ballou, M. A., 2011. Partitioning innate immune response variation: How much variation is due to the animal? *Journal of Animal Science*, 89 (E-Suppl.1), 18–19.

Scott, P. R., Penny, C. D. and Macrae, A., 2011. *Cattle Medicine*, 1st Edn. CRC Press, pp. 224-236.

Shaw, D. J., Vercruysse, J., Claerebout, E. and Dorny, P., 1998a. Gastrointestinal nematode infections of first-grazing season calves in Western Europe: general patterns and the effect of chemoprophylaxis. *Veterinary Parasitology*, 75, 115–131.

Shaw, D. J., Vercruysse, J., Claerebout, E. and Dorny, P., 1998b. Gastrointestinal nematode infections of first-grazing season calves in Western Europe: Associations between parasitological, physiological and physical factors. *Veterinary Parasitology*, 75, 133–151.

Shaw, D. J., Vercruysse, J., Claerebout, E., Agneessens, J. and Dorny, P., 1997. Gastrointestinal nematode infections of first-season grazing calves in Belgium: general patterns and the effect of chemoprophylaxis. *Veterinary Parasitology*, 69, 103–116.

Sibbald, A. M., Shellard, L. J. F., Smart, T. S., 2000. Effects of space allowance on the grazing behaviour and spacing of sheep. *Applied Animal Behaviour Science*, 70, 49–62.

Skuce, P. J., Morgan, E. R., van Dijk, J. and Mitchell, M., 2013. Animal health aspects of adaptation to climate change: beating the heat and parasites in a warming Europe. *Animal*, 7, 333–345.

Smith, B. P., 2014. *Large Animal Internal Medicine*, 5th Edn. Mosby Inc., Elsevier Health Sciences, Missouri, US

Smith, G., 1997. The economics of parasite control: obstacles to creating reliable models. *Veterinary Parasitology,* 72, 437–449.

Smith, G. and Galligan, D. T., 1988. Mathematical models of the population biology of *Ostertagia ostertagi* and *Teladorsagia circumcincta*, and the economic evaluation of disease control strategies. *Veterinary Parasitology*, 27, 73–83.

Smith, G. and Grenfell, B. T., 1985. The population biology of *Ostertagia ostertagi*. *Parasitology Today*, 1, 76–81.

Smith, G., Grenfell, B. T., 1994. Modelling of parasite populations: gastrointestinal nematode models. *Veterinary Parasitology,* 54, 127–143.

Smith, G., Grenfell, B. T. and Anderson, R. M., 1986. The development and mortality of the non-infective free-living stages of *Ostertagia ostertagi* in the field and in laboratory culture. *Parasitology*, 92, 471–482.

Smith, G., Grenfell, B. T. and Anderson, R. M., 1987a. The regulation of *Ostertagia ostertagi* populations in calves: density-dependent control of fecundity. *Parasitology* 95, 373–388.

Smith, G., Grenfell, B. T., Anderson, R. M. and Beddington, J., 1987b. Population biology of *Ostertagia ostertagi* and anthelmintic strategies against ostertagiasis in calves. *Parasitology*, 95, 407–420.

Smith, G., Grenfell, B. T., Isham, V., Cornell, S., 1999. Anthelmintic resistance revisited: under-dosing, chemoprophylactic strategies, and mating probabilities. *International Journal of Parasitology,* 29, 77–94.

Smith, G. and Guerrero, J., 1993. Mathematical models for the population biology of *Ostertagia ostertagi* and the significance of aggregated parasite distributions. *Veterinary Parasitology*, 46, 243–257.

Smith G. and Wilson R. A., 1980. Seasonal variations in the microclimate of habitats. *Journal Applied Ecological*, 17, 329–342.

Snider, T. G., Williams, J. C., Sheehan, D. S. and Fuselier, R. H., 1981. Plasma pepsinogen, inhibited larval development, and abomasal lesions in experimental infections of calves with *Ostertagia ostertagi*. *Veterinary Parasitology,* 8, 173–183.

Soulsby, E. J. L., 1965. Vol.I. Helminths *In Textbook of Veterinary Clinical Parasitology*, Blackwell Scientific Publications, Oxford, UK.

Stafford, K. and Coles, G. C., 1999. Nematode control practices and anthelmintic resistance in dairy calves in the south west of England. *Veterinary Record*, 144, 659-661.

Stafford, K. A., Morgan, E. R. and Coles, G. C., 2009. Weight-based targeted selective treatment of gastrointestinal nematodes in a commercial sheep flock. *Veterinary Parasitology*, 164, 59–65.

Stear, M. J. and Bishop, S. C., 1999. The curvilinear relationship between worm length and fecundity of *Teladorsagia circumcincta*. *International Journal of Parasitology,* 29, 777–780.

Stear, M. J., Bishop, S. C., Doligalska, M., Duncan, J. L., Holmes, P. H., Irvine, J., McCririe, L., McKellar, Q. A., Sinski, E. and Murray, M., 1995. Regulation of egg production, worm burden, worm length and worm fecundity by host responses in sheep infected with *Ostertagia circumcincta*. *Parasite Immunology*, 17, 643–652.

Stromberg, B. E., 1997. Environmental factors influencing transmission. *Veterinary Parasitology*, 72, 247–264.

Suarez, V. H. and Cristel, S. L., 2007. Anthelmintic resistance in cattle nematode in the western Pampeana Region of Argentina. *Veterinary Parasitology*, 144, 111–117.

Sutherland, I. A. and Bullen, S. L., 2014. Parasite control–are we at the edge of a precipice? *Proceedings of the 5th Australasian Dairy Science Symposium*. 298–303.

Sutherland, I. A. and Leathwick, D. M., 2011. Anthelmintic resistance in nematode parasites of cattle: a global issue? *Trends in Parasitology*, 27, 176–181.

Sutherland, I. A. and Scott, I., 2010. *Gastrointestinal Nematodes of Sheep and Cattle*, 1st Edn. Wiley-Blackwell, Chichester, UK.

Sykes, A. R., 2000. Environmental effects on animal production: the nutritional demands of nematode parasite exposure in sheep. *Asian-Australasian Journal of Animal Sciences*, 13, 343-350.

Symeou, V., Leinonen, I. and Kyriazakis, I., 2014. Modelling phosphorus intake, digestion, retention and excretion in growing and finishing pig: model evaluation. *Animal*, 8, 1622–1631.

Symeou V., Leinonen I. and Kyriazakis I., 2016. Quantifying the consequences of nutritional strategies aimed at decreasing phosphorus excretion from pig populations: a modelling approach. *Animal*, 10, 578-591.

Szyszka, O. and Kyriazakis, I., 2013. What is the relationship between level of infection and "sickness behaviour" in cattle? *Applied Animal Behaviour Science*, 147, 1–10.

Taylor, L. M., Parkins, J. J., Armour, J., Holmes, K., Bairden, K., Ibarra-silva, A. M., Salman, S. K. and McWilliams, P. N., 1989. Pathophysiological and Parasitological studies on *Ostertagi ostertagi* infections in calves. *Research in Veterinary Science,* 46, 218–225.

Taylor, M. A., 2010. FEC monitoring to optimize the timing of anthelmintics use. In: *A Technical Manual for Veterinary Surgeons and Advisors*. COWS, ADBH http://beefandlamb.ahdb.org.uk/wp/wp-content/uploads/2013/04/sustainable_control_of_worms_in_cattle_cows_manual_final_report_210710.pdf

Taylor, M. A., Coop, R. L and Wall, L., 2015. *Veterinary Parasitology*, 4th Edn. John Wiley & Sons, Chichester, UK.

Taylor, N., 2003. *Review of the use of models in informing disease control policy development and adjustment . A report for DEFRA*.

Taylor, S. M., Mallon, T. R., Kenny, J. and Edgar, H., 1995. A comparison of early and mid grazing season suppressive anthelmintic treatments for first year grazing calves and their effects on natural and experimental infection during the second year. *Veterinary Parasitology*, 56, 75–90.

Thamsborg, S. M., Jørgensen, R. J. and Nansen, P., 1998. Internal parasitism of steers grazing at different stocking rates. *Acta Agriculturæ Scandinavica*, 39, 311-323.

The British Limousin Cattle Society (2010) http://limousin.co.uk/the-breed/breed-standard/ [Accessed: April 2016]

Tisdell, C. A., Harrison, S. R., Ramsay, G. C., 1999. The economic impacts of endemic diseases and disease control programmes. *Revue Scientifique et Technique*, 18, 380–398.

Todd, D. L., Woolliams, J. A. and Roughsedge, T., 2011. Gene flow in a national cross-breeding beef population. *Animal*, 5, 1874–1886.

Torgerson, P. R., Paul, M., Lewis, F. I., 2012. The contribution of simple random sampling to observed variations in faecal egg counts. *Veterinary Parasitology,* 188, 397–401.

Toutain, P. L., Upson, D. W., Terhune, T. N., Mckenzie, M. E., 1997. Comparative pharmacokinetics of doramectin and ivermectin in cattle. *Veterinary Parasitology,* 72, 3–8.

Trouw Nutrition (2010). Milk Yield from Grazing Update 2010. Frank Wright Trouw Nutrition International, UK.

Urquhart, G. M., Armour, J., Duncan, J. L., Dunn, A. M. and Jennings F. W., 1996. *Veterinary Parasitology*, 2nd Edn. Blackwell Publishing, Oxford, UK.

Vagenas, D., Bishop, S. C. and Kyriazakis, I., 2007a. A model to account for the consequences of host nutrition on the outcome of gastrointestinal parasitism in sheep: logic and concepts. *Parasitology*, 134, 1263–1277.

Vagenas, D., Bishop, S. C. and Kyriazakis, I., 2007b. A model to account for the consequences of host nutrition on the outcome of gastrointestinal parasitism in sheep: model evaluation. *Parasitology*, 134, 1279–1289.

Vagenas, D., Doeschl-Wilson, A., Bishop, S. C. and Kyriazakis, I., 2007c. In silico exploration of the effects of host genotype and nutrition on the genetic parameters of lambs challenged with gastrointestinal parasites. *International Journal of Parasitology,* 37, 1617-1630.

