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**PARASITIC GASTROENTERITIS IN CALVES DURING
THEIR FIRST SEASON AT GRASS ON ORGANIC AND
CONVENTIONAL FARMS IN SCOTLAND - THE
POTENTIAL FOR A PERFORMANCE-BASED TARGETED
SELECTIVE ANTHELMINTIC TREATMENT PROGRAMME**

A. JACKSON

PARASITIC GASTROENTERITIS IN CALVES DURING THEIR FIRST SEASON AT GRASS ON ORGANIC AND CONVENTIONAL FARMS IN SCOTLAND - THE POTENTIAL FOR A PERFORMANCE-BASED TARGETED SELECTIVE ANTHELMINTIC TREATMENT PROGRAMME

By

Abigail Jackson (B.V.M.S. BSc.(Hons). M.R.C.V.S)

A thesis submitted for the degree of doctor of philosophy in the
School of Veterinary Medicine, University of Glasgow



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DECLARATION

I declare that the work described in this thesis is my original work, any collaboration and assistance having been duly acknowledged.

Some of the work described in this thesis has been the subject of the following presentations:

Investigating the feasibility of targeted selected anthelmintic treatment in first season grazing calves on Scottish organic and conventional dairy farms. (2011). Jackson, A., Forbes, A., Stear, M., Jonsson, N., Ellis, K. 23rd. International Conference of the World Association for the Advancement of Veterinary Parasitology, Buenos Aires, Argentina. pp 18.

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LIST OF ABBREVIATIONS

AAD	Amino