Van Bruchem, J., Bosch, M. W., Lammers-Weinhoven, S. C. W. and Bangma, G. A., 1991. Intake, rumination, reticulo-rumen fluid and particle kinetics, and faecal particle size in heifers and cattle fed on grass hay and wilted grass silage. *Livestock Production Science*, 27, 297–308.

van Dijk, J., Sargison, N. D., Kenyon, F. and Skuce, P. J., 2010. Climate change and infectious disease: helminthological challenges to farmed ruminants in temperate regions. *Animal*, 4, 377–392.

Van Souest, P. J., 1994. Body Size and Limitations of Ruminants, in: *Nutritional Ecology of the Ruminant*., 1st Edn. Conrell University Press, Ithaca and London, pp. 40–56.

van Wyk, J. A., 2001. Refugia – overlooked as perhaps the most potent factor concerning the development of anthelmintic resistance. *Onderstepoort Journal of Veterinary Research*, 68, 55–67.

van Wyk, J. A., Hoste, H., Kaplan, R. M. and Besier, R. B., 2006. Targeted selective treatment for worm management-How do we sell rational programs to farmers? *Veterinary Parasitology,* 139, 336–346.

Vercruysse, J. and Claerebout, E., 1997. Immunity development against *Ostertagia ostertagi* and other gastrointestinal nematodes in cattle. *Veterinary Parasitology*, 72, 309–326.

Vercruysse, J. and Claerebout, E., 2001. Treatment vs non-treatment of helminth infections in cattle: defining the threshold. *Veterinary Parasitology,* 98, 195–214.

Vercruysse, J., Dorny, P., Claerebout, E., Demeulenaere, D., Smets, K. and Agneessens, J., 2000. Evaluation of the persistent efficacy of doramectin and ivermectin injectable against *Ostertagia ostertagi* and *Cooperia oncophora* in cattle. *Veterinary Parasitology,* 56, 63–69.

Vercruysse, J., Hilderson, H. and Claerebout, E., 1995. Effect of chemoprophylaxis with avermectins on the immune response to gastrointestinal nematodes in first-season grazing calves. *Veterinary Parasitology*, 58, 35-48.

Vercruysse, J., Hilderson, H., Dorny, P. and Berghen, P., 1988. Efficacy of early season anthelmintic treatment against gastrointestinal nematodes. *The Veterinary Quarterly*, 10, 225–229.

Verschave, S. H., 2015. Development of a transmission model for gastro-intestinal nematode infections in cattle. Thesis (PhD). Ghent University, Belgium.

Verschave, S. H., Charlier, J., Rose, H., Claerebout, E. and Morgan, E. R., 2016a. Cattle and Nematodes Under Global Change: Transmission Models as an Ally. *Trends in Parasitology*, 32, 724-738.

Verschave, S. H., Levecke, B., Duchateau, L., Vercruysse, J. and Charlier, J., 2015. Measuring larval nematode contamination on cattle pastures: Comparing two herbage sampling methods. *Veterinary Parasitology*, 210, 159–166.

Verschave, S. H., Rose, H., Morgan, E. R., Claerebout, E., Vercruysse, J. and Charlier, J., 2016b. Modelling *Cooperia oncophora*: Quantification of key parameters in the parasitic phase. *Veterinary Parasitology*, 223, 111–114.

Verschave, S. H., Vercruysse, J., Claerebout, E., Rose, H., Morgan, E. R. and Charlier, J., 2014. The parasitic phase of *Ostertagia ostertagi*: quantification of the main life history traits through systematic review and meta-analysis. *International Journal for Parasitology*, 44, 1091-1104.

Viney, M. E., Riley, E. M. and Buchanan, K. L., 2005. Optimal immune responses: immunocompetence revisited. *Trends in Ecology and Evolution*, 20, 665–669.

Waghorn, T. S., Leathwick, D. M., Miller, C. M. and Atkinson D. S., 2008. Brave or gullible: testing the concept that leaving susceptible parasites in refugia will slow the development of anthelmintic resistance. *New Zealand Veterinary Journal*, 56, 158–163.

Waghorn, T. S., Miller, C. M., Oliver, A. M. and Leathwick, D. M., 2009. Drench-and-shift is a high-risk practice in the absence of refugia. *New Zealand Veterinary Journal*, 57, 359–363.

Waller, P. J., 2006. Sustainable nematode parasite control strategies for ruminant livestock by grazing management and biological control. *Animal Feed Science and Technology*, 126, 277–289.

Waller, P. J. and Thamsborg, S.M., 2004. Nematode control in 'green' ruminant production systems. *Trends in Parasitology*, 20, 493–497.

Ward, C. J., 2006a. Mathematical models to assess strategies for the control of gastrointestinal roundworms in cattle 1. Construction. *Veterinary Parasitology,* 138, 247–267.

Ward, C. J., 2006b. Mathematical models to assess strategies for the control of gastrointestinal roundworms in cattle 2. Validation. *Veterinary Parasitology,* 138, 268–279.

Wellock, I. J., Emmans, G. C. and Kyriazakis, I., 2003. Modelling the effects of thermal environment and dietary composition on pig performance: model logic and concepts. *Animal Science*, 77, 255–266.

Wellock, I.J., Emmans, G.C., Kyriazakis, I., 2004. Describing and predicting potential growth in the pig. *Animal Science*, 78, 379–388.

Wiggin, C. J. and Gibbs, H. C., 1989. Studies of the immunomodulatory effects of low-level infection with *Ostertagia ostertagi* in calves. *American Journal of Veterinary Research*, 50, 1764–1770.

Williams, C. B. and Jenkins, T. G., 1997. Predicting empty body composition and composition of empty body weight changes in mature cattle. *Agricultural Systems*, 53, 1–25.

Williams, J. C., Roberts, E. D. and Todd, J. M., 1974. *Ostertagia ostertagi*: establishment of patent infections in calves by intravenous inoculation. *International Journal for Parasitology*, 4, 55–61.

Williams, J.C., Broussard, S.D., 1995. Persistent anthelmintic activity of ivermectin against gastrointestinal nematodes of cattle. *American Journal of Veterinary Research*, 56, 1169–1175.

Williams, J.C., Knox, J.W., Marbury, K.S., Kimball, M.D., Baumann, B. A, Snider, T.G., 1987. The epidemiology of *Ostertagia ostertagi* and other gastrointestinal nematodes of cattle in Louisiana. *Parasitology*, 95, 135–53.

Williams, J. C., Knox, J. W., Marbury, K. S., Swalley, R. A. and Eddi, C. S., 1991. Efficacy of levamisole against *Ostertagia ostertagi* in Louisiana cattle during maturation of inhibited larvae (September) and during minimal inhibition. *Veterinary Parasitology*, 40, 73–85.

Wisse, B. E., Ogimoto, K., Morton, G. J., Wilkinson, C. W., Frayo, R. S., Cummings, D. E. and Schwartz, M. W., 2004. Physiological regulation of hypothalamic IL-1beta gene expression by leptin and glucocorticoids: implications for energy homeostasis. *American Journal of Physiology- Endocrinology and Metabolism*, 287, E1107-1113.

Wolstenholme, A. J., Fairweather, I., Prichard, R., von Samson-himmelstjerna, G. and Sangster, N. C., 2004. Drug resistance in veterinary helminths. *Trends in Parasitology*, 20, 469–476.

Woodgate, R. G. and Love, S., 2012. WormKill to WormBoss-Can we sell sustainable sheep worm control? *Veterinary Parasitology*, 186, 51–57.

Woodward, T. E., Shepherd, J. B. and Hein, M. A., 1938. *The Hohenheim system in the management of permanent pastures for dairy cattle, Volumes 651-675*. United States Department of Agriculture, US.

Worthington, J. J., Samuelson, L. C., Grencis, R. K. and McLaughlin, J. T., 2013. Adaptive immunity alters distinct host feeding pathways during nematode induced inflammation, a novel mechanism in parasite expulsion. *PLoS pathogens* 9, e1003122.

Xiao, L. and Gibbs, H. C., 1992. Nutritional and pathophysiologic effects of clinically apparent and subclinical infections of *Ostertagia ostertagi* in calves. *American Journal of Veterinary Research*, 53, 2013–2018.

Yazwinski, T. A., Tucker, C. A., Powell, J., Reynolds, J., Hornsby, P. and Johnson, Z., 2009. Fecal egg count reduction and control trial determinations of anthelmintic efficacies for several parasiticides utilizing a single set of naturally infected calves. *Veterinary Parasitology,* 164, 232–241.

Yazwinski, T. A., Tucker, C. A., Wray, E., Jones, L., Reynolds, J., Hornsby, P. and Powell, J., 2013. Control trial and fecal egg count reduction test determinations of nematocidal efficacies of moxidectin and generic ivermectin in recently weaned, naturally infected calves. *Veterinary Parasitology,* 195, 95–101.

Young, R. and Anderson, N., 1981. The ecology of the free-living stages of *Ostertagia ostertagi* in a winter rainfall region. *Australian Journal of Agricultural Research* 32, 371–388.

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

Zaralis, K., Tolkamp, B. and Wylie, A., 2011. Parasite-induced anorexia and its association with the immune response and plasma leptin concentrations in lambs of two genotypes. *Options Méditerranéennes*, A No. 99, 305–311.

Zarlenga, D. S., Hoberg, E. P., Stringfellow, F. and Lichtenfels, J. R., 1998. Comparisons of Two Polymorphic Species of *Ostertagia* and Phylogenetic relationships within the Ostertagiinae (Nematoda: Trichostrongyloidea) inferred from Ribosomal DNA Repeat and Mitochondrial DNA Sequences. *Journal of Parasitology*, 84, 806–812.

Chapter 8: Appendices

8.1 Appendix A

Previously published generic equations and relationships used as the basis for the model are presented below, along with more detailed justifications for determining intrinsic calf growth rate and body composition.

8.1.1 *Calf body composition*

The live bodyweight of a calf consists of gut fill (GF) and empty body weight (EBW). EBW composition comprises of four main components; protein, lipid, ash and water. The methodology for the calculation of the mature body composition is presented below.

The calf intrinsic growth rate parameter (B) can be calculated by the method of Kyriazakis and Emmans (1990) as:

$$B(t_1 - t_2) = -\ln(-\ln\frac{w_2}{w_m}) + \ln(-\ln\frac{w_1}{w_m}) \quad \text{(day}^{-1}\text{)} \tag{A.1}$$

Where B is the intrinsic growth rate, $w_1$is the calf body weight recorded at time t_1 and $w_2$is the bodyweight recorded at time t_2; $w_m$is the calf bodyweight at maturity.

Calf 'target' live body weights (w) of the genotype used were taken from EBLEX (2005). An average Limousin x Holstein-Friesian bull is expected to reach a mature weight (w_m) of 1000kg (The British Limousin Cattle Society, 2010). Steers are expected to achieve a w_m of 800kg, consistent with AFRC (1993).

Using the above values an intrinsic growth rate (B) of 0.00711 day^{-1} was calculated. From this a mature protein content (P_M) of 106kg was predicted, assuming a constant B* (B x P_M) value of 0.025 (Emmans and Kyriazakis, 1997):

$$P_M = \sqrt[0.27]{\left(\frac{B^*}{B}\right)} \quad \text{(kg)} \tag{A.2}$$

where P_M is the mature protein content of the calf (kg) and B* is a constant relationship between mature protein content and growth across mammals (Emmans, 1997).

Mature water and ash content were both assumed to be functions of the body protein content (Emmans and Kyriazakis, 1997), hence the remaining components of the mature empty body weight (EBW_M) can be assumed to be lipid. The gutfill of a steer can range from 5-25% depending on the quality of the feed available (Louw, 1988; NCR, 2001). For an average quality feed the EBW was assumed to be 85% of body weight (Van Souest, 1994; Williams and Jenkins, 1997); hence a Limousin X Holstein-Friesian steer was assumed to have EBW_M of 680kg. The mature lipid content was calculated at 207kg; this is consistent with literature reports of EBW fat percentages at slaughter of 25 and 30% w_m (Williams and Jenkins, 1997).

8.1.2 ***Basic Intrinsic Growth Model***

The intrinsic (maximum) body protein growth ($\Delta PGrowth_{max}$) was estimated (Emmans, 1997) as:

$$\Delta PGrowth_{max} = P.B.\ln\left(\frac{P_M}{P}\right) \quad \text{(kg/day)} \qquad \text{(A.3)}$$

where P is the current protein mass.

The daily ash accretion (ΔAsh) was estimated (Emmans and Kyriazakis, 1997) as:

$$\Delta Ash = 0.211.\Delta PGrowth \quad \text{(kg/day)} \qquad \text{(A.4)}$$

and the daily water accretion ($\Delta Water$) as:

$$\Delta Water = 2.65.\Delta PGrowth\left(\frac{P}{P_M}\right)^{-0.185} \quad \text{(kg/day)} \qquad \text{(A.5)}$$

The desired daily lipid deposition ($\Delta PLipid_{des}$) was estimated (Emmans, 1997) as:

$$\Delta PLipid_{des} = \Delta PGrowth_{max}.\left(\frac{L_M}{P_M}\right).d.\left(\frac{P}{P_M}\right)^{(d-1)} \quad \text{(kg/day)} \qquad \text{(A.6)}$$

where L_M is the lipid at maturity (kg) and d is given as (Emmans, 1997):

$$d = 1.46.\left(\frac{L_M}{P_M}\right)^{0.23} \tag{A.7}$$

The gutfill (GF) of the calf depends largely on the feed intake and the Metabolisable Energy (ME) content of the feed (MJ/kg DM) (Coffey et al., 2001):

$$GF = FI\left(11 - \frac{7.ME}{15}\right) \quad \text{(kg/day)} \tag{A.8}$$

where FI is the feed intake (kg DM/day).

8.1.3 *Resource requirement and Feed Intake*

The maintenance requirements for protein and energy, $PR_{maint}\ and\ ER_{maint}$ respectively, were both estimated as functions of P and Pm (Emmans and Fisher, 1986; Wellock et al., 2003):

$$PR_{maint} = 0.004\ \frac{P}{{P_M}^{0.27}} \quad \text{(kg/day)} \tag{A.9}$$

$$ER_{maint} = 1.63\ \frac{P}{{P_M}^{0.27}} \quad \text{(MJ/day)} \tag{A.10}$$

The growth requirement for protein (PR_{growth}) was estimated by Wellock et al. (2003):

$$PR_{growth} = \frac{\Delta PGrowth_{max}}{ep} \quad \text{(kg/day)} \tag{A.11}$$

where *ep* is the efficiency of protein deposition, assumed to be 0.26 (AFRC, 1993).

The growth requirement for energy (ER_{growth}) was estimated by Wellock et al (2003):

$$ER_{growth} = (bl.\Delta PLipid_{des}) + (bp.\Delta PGrowth_{max}) \quad \text{(MJ/day)} \quad \text{(A.12)}$$

Where *bl* is the energetic cost per kg of lipid deposition of 56 MJ/kg (Emmans, 1994) and *bp* is the energetic cost of per kg protein deposition of 50 MJ/kg (Emmans, 1994).

The desired feed intake to meet the total energy requirements of the calf (FI_E) was estimated as (Vagenas et al., 2007a):

$$FI_E = \frac{ER}{EEC} \quad \text{(kg DM/day)} \quad \text{(A.13)}$$

where ER is the total daily energy requirement and EEC is the effective energy content of the feed given as (Emmans, 1994):

$$EEC = 1.15ME - 3.84 - 4.67(0.9CP - 0.032) \quad \text{(MJ/kg DM)} \quad \text{(A.14)}$$

where *ME* is the metabolisable energy content of the feed (MJ/kg DM), and *CP* is the crude protein content of the feed (g/kg DM).The desired feed intake to meet the total protein requirements of the calf (FI_P) was estimated by Laurenson et al (2011) as:

$$FI_P = \frac{PR}{0.9CP - 0.032} \quad \text{(kg DM/day)} \quad \text{(A.15)}$$

where PR is the total daily protein requirement and CP is the crude protein content of the feed (g/kg DM).

8.1.4 *Constrained Resources*

Constrained feed intake (CFI) was defined as follows (Lewis et al., 2004):

$$CFI = \frac{CAP}{0.93 - \left(\frac{ME}{15.58}\right)} \quad \text{(kg/day)} \qquad \text{(A.16)}$$

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

The capacity of the animal for daily indigestible organic matter (*CAP*) was estimated as the smaller of: $CAP = 0{\cdot}0223 \cdot BW$ or $CAP = 0{\cdot}0223 \cdot 0{\cdot}51 \cdot BW_M$ (kg/day) where BW is the current body weight of the calf (kg) and BW_M is the body weight of the calf at maturity (kg).

8.1.5 *Allocation of Nutrient resources*

The daily lipid deposited was described by the following equation (Vagenas et al., 2007a).

$$\Delta Lipid = \frac{((FI.EEC) - E_{maint} - E_{protein})}{bl} \quad \text{(kg/day)} \qquad \text{(A.17)}$$

where E_{maint} is the energy given to maintenance, $E_{protein}$ is the energy given to protein growth ($bp.\Delta PGrowth_{max}$).

When there are insufficient resources, and in the case of lipid catabolism, the *bl* parameter was replaced by the heat combustion of lipid (bp_c) assumed to be 39 MJ/kg (AFRC, 1993).

Labile protein (maximum amount of protein the animal can mobilize from its body) was defined by (Houdijk et al., 2001; Sykes, 2000):

$$P_{Labile} = 0.2.P_{max} \quad \text{(kg)} \qquad \text{(A.18)}$$

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

The baseline lipid level (the minimum body lipid level required for survival) is defined as (Vagenas et al., 2007a):

$$L_{Base} = 0.2.P \quad \text{(kg)} \qquad \text{(A.19)}$$

8.1.6 ***Protein Loss***

The protein loss associated with both larval burden and worm mass was described in the paper. This loss is prior to any immune response and hence the protein loss was re-calculated following this consideration. The actual protein loss caused by larval burden after considering this effect has been accounted for (PLB) (Vagenas et al., 2007a):

$$PLB = PLB_{Pot}\left(\frac{PLB_{Pot}.e^{-K_{Imm}.PRQ_{Imm}}}{Ploss_{max}}\right)^{\left(\frac{PAC_{Imm}}{(PAC_{Imm})_{max}}\right)} \quad \text{(kg/day)} \qquad \text{(A.20)}$$

where $PRQ_{Imm}$is the protein required by the immune response, PAC_{Imm} is the protein allocated to the immune response, $(PAC_{Imm})_{max}$ is the maximum protein allocated to immunity ($0.2P_{maint}$), K_{Imm} is an the immune exponent detailed in equation (A.21), PLB_{Pot} is the potential protein associated with larval burden as described in the paper (equation 12), and $Ploss_{max}$ is the maximum protein loss (0.5kg/d) as described in the paper (equation 14).

Protein loss has been calculated prior to consideration of the immune response. The actual protein loss caused by worm mass after considering this effect has been accounted for (PWM) (Vagenas et al., 2007a):

$$PWM = PWM_{Pot}\left(\frac{PWM_{Pot}.e^{-K_{Imm}.PRQ_{Imm}}}{Ploss_{max}}\right)^{\left(\frac{PAC_{Imm}}{(PAC_{Imm})_{max}}\right)} \quad \text{(kg/day)} \qquad \text{(A.21)}$$

where PWM_{Pot} is the potential protein associated with worm mass as described in the paper (equation 13).

The potential protein loss was affected by the immune exponent (K_{Imm}) (Vagenas et al., 2007a):

$$K_{Imm} = \frac{\ln\left(\frac{Ploss_{target}}{Ploss_{max}}\right)}{(PAC_{Imm})_{max}} \quad \text{(A.22)}$$

where $Ploss_{target}$ is the value at which the animal stops allocating protein to immunity (0.001kg/d).

8.1.7 *Immune Requirements*

The protein required for immunity for larval burden ($PRQLB_{Imm}$) is estimated by Vagenas (2007a) as:

$$PRQLB_{Imm} = (PAC_{Imm})_{max} \cdot \frac{\ln\left(\frac{Ploss_{target}}{PLB_{pot}}\right)}{\ln\left(\frac{Ploss_{target}}{PLoss_{max}}\right)} \quad \text{(kg/day)} \quad \text{(A.23)}$$

where $Ploss_{target}$ is the minimum damage for which there is no immune response (0·0001(Vagenas et al., 2007a;2007b))

The protein required for immunity for worm mass ($PRQWM_{Imm}$) was also estimated by Vagenas et al. (2007a) as:

$$PRQWM_{Imm} = -\frac{\ln\left(\frac{Ploss_{target}}{PWM}\right)}{-K_{Imm}} \quad \text{(kg/day)} \quad \text{(A.24)}$$

8.1.8 ***Protein Partitioning***

Protein allocated to growth depends on the requirements for both immunity (PR_{Imm}) and growth (PR_{Growth}), the proportion of protein allocated to growth (PAC_{Growth}) is given as (Vagenas et al., 2007a):

$$PAC_{Growth} = \frac{PR_{Growth}}{PR_{Growth} + PR_{Imm}} \quad \text{(kg/day)} \tag{A.25}$$

The proportion of protein allocated to immunity (PAC_{Imm}) (Vagenas et al., 2007a):

$$PAC_{Imm} = \frac{PR_{Imm}}{PR_{Growth} + PR_{Imm}} \quad \text{(kg/day)} \tag{A.26}$$

The efficiency of metabolisable protein use in immunity is considered to be 0.59 (Laurenson et al., 2011)

8.2 **Appendix B**

8.2.1 ***The consequences of different modes of infection***

As many experiments, for practical reasons, challenge animals with single or weekly doses of larvae, the model was investigated for its behaviour under such scenarios. The worm burdens (WB) of a single calf infected over the course a three week period with a total of 210,000 *O. ostertagi* larvae administered either daily (10,000 L_3 per day trickle challenge), or in three weekly doses of 70,000 L_3, or as a single dose at the start of the period are shown in figure B1.

The single infection resulted in a higher and an earlier peak WB, which was observed on d24 post infection (pi) (figure B1.A); the peak WBs for the weekly and trickle infections were observed on d37 and 40 pi respectively. This was a reflection of both the mode of infection and the associated development of the immune response. WBs started to decline at a faster rate for single infections. WBs declined to negligible levels for all modes of administration, as no new larvae were administered after week 3 pi.

The WB patterns of the different modes of administration were reflected in the numbers of total egg outputs produced (figure B1.B), with single infections resulting in a higher and earlier maximum total egg output. Differences in the patterns of WB and total egg outputs reflect the impact of immunity of worm fecundity and the density-dependent effects on fecundity.

A reduction in feed intake was observed for all modes of larval administration; the point of maximum intake reduction was observed to be earlier and recovery to be slightly faster for infections administered through fewer doses (figure B1.C). The recovery began at d29, 36 and 39 dpi for single, weekly and daily modes of infection respectively; this corresponded closely to the peak timing of the WBs, as the model assumes for both to be dependent on the development of the immune response. Feed intake remains slightly below that of a healthy host for all methods of administration, due to the slow continual development of immunity.

The reductions in calf bodyweight, comparative to the uninfected control calf, for the different modes of larval administration are shown in figure B1.D. Although the reduction in

bodyweight reached its maximum value earlier in the single infection, the final bodyweight loss was more pronounced in trickle infections.

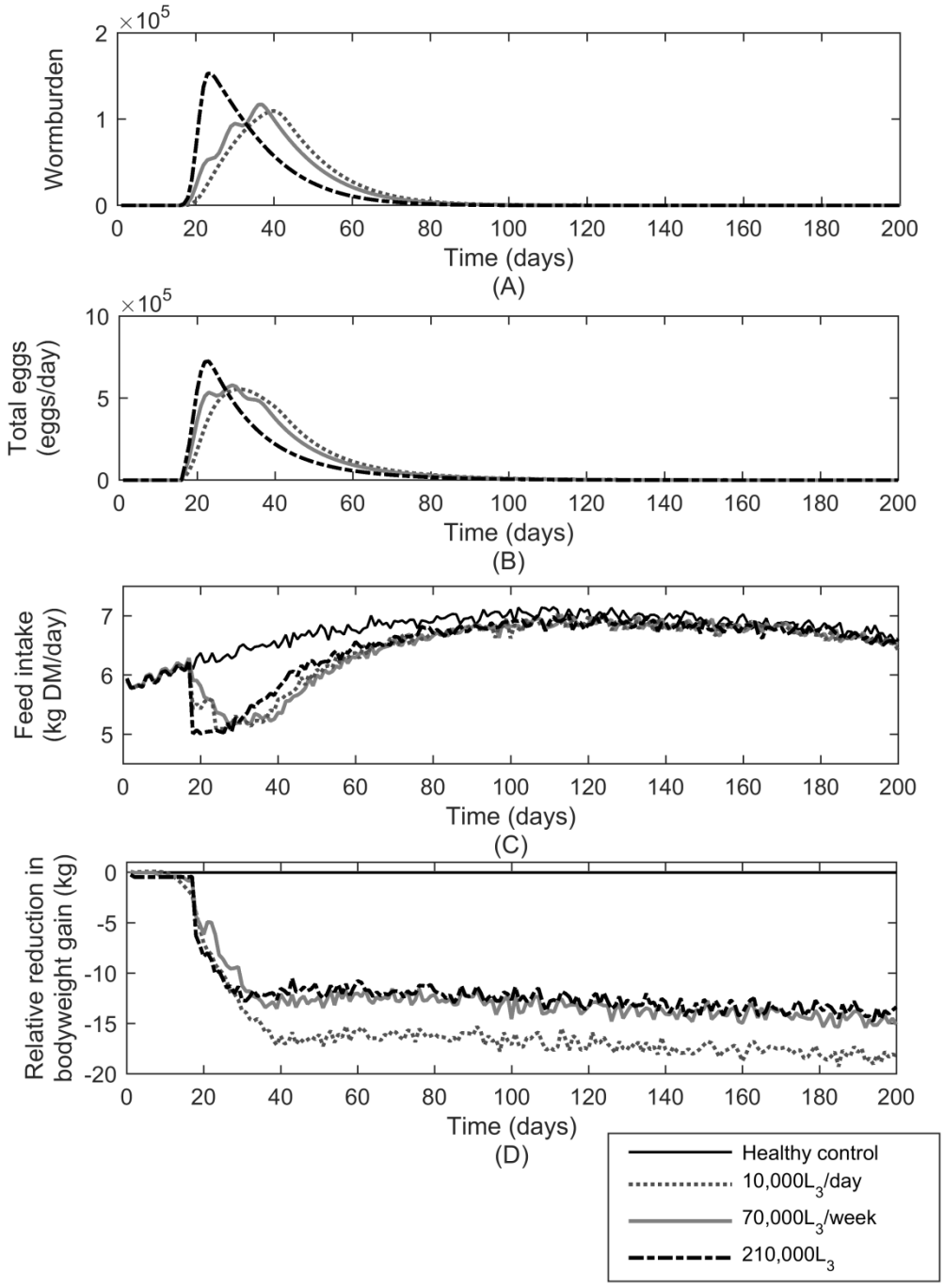

Figure B1: Worm burden (A), daily faecal egg output (B), daily feed intake (C) and total relative bodyweight loss (in comparison to uninfected controls, losses are cumulative over time) (D) incurred over time in calves given a total of 210,000 *Ostertagia ostertagi* larvae over three weeks administered either daily (10,000 per day trickle challenge), as three weekly doses of 70,000, or as a single dose at the start of the period.

8.3 **Appendix C**

8.3.1 ***Sensitivity analysis: P values***

Table C1: Tables of P values for ANOVA tests conducted for the 5 key outputs (peak worm burden, time of the peak worm burden (days), the peak total egg count (eggs/d), the maximum reduction in feed intake (%) and the peak body weight loss) and the significance of 12 key model parameters as described in the text. The values are given for the 3 infection levels explored of A)3500 L3 larvae/day, B)7000 L3 larvae/day and C)14000 L3 larvae/day. The significant parameters are reported in the main text.

Challenge Dose (L3/day)	Parameter	Peak worm burden (worms)	Time of peak worm burden (days)	Peak total egg count (eggs/d)	Maximum reduction in feed intake (%)	Peak BW loss (kg)
3,500	**Max. estabmort**	0.000	0.116	0.000	0.867	0.037
	Min. estabmort	0.885	0.831	0.671	0.353	0.573
	Rate estabmort	0.000	0.000	0.000	0.000	0.000
	Max. mortality	0.240	0.003	0.022	0.360	0.465
	Min. mortality	0.085	0.500	0.023	0.245	0.443
	Rate mortality	0.000	0.000	0.000	0.745	0.080
	Max. fecundity	0.029	0.200	0.000	0.580	0.648
	Min. fecundity	0.777	0.867	0.869	0.865	0.947
	Rate fecundity	0.296	0.653	0.263	0.237	0.762
	Rate anorexia	0.489	0.502	0.639	0.000	0.921
	PlossWM	0.952	0.996	0.937	0.985	0.000
	PlossLM	0.070	0.338	0.434	0.823	0.000

7,000	**Max. estabmort**	0.000	0.210	0.000	0.865	0.146
	Min. estabmort	0.879	0.811	0.650	0.347	0.421
	Rate estabmort	0.000	0.000	0.000	0.000	0.000
	Max. mortality	0.224	0.000	0.015	0.356	0.971
	Min. mortality	0.170	0.409	0.054	0.250	0.117
	Rate mortality	0.000	0.000	0.000	0.746	0.039
	Max. fecundity	0.032	0.424	0.000	0.574	0.118
	Min. fecundity	0.798	0.973	0.899	0.869	0.895
	Rate fecundity	0.293	0.645	0.229	0.236	0.573
	Rate anorexia	0.489	0.475	0.632	0.000	0.994
	PlossWM	0.955	0.949	0.955	0.984	0.000
	PlossLM	0.062	0.454	0.348	0.824	0.000
14,000	**Max. estabmort**	0.000	0.448	0.000	0.863	0.147
	Min. estabmort	0.872	0.517	0.609	0.347	0.774
	Rate estabmort	0.000	0.000	0.000	0.000	0.000
	Max. mortality	0.184	0.000	0.007	0.352	0.457
	Min. mortality	0.256	0.348	0.093	0.252	0.125
	Rate mortality	0.000	0.000	0.000	0.748	0.020
	Max. fecundity	0.038	0.751	0.000	0.572	0.142
	Min. fecundity	0.815	1.000	0.924	0.868	0.886
	Rate fecundity	0.279	0.786	0.187	0.239	0.700
	Rate anorexia	0.481	0.407	0.625	0.000	0.658
	PlossWM	0.957	0.584	0.961	0.984	0.000
	PlossLM	0.055	0.770	0.282	0.825	0.000

8.4 **Appendix D**

Output	Determinant criteria	Percentage of calves treated		
		10%	25%	50%
Cumulative FEC (eggs/g)	ADG	0.132	0.000	0.000
	FEC	0.429	0.038	0.024
	Pepsinogen	0.996	0.721	0.265
Reduction in bodyweight gain (kg)	ADG	0.832	0.510	0.433
	FEC	0.108	0.019	0.382
	Pepsinogen	0.445	0.114	0.132
Frequency of R	ADG	0.000	0.000	0.000
	FEC	0.000	0.000	0.000
	Pepsinogen	0.218	0.529	0.674
BPR (kg/R)	ADG	0.000	0.000	0.000
	FEC	0.019	0.000	0.000
	Pepsinogen	0.584	0.390	0.216

Table D1: Table of P values for statistical comparisons made for treatments according to determinant criteria of ADG (kg/d), FEC (eggs/g) and pepsinogen (IUT/l) in relation to random selection for a range of model outputs for calves kept at conventional stocking rates and exposed to medium pasture contamination levels. These were the final predicted values at the end of the grazing season (day 180) for: A) cumulative faecal egg counts as a measure of parasitism; B) relative reductions in bodyweight gain as a measure of performance; C) frequency of R on pasture as a measure of resistance and D) BPR value. A two-tailed Z test was carried out to assess the statistical significance of treatments according to each determinant criterion, with the exception of relative reductions in bodyweight gain which were assessed using the Mann-Whitney U test due to the skewed data distribution. Assessments were made for each of the investigated percentage of calves treated.

8.5 **Appendix E**

Output	Determinant criteria	Percentage of calves treated	
		10%	25%
Cumulative FEC (eggs/g)	ADG	0.085	0.002
	FEC	0.322	0.184
	Pepsinogen	0.956	0.673
Reduction in bodyweight gain (kg)	ADG	0.523	0.927
	FEC	0.294	0.257
	Pepsinogen	0.277	0.658
Frequency of R	ADG	0.000	0.000
	FEC	0.000	0.000
	Pepsinogen	0.478	0.044
BPR (kg/R)	ADG	0.000	0.000
	FEC	0.000	0.000
	Pepsinogen	0.280	0.008

Table E1: Table of P values for statistical comparisons made for treatments according to determinant criteria of ADG (kg/d), FEC (eggs/g) and pepsinogen (IUT/l) in relation to random selection for a range of model outputs for calves kept at conventional stocking rates and exposed to low pasture contamination levels. These were the final predicted values at the end of the grazing season (day 180) for: A) cumulative faecal egg counts as a measure of parasitism; B) relative reductions in bodyweight gain as a measure of performance; C) frequency of R on pasture as a measure of resistance and D) BPR value. A two-tailed Z test was carried out to assess the statistical significance of treatments according to each determinant criterion, with the exception of relative reductions in bodyweight gain which were assessed using the Mann-Whitney U test due to the skewed data distribution. Assessments were made for each of the investigated percentage of calves treated.

Output	Determinant criteria	Percentage of calves treated	
		10%	25%
Cumulative FEC (eggs/g)	ADG	0.147	0.001
	FEC	0.452	0.033
	Pepsinogen	0.778	0.214
Reduction in bodyweight gain (kg)	ADG	0.437	0.576
	FEC	0.442	0.093
	Pepsinogen	0.648	0.038
Frequency of R	ADG	0.000	0.000
	FEC	0.000	0.000
	Pepsinogen	0.007	0.380
BPR (kg/R)	ADG	0.000	0.000
	FEC	0.000	0.000
	Pepsinogen	0.549	0.823

Table E2: Table of P values for statistical comparisons made for treatments according to determinant criteria of ADG (kg/d), FEC (eggs/g) and pepsinogen (IUT/l) in relation to random selection for a range of model outputs for calves kept at conventional stocking rates and exposed to high pasture contamination levels. These were the final predicted values at the end of the grazing season (day 180) for: A) cumulative faecal egg counts as a measure of parasitism; B) relative reductions in bodyweight gain as a measure of performance; C) frequency of R on pasture as a measure of resistance and D) BPR value. A two-tailed Z test was carried out to assess the statistical significance of treatments according to each determinant criterion, with the exception of relative reductions in bodyweight gain which were assessed using the Mann-Whitney U test due to the skewed data distribution. Assessments were made for each of the investigated percentage of calves treated.

Output	Determinant criteria	Percentage of calves treated	
		10%	25%
Cumulative FEC (eggs/g)	ADG	0.103	0.000
	FEC	0.496	0.113
	Pepsinogen	0.699	0.707
Reduction in bodyweight gain (kg)	ADG	0.170	0.230
	FEC	0.880	0.507
	Pepsinogen	0.931	0.621
Frequency of R	ADG	0.000	0.000
	FEC	0.000	0.000
	Pepsinogen	0.595	0.584
BPR (kg/R)	ADG	0.000	0.000
	FEC	0.000	0.000
	Pepsinogen	0.551	0.974

Table E3: Table of P values for statistical comparisons made for treatments according to determinant criteria of ADG (kg/d), FEC (eggs/g) and pepsinogen (IUT/l) in relation to random selection for a range of model outputs for calves kept at low stocking rates and exposed to medium pasture contamination levels. These were the final predicted values at the end of the grazing season (day 180) for: A) cumulative faecal egg counts as a measure of parasitism; B) relative reductions in bodyweight gain as a measure of performance; C) frequency of R on pasture as a measure of resistance and D) BPR value. A two-tailed Z test was carried out to assess the statistical significance of treatments according to each determinant criterion, with the exception of relative reductions in bodyweight gain which were assessed using the Mann-Whitney U test due to the skewed data distribution. Assessments were made for each of the investigated percentage of calves treated.

Output	Determinant criteria	Percentage of calves treated	
		10%	25%
Cumulative FEC (eggs/g)	ADG	0.157	0.000
	FEC	0.294	0.015
	Pepsinogen	0.873	0.344
Reduction in bodyweight gain (kg)	ADG	0.692	0.417
	FEC	0.724	0.019
	Pepsinogen	0.487	0.012
Frequency of R	ADG	0.000	0.000
	FEC	0.000	0.000
	Pepsinogen	0.038	0.118
BPR (kg/R)	ADG	0.000	0.000
	FEC	0.000	0.000
	Pepsinogen	0.026	0.681

Table E4: Table of P values for statistical comparisons made for treatments according to determinant criteria of ADG (kg/d), FEC (eggs/g) and pepsinogen (IUT/l) in relation to random selection for a range of model outputs for calves kept at high stocking rates and exposed to medium pasture contamination levels. These were the final predicted values at the end of the grazing season (day 180) for: A) cumulative faecal egg counts as a measure of parasitism; B) relative reductions in bodyweight gain as a measure of performance; C) frequency of R on pasture as a measure of resistance and D) BPR value. A two-tailed Z test was carried out to assess the statistical significance of treatments according to each determinant criterion, with the exception of relative reductions in bodyweight gain which were assessed using the Mann-Whitney U test due to the skewed data distribution. Assessments were made for each of the investigated percentage of calves treated.

8.6 **Appendix F**

An overview of model code is provided for those equations not explicit within the thesis text.

8.6.1 ***Calf initial protein***

```
 %% Starting protein iterations
if L==1
   Starting_protein(G)=0.16.*Initial_EBW(G);
   for I= 1:ITER;
     if I==1
      T1(G,1)=Starting_protein(G)+(Ratio_water_protein(G)*(Starting_protein(G)
       ^Constantw))+(Constantratio_ash_protein*Starting_protein(G))+...
       (Lipid_maturity(G)*(Starting_protein(G)^D(G))/(Protein_maturity(G)^D(G)))-...
       Initial_EBW(G);
      T5(G,1) = (1+(Constantw*Ratio_water_protein(G)*(Starting_protein(G)^...
       (Constantw-1)))+Constantratio_ash_protein)+(((Lipid_maturity(G)...
       /Protein_maturity(G))^D(G))*(Starting_protein(G)^(D(G)-1)));
      Birthprotein(G,I)=Starting_protein(G)-(T1(G,1)/T5(G,1));
      else
      T1(G,I) = Birthprotein(G,I-1)+(Ratio_water_protein(G)*(Birthprotein(G,I-1)^Constantw))
       + (Constantratio_ash_protein*Birthprotein(G,I-1))+(Lipid_maturity(G)*…
         (Birthprotein(G,I-1)^D(G))/(Protein_maturity(G)^D(G)))-Initial_EBW(G);
      T5(G,I)=(1+(Constantw*Ratio_water_protein(G)*(Birthprotein(G,I-1)^...
       (Constantw-1)))+Constantratio_ash_protein)+((Lipid_maturity(G)/...
       Protein_maturity(G))^D(G))*(Birthprotein(G,I-1)^(D(G)-1));
      Birthprotein(G,I)=Birthprotein(G,I-1)-(T1(G,I)/T5(G,I));
      end
    end
   Initialprotein(G)=Birthprotein(G,ITER)';
end
```

8.6.2 ***Seasonal grass quality and growth***

```
Metabolisable_energy(L)= 4.3e-05*(L+startdate)^2 - 0.012*(L+startdate) + 12;
if (L+startdate)<73
Crudeprotein(L)= -0.35*(L+startdate) + 190;
elseif (L+startdate)>72
Crudeprotein(L)= 0.34*(L+startdate) + 140;
if (L+startdate)>172
Crudeprotein(L)= 0.05*(L+startdate) + 190;
end
end
Digestable_crudeprotein(L)=(0.9*Crudeprotein(L)/1000)-0.032;
Digestable_OM(L)=Metabolisable_energy(L)/15.58;
Indigestable_OM(L)=0.93-Digestable_OM(L);
Digestability_OM(L)=Digestable_OM(L)/(Digestable_OM(L)+Indigestable_OM(L));
DM_digestibility(L)=(Digestability_OM(L)-0.0169)/1.01;
```

```
Effective_energycontent(L)=(1.15*Metabolisable_energy(L))-(4.67*
Digestable_crudeprotein(L))-((1-Ashcontent)*3.84);

 if (L+startdate)<=135          %Grass growth based on EBLEX better returns
        Grassgrowth_ha(L)=(1/3)*(L+startdate)+5;
         if (L+startdate)<=105
            Grassgrowth_ha(L)=75-(1/3)*(L+startdate);
            if (L+startdate)<=45
               Grassgrowth_ha(L)=(2/3)*(L+startdate)+30;
            end
         end
     else
         Grassgrowth_ha(L)=95-(1/3)*(L+startdate);   %0.8
   end

% Feed quality (variable, dependent on CP and ME)
Fermentable_ME=Metabolisable_energy(L)-
((Fatcontent/1000)*Metabolisable_energy_offat);
Level_feeding(L,G)=(Fermentable_ME*FI_actual(L,G))/Energyreq_maintenance(L,G);
Rumen_outflowrate(L,G)=-0.024+(0.179*(1-exp(-0.278*Level_feeding(L,G))));
Degradableprotein_quick(L,G)=Watersoluble_N*Crudeprotein(L);
Degradableprotein_slow(L,G)=((Potentiallydegradable_N*Fractionalratedegredation_N)/(Fra
ctionalratedegredation_N+Rumen_outflowrate(L,G)))*Crudeprotein(L);
Rumen_degradableprotein(L,G)=(0.8*Degradableprotein_quick(L,G))+Degradableprotein_slo
w(L,G);
Undegradableprotein(L,G)=Crudeprotein(L)-
(Degradableprotein_quick(L,G)+Degradableprotein_slow(L,G));
Digestable_undegradableprotein(L,G)=0.9*(Undegradableprotein(L,G)-
(6.25*Aciddetergentinsoluble_N));
Microbial_crudeproteinyeild(L,G)=7+(6*(1-exp(-0.35*Level_feeding(L,G))));
Fermentablemetabolisableenergy_digested(L,G)=Fermentable_ME*Microbial_crudeproteiny
eild(L,G);
if Fermentablemetabolisableenergy_digested(L,G)<Rumen_degradableprotein(L,G)
   Rumen_degradableprotein(L,G)=Fermentablemetabolisableenergy_digested(L,G);
end
Metabolisableprotein(L,G)=(0.6375*Rumen_degradableprotein(L,G)+
Digestable_undegradableprotein(L,G))/1000;
Proteinintake(L,G)=FI(L,G)*Metabolisableprotein(L,G);
```

8.6.3 *Gene proportions on pasture*

```
  if L==1 %For day 1 set the inititial pasture contamination
  %calculate the initial allele frequencies of gene 1 according to Hardy-Weinberg
     p_RR(L)=Init_R^2;
     p_SR(L)=2*Init_R*(1-Init_R);
     p_SS(L)=(1-Init_R)^2;
     %calculate total numbers of each genotype on pasture
     RR_PastureL3_current(L)=PastureL3_current(L)*p_RR(L);
```

```
      SR_PastureL3_current(L)=PastureL3_current(L)*p_SR(L);
      SS_PastureL3_current(L)=PastureL3_current(L)*p_SS(L);
    %Repeat- calculate the initial allele frequencies of gene 2 according to Hardy-Weinberg
      p_QQ(L)=Init_Q^2;
      p_PQ(L)=2*Init_Q*(1-Init_Q);
      p_PP(L)=(1-Init_Q)^2;
      %calculate total numbers of eaach genotype on pasture
      QQ_PastureL3_current(L)=PastureL3_current(L)*p_QQ(L);
      PQ_PastureL3_current(L)=PastureL3_current(L)*p_PQ(L);
      PP_PastureL3_current(L)=PastureL3_current(L)*p_PP(L);
     %Caluculate the proportion of each genotype (2 genes)
      p_QQRR(L)=p_RR(L)*p_QQ(L);
      p_PQRR(L)=p_RR(L)*p_PQ(L);
      p_PPRR(L)=p_RR(L)*p_PP(L);
      p_QQSR(L)=p_SR(L)*p_QQ(L);
      p_PQSR(L)=p_SR(L)*p_PQ(L);
      p_PPSR(L)=p_SR(L)*p_PP(L);
      p_QQSS(L)=p_SS(L)*p_QQ(L);
      p_PQSS(L)=p_SS(L)*p_PQ(L);
      p_PPSS(L)=p_SS(L)*p_PP(L);
   else          % calculate the pasture contamintion on subsequent days for each genotype
      RR_PastureL3_current(L)=(RR_PastureL3_total(L-1)-RR_PastureL3_consumed(L-1))*(1-
PastureL3_deathrate(L));
      SR_PastureL3_current(L)=(SR_PastureL3_total(L-1)-SR_PastureL3_consumed(L-1))*(1-
PastureL3_deathrate(L));
      SS_PastureL3_current(L)=(SS_PastureL3_total(L-1)-SS_PastureL3_consumed(L-1))*(1-
PastureL3_deathrate(L));
      QQ_PastureL3_current(L)=(QQ_PastureL3_total(L-1)-QQ_PastureL3_consumed(L-1))*(1-
PastureL3_deathrate(L));
      PQ_PastureL3_current(L)=(PQ_PastureL3_total(L-1)-PQ_PastureL3_consumed(L-1))*(1-
PastureL3_deathrate(L));
      PP_PastureL3_current(L)=(PP_PastureL3_total(L-1)-PP_PastureL3_consumed(L-1))*(1-
PastureL3_deathrate(L));

PastureL3_current(L)=RR_PastureL3_current(L)+SR_PastureL3_current(L)+SS_PastureL3_cur
rent(L); % (or QQ+PQ+PP)
  end
```

8.6.4 ***Feed intake***

```
if L==1
   EmptyBW_gut(L,G)=Empty_BW(L,G)/0.85; %Empty bodyweight plus gutfill
else
   EmptyBW_gut(L,G)=EmptyBW_gut(L-1,G);
end
 if L>1
   Ratio_emptyBW_maturity(L,G)=(EmptyBW_gut(L-1,G))/(BW_maturity(G));
  if Ratio_emptyBW_maturity(L,G)>=0.51
     Max_FI(L,G)=(0.51*BW_maturity(G)*Bulkconstraint)/Indigestable_OM(L);
```

```
  else
     Max_FI(L,G)=(EmptyBW_gut(L-1,G)*Bulkconstraint)/Indigestable_OM(L);
  end
else
   Max_FI(L,G)=(EmptyBW_gut(L,G)*Bulkconstraint)/Indigestable_OM(L);
end
% Estimate random variation in desired food intake as follows:
 X= rand(L,G);
 Y= rand(L,G);
 RN1(L,G)=sqrt(-2*log(X(L,G)));
 RN2(L,G)=Y(L,G)*6.2831853;
Randvar_FI(L,G)=RN1(L,G)* cos(RN2(L,G))*Variation_FI;
 FI(1,G)=FI_desired(1,G);

if L>1
  FI(L,G)=FI_desired(L,G).*RED(L,G);
end
if FI(L,G)>Max_FI(L,G)  %Cap maximum feed intake
   FI_actual(L,G)=Max_FI(L,G)*(1-Randvar_FI(L,G));
else
    FI_actual(L,G)=FI(L,G)*(1+Randvar_FI(L,G));
end
```

8.6.5 *New larvae developed from eggs*

Code is provided for one gene only as an example, the same principles apply for the second gene.

```
  if L>=(JUVmin+DT(1))
     RR_Totalnew=0;  %No new L3 larvae when minimun DT has not been acheived
     SR_Totalnew=0;
     SS_Totalnew=0;
     for i=(L-40):(L-2)     % New L3 larvae developing from eggs summed over previous days
        if L==i+DT(i)
           RR_NewL3(i)=RR_Eggs_total(L-DT(i))*Prop_eggstoL3*DT_distrib5;
           RR_Totalnew= RR_Totalnew+RR_NewL3(i);
           SR_NewL3(i)=SR_Eggs_total(L-DT(i))*Prop_eggstoL3*DT_distrib5;
           SR_Totalnew= SR_Totalnew+SR_NewL3(i);
           SS_NewL3(i)=SS_Eggs_total(L-DT(i))*Prop_eggstoL3*DT_distrib5;
           SS_Totalnew= SS_Totalnew+SS_NewL3(i);
        end
        if L==i+DT_min(i)
           RR_NewL3(i)=RR_Eggs_total(L-DT_min(i))*Prop_eggstoL3*DT_distrib1;
           RR_Totalnew= RR_Totalnew+RR_NewL3(i);
           SR_NewL3(i)=SR_Eggs_total(L-DT_min(i))*Prop_eggstoL3*DT_distrib1;
           SR_Totalnew= SR_Totalnew+SR_NewL3(i);
           SS_NewL3(i)=SS_Eggs_total(L-DT_min(i))*Prop_eggstoL3*DT_distrib1;
           SS_Totalnew= SS_Totalnew+SS_NewL3(i);
```

```
end
if L==i+DT_max(i)
   RR_NewL3(i)=RR_Eggs_total(L-DT_max(i))*Prop_eggstoL3*DT_distrib1;
   RR_Totalnew= RR_Totalnew+RR_NewL3(i);
   SR_NewL3(i)=SR_Eggs_total(L-DT_max(i))*Prop_eggstoL3*DT_distrib1;
   SR_Totalnew= SR_Totalnew+SR_NewL3(i);
   SS_NewL3(i)=SS_Eggs_total(L-DT_max(i))*Prop_eggstoL3*DT_distrib1;
   SS_Totalnew= SS_Totalnew+SS_NewL3(i);
end
if L==i+DT2(i)
   RR_NewL3(i)=RR_Eggs_total(L-DT2(i))*Prop_eggstoL3*DT_distrib2;
   RR_Totalnew= RR_Totalnew+RR_NewL3(i);
   SR_NewL3(i)=SR_Eggs_total(L-DT2(i))*Prop_eggstoL3*DT_distrib2;
   SR_Totalnew= SR_Totalnew+SR_NewL3(i);
   SS_NewL3(i)=SS_Eggs_total(L-DT2(i))*Prop_eggstoL3*DT_distrib2;
   SS_Totalnew= SS_Totalnew+SS_NewL3(i);
end
if L==i+DT8(i)
   RR_NewL3(i)=RR_Eggs_total(L-DT8(i))*Prop_eggstoL3*DT_distrib2;
   RR_Totalnew= RR_Totalnew+RR_NewL3(i);
   SR_NewL3(i)=SR_Eggs_total(L-DT8(i))*Prop_eggstoL3*DT_distrib2;
   SR_Totalnew= SR_Totalnew+SR_NewL3(i);
   SS_NewL3(i)=SS_Eggs_total(L-DT8(i))*Prop_eggstoL3*DT_distrib2;
   SS_Totalnew= SS_Totalnew+SS_NewL3(i);
end
if L==i+DT3(i)
   RR_NewL3(i)=RR_Eggs_total(L-DT3(i))*Prop_eggstoL3*DT_distrib3;
   RR_Totalnew= RR_Totalnew+RR_NewL3(i);
   SR_NewL3(i)=SR_Eggs_total(L-DT3(i))*Prop_eggstoL3*DT_distrib3;
   SR_Totalnew= SR_Totalnew+SR_NewL3(i);
   SS_NewL3(i)=SS_Eggs_total(L-DT3(i))*Prop_eggstoL3*DT_distrib3;
   SS_Totalnew= SS_Totalnew+SS_NewL3(i);
end
if L==i+DT7(i)
   RR_NewL3(i)=RR_Eggs_total(L-DT7(i))*Prop_eggstoL3*DT_distrib3;
   RR_Totalnew= RR_Totalnew+RR_NewL3(i);
   SR_NewL3(i)=SR_Eggs_total(L-DT7(i))*Prop_eggstoL3*DT_distrib3;
   SR_Totalnew= SR_Totalnew+SR_NewL3(i);
   SS_NewL3(i)=SS_Eggs_total(L-DT7(i))*Prop_eggstoL3*DT_distrib3;
   SS_Totalnew= SS_Totalnew+SS_NewL3(i);
end
if L==i+DT4(i)
   RR_NewL3(i)=RR_Eggs_total(L-DT4(i))*Prop_eggstoL3*DT_distrib4;
   RR_Totalnew= RR_Totalnew+RR_NewL3(i);
   SR_NewL3(i)=SR_Eggs_total(L-DT4(i))*Prop_eggstoL3*DT_distrib4;
   SR_Totalnew= SR_Totalnew+SR_NewL3(i);
   SS_NewL3(i)=SS_Eggs_total(L-DT4(i))*Prop_eggstoL3*DT_distrib4;
   SS_Totalnew= SS_Totalnew+SS_NewL3(i);
end
```

```
        if L==i+DT6(i)
            RR_NewL3(i)=RR_Eggs_total(L-DT6(i))*Prop_eggstoL3*DT_distrib4;
            RR_Totalnew= RR_Totalnew+RR_NewL3(i);
            SR_NewL3(i)=SR_Eggs_total(L-DT6(i))*Prop_eggstoL3*DT_distrib4;
            SR_Totalnew= SR_Totalnew+SR_NewL3(i);
            SS_NewL3(i)=SS_Eggs_total(L-DT6(i))*Prop_eggstoL3*DT_distrib4;
            SS_Totalnew= SS_Totalnew+SS_NewL3(i);
        end
    end
    RR_PastureL3_new(L)= RR_Totalnew;
    SR_PastureL3_new(L)=SR_Totalnew;
    SS_PastureL3_new(L)=SS_Totalnew;
    PastureL3_new(L)=SS_PastureL3_new(L)+SR_PastureL3_new(L)+RR_PastureL3_new(L);
end
```

8.6.6 *Larvaldays exposure*

```
%% STEP 11: Cumulative LI, larvaldays and gene frequencies
if L==1
    Larvaldays(L,G)=Larvalintake(L,G);
    CumLI(L,G)=Larvalintake(L,G);   %cumulative larval intake

    % Genotypes of the larvalintake
    RR_QQ_Larvalintake(L,G)=Larvalintake(L,G)*p_RR(L)*p_QQ(L);
    SR_QQ_Larvalintake(L,G)=Larvalintake(L,G)*p_SR(L)*p_QQ(L);
    SS_QQ_Larvalintake(L,G)=Larvalintake(L,G)*p_SS(L)*p_QQ(L);
    RR_PQ_Larvalintake(L,G)=Larvalintake(L,G)*p_RR(L)*p_PQ(L);
    SR_PQ_Larvalintake(L,G)=Larvalintake(L,G)*p_SR(L)*p_PQ(L);
    SS_PQ_Larvalintake(L,G)=Larvalintake(L,G)*p_SS(L)*p_PQ(L);
    RR_PP_Larvalintake(L,G)=Larvalintake(L,G)*p_RR(L)*p_PP(L);
    SR_PP_Larvalintake(L,G)=Larvalintake(L,G)*p_SR(L)*p_PP(L);
    SS_PP_Larvalintake(L,G)=Larvalintake(L,G)*p_SS(L)*p_PP(L);

    RR_QQ_CumLI(L,G)=RR_QQ_Larvalintake(L,G);  % calculate cumulative LI of each gtype
    SR_QQ_CumLI(L,G)=SR_QQ_Larvalintake(L,G);
    SS_QQ_CumLI(L,G)=SS_QQ_Larvalintake(L,G);
    RR_PQ_CumLI(L,G)=RR_PQ_Larvalintake(L,G);
    SR_PQ_CumLI(L,G)=SR_PQ_Larvalintake(L,G);
    SS_PQ_CumLI(L,G)=SS_PQ_Larvalintake(L,G);
    RR_PP_CumLI(L,G)=RR_PP_Larvalintake(L,G);
    SR_PP_CumLI(L,G)=SR_PP_Larvalintake(L,G);
    SS_PP_CumLI(L,G)=SS_PP_Larvalintake(L,G);

else       % on subsequent days calculate cumulative larval intake (for each genotype)
    RR_QQ_CumLI(L,G)=(RR_QQ_CumLI(L-1,G)+RR_QQ_Larvalintake(L-1,G)).*(1-
EfficacyRR_QQ_Ad(L,G));
    RR_PQ_CumLI(L,G)=(RR_PQ_CumLI(L-1,G)+RR_PQ_Larvalintake(L-1,G)).*(1-
EfficacyRR_PQ_Ad(L,G));
```

```
   RR_PP_CumLI(L,G)=(RR_PP_CumLI(L-1,G)+RR_PP_Larvalintake(L-1,G)).*(1-
EfficacyRR_PP_Ad(L,G));
   SR_QQ_CumLI(L,G)=(SR_QQ_CumLI(L-1,G)+SR_QQ_Larvalintake(L-1,G)).*(1-
EfficacySR_QQ_Ad(L,G));
   SR_PQ_CumLI(L,G)=(SR_PQ_CumLI(L-1,G)+SR_PQ_Larvalintake(L-1,G)).*(1-
EfficacySR_PQ_Ad(L,G));
   SR_PP_CumLI(L,G)=(SR_PP_CumLI(L-1,G)+SR_PP_Larvalintake(L-1,G)).*(1-
EfficacySR_PP_Ad(L,G));
   SS_QQ_CumLI(L,G)=(SS_QQ_CumLI(L-1,G)+SS_QQ_Larvalintake(L-1,G)).*(1-
EfficacySS_QQ_Ad(L,G));
   SS_PQ_CumLI(L,G)=(SS_PQ_CumLI(L-1,G)+SS_PQ_Larvalintake(L-1,G)).*(1-
EfficacySS_PQ_Ad(L,G));
   SS_PP_CumLI(L,G)=(SS_PP_CumLI(L-1,G)+SS_PP_Larvalintake(L-1,G)).*(1-
EfficacySS_PP_Ad(L,G));

   % Calculate the total cumulative larval intake
   CumLI(L,G)=RR_QQ_CumLI(L,G)+ RR_PQ_CumLI(L,G)+RR_PP_CumLI(L,G)+
SR_QQ_CumLI(L,G)+ SR_PQ_CumLI(L,G)+ SR_PP_CumLI(L,G)+SS_QQ_CumLI(L,G)+ …
SS_PQ_CumLI(L,G)+SS_PP_CumLI(L,G);
   % Calculate the larvaldays as a measure of exposure
   Larvaldays(L,G)=Larvaldays(L-1,G)+ CumLI(L,G);
```

8.6.7 *Larval and worm burdens*

Code is provided for one genotype (SSPP) only as an example, the same principles apply for the remaining genotypes.

```
%% Larval/Adult stages and immune/anthelmintic effects
if L>1
  %% Step 13a: Larval stages and effects of immunity/anthelmintic
  % Calculate the number of larvae in each cohort for each genotype and
  % the effects of anthelmintic on each
   LarvaeSSPP01d(L,G)=SS_PP_Larvalintake(L-1,G)*Establishment(L-1,G)*(1-
EfficacySS_PP_L3(L,G));
   LarvaeSSPP02d(L,G)=LarvaeSSPP01d(L-1,G)*(1-EfficacySS_PP_L3(L,G));
   LarvaeSSPP03d(L,G)=LarvaeSSPP02d(L-1,G)*(1-EfficacySS_PP_L3(L,G));
   LarvaeSSPP04d(L,G)=LarvaeSSPP03d(L-1,G)*(1-EfficacySS_PP_EL4(L,G));
   LarvaeSSPP05d(L,G)=LarvaeSSPP04d(L-1,G)*(1-EfficacySS_PP_EL4(L,G));
   LarvaeSSPP06d(L,G)=LarvaeSSPP05d(L-1,G)*(1-EfficacySS_PP_EL4(L,G));
   LarvaeSSPP07d(L,G)=LarvaeSSPP06d(L-1,G)*(1-EfficacySS_PP_EL4(L,G));
   LarvaeSSPP08d(L,G)=LarvaeSSPP07d(L-1,G)*(1-EfficacySS_PP_L4(L,G));
   LarvaeSSPP09d(L,G)=LarvaeSSPP08d(L-1,G)*(1-EfficacySS_PP_L4(L,G));
   LarvaeSSPP10d(L,G)=LarvaeSSPP09d(L-1,G)*(1-EfficacySS_PP_L4(L,G));
   LarvaeSSPP11d(L,G)=LarvaeSSPP10d(L-1,G)*(1-EfficacySS_PP_L4(L,G));
   LarvaeSSPP12d(L,G)=LarvaeSSPP11d(L-1,G)*(1-EfficacySS_PP_L4(L,G));
   LarvaeSSPP13d(L,G)=LarvaeSSPP12d(L-1,G)*(1-EfficacySS_PP_L4(L,G));
   LarvaeSSPP14d(L,G)=LarvaeSSPP13d(L-1,G)*(1-EfficacySS_PP_L4(L,G));
   LarvaeSSPP15d(L,G)=LarvaeSSPP14d(L-1,G)*(1-EfficacySS_PP_L4(L,G));
```

```
  if L>=16
   LarvaeSSPP16d(L,G)=LarvaeSSPP15d(L-1,G)*(1-EfficacySS_PP_Ad(L,G));
   LarvaeSSPP17d(L,G)=LarvaeSSPP16d(L-1,G)*(1-JS4)*(1-EfficacySS_PP_Ad(L,G));
   LarvaeSSPP18d(L,G)=LarvaeSSPP17d(L-1,G)*(1-JS3)*(1-EfficacySS_PP_Ad(L,G));
   LarvaeSSPP19d(L,G)=LarvaeSSPP18d(L-1,G)*(1-JS2)*(1-EfficacySS_PP_Ad(L,G));
   LarvaeSSPP20d(L,G)=LarvaeSSPP19d(L-1,G)*(1-JS1)*(1-EfficacySS_PP_Ad(L,G));
   LarvaeSSPP21d(L,G)=LarvaeSSPP20d(L-1,G)*(1-JS0)*(1-EfficacySS_PP_Ad(L,G));
   LarvaeSSPP22d(L,G)=LarvaeSSPP21d(L-1,G)*(1-JS1)*(1-EfficacySS_PP_Ad(L,G));
   LarvaeSSPP23d(L,G)=LarvaeSSPP22d(L-1,G)*(1-JS2)*(1-EfficacySS_PP_Ad(L,G));
   LarvaeSSPP24d(L,G)=LarvaeSSPP23d(L-1,G)*(1-JS3)*(1-EfficacySS_PP_Ad(L,G));
   % All larvae should have turned into adult worms by this point.
   SS_PP_Mature17d(L,G)=LarvaeSSPP16d(L-1,G)*JS4*(1-EfficacySS_PP_Ad(L,G));
   SS_PP_Mature18d(L,G)=LarvaeSSPP17d(L-1,G)*JS3*(1-EfficacySS_PP_Ad(L,G));
   SS_PP_Mature19d(L,G)=LarvaeSSPP18d(L-1,G)*JS2*(1-EfficacySS_PP_Ad(L,G));
   SS_PP_Mature20d(L,G)=LarvaeSSPP19d(L-1,G)*JS1*(1-EfficacySS_PP_Ad(L,G));
   SS_PP_Mature21d(L,G)=LarvaeSSPP20d(L-1,G)*JS0*(1-EfficacySS_PP_Ad(L,G));
   SS_PP_Mature22d(L,G)=LarvaeSSPP21d(L-1,G)*JS1*(1-EfficacySS_PP_Ad(L,G));
   SS_PP_Mature23d(L,G)=LarvaeSSPP22d(L-1,G)*JS2*(1-EfficacySS_PP_Ad(L,G));
   SS_PP_Mature24d(L,G)=LarvaeSSPP23d(L-1,G)*JS3*(1-EfficacySS_PP_Ad(L,G));
   SS_PP_Mature25d(L,G)=LarvaeSSPP24d(L-1,G)*JS4*(1-EfficacySS_PP_Ad(L,G));

%total mature worms for each genotype summed across cohorts
SS_PP_MaturedL(L,G)=SS_PP_Mature17d(L,G)+SS_PP_Mature18d(L,G)+
        SS_PP_Mature19d(L,G)+SS_PP_Mature20d(L,G)+SS_PP_Mature21d(L,G)+
        SS_PP_Mature22d(L,G)+SS_PP_Mature23d(L,G)+SS_PP_Mature24d(L,G)+
        SS_PP_Mature25d(L,G);
% Calculate WBs of each genotype
SS_PP_Wormburden(L,G)=(((1-Mortality(L-16,G)).*SS_PP_Wormburden(L,G)).*(1-
EfficacySS_PP_Ad(L,G)))+SS_PP_MaturedL(L,G);
end

   %sum the total larvae in each cohort over all genotypes (larvae01d up to larvae24d)
   Larvae01d(L,G)=LarvaeSSPP01d(L,G)+ LarvaeSRPP01d(L,G)+ LarvaeRRPP01d(L,G)+
LarvaeSSPQ01d(L,G)+LarvaeSRPQ01d(L,G)+ LarvaeRRPQ01d(L,G)+ LarvaeSSQQ01d(L,G)+
LarvaeSRQQ01d(L,G)+ LarvaeRRQQ01d(L,G);
…
   Larvae24d(L,G)=LarvaeSSPP24d(L,G)+ LarvaeSRPP24d(L,G)+LarvaeRRPP24d(L,G)+
LarvaeSSPQ24d(L,G)+LarvaeSRPQ24d(L,G)+LarvaeRRPQ24d(L,G)+LarvaeSSQQ24d(L,G)+
LarvaeSRQQ24d(L,G)+ LarvaeRRQQ24d(L,G);

%calculate total WB
Wormburden(L,G)=RR_QQ_Wormburden(L,G)+RR_PQ_Wormburden(L,G)+RR_PP_Wormbur
den(L,G)+SR_QQ_Wormburden(L,G)+SR_PQ_Wormburden(L,G)+SR_PP_Wormburden(L,G)+S
S_QQ_Wormburden(L,G)+SS_PQ_Wormburden(L,G)+SS_PP_Wormburden(L,G);


% The total number of larvae, hence larval burden
```

```
Alllarvae(L,G)=Larvae01d(L,G)+Larvae02d(L,G)+Larvae03d(L,G)+Larvae04d(L,G)+Larvae05d(L,
G)+Larvae06d(L,G)+Larvae07d(L,G)+Larvae08d(L,G)+Larvae09d(L,G)+Larvae10d(L,G)+...
Larvae11d(L,G)+Larvae12d(L,G)+Larvae13d(L,G)+Larvae14d(L,G)+ Larvae15d(L,G)+...
Larvae16d(L,G)+Larvae17d(L,G)+Larvae18d(L,G)+Larvae19d(L,G)+Larvae20d(L,G)+...
Larvae21d(L,G)+Larvae22d(L,G)+Larvae23d(L,G)+Larvae24d(L,G);
 end
```

8.6.8 *Frequency of R within host*

```
  FreqR(L,G)=(2*RR_Wormburden(L,G)+SR_Wormburden(L,G))/(2*Wormburden(L,G));
  FreqQ(L,G)=(2*QQ_Wormburden(L,G)+PQ_Wormburden(L,G))/(2*Wormburden(L,G));

    RR_QQ_Wormmass(L,G)=Wormmass(L,G)*(FreqR(L,G)^2)*(FreqQ(L,G)^2);
    SR_QQ_Wormmass(L,G)=Wormmass(L,G)*(2*FreqR(L,G)*(1-FreqR(L,G)))*(FreqQ(L,G)^2);
    SS_QQ_Wormmass(L,G)=Wormmass(L,G)*((1-FreqR(L,G))^2)*(FreqQ(L,G)^2);
    RR_PQ_Wormmass(L,G)=Wormmass(L,G)*(FreqR(L,G)^2)*(2*FreqQ(L,G)*(1-FreqQ(L,G)));
    SR_PQ_Wormmass(L,G)=Wormmass(L,G)*(2*FreqR(L,G)*(1-FreqR(L,G)))*(2*FreqQ(L,G)*
(1-FreqQ(L,G)));
    SS_PQ_Wormmass(L,G)=Wormmass(L,G)*((1-FreqR(L,G))^2)*(2*FreqQ(L,G)*(1-
FreqQ(L,G)));
    RR_PP_Wormmass(L,G)=Wormmass(L,G)*(FreqR(L,G)^2)*((1-FreqQ(L,G))^2);
    SR_PP_Wormmass(L,G)=Wormmass(L,G)*(2*FreqR(L,G)*(1-FreqR(L,G)))*((1-
FreqQ(L,G))^2);
    SS_PP_Wormmass(L,G)=Wormmass(L,G)*((1-FreqR(L,G))^2)*((1-FreqQ(L,G))^2);

%Calculate FEO and FEC
Faecaloutput(L,G)=((1-DM_digestibility(L))*FI_actual(L,G)*1000); %
SS_Eggs_femaleworms(L,G)=SS_Wormmass(L,G)*0.55; %0.55 ratio female eggs
SR_Eggs_femaleworms(L,G)=SR_Wormmass(L,G)*0.55;
RR_Eggs_femaleworms(L,G)=RR_Wormmass(L,G)*0.55;
PP_Eggs_femaleworms(L,G)=PP_Wormmass(L,G)*0.55;
PQ_Eggs_femaleworms(L,G)=PQ_Wormmass(L,G)*0.55;
QQ_Eggs_femaleworms(L,G)=QQ_Wormmass(L,G)*0.55;
Eggs_femaleworms(L,G)=Wormmass(L,G)*0.55;

FEO (L,G)= Eggs_femaleworms(L,G)/(1/.25*Faecaloutput(L,G)); % Assume dry matter content
25% for grass (or 30% for dry hay)
dilution = 25;
lambda=FEO(L,G)/dilution;
Sampled_FEC(L,G) = dilution*poissrnd(lambda);
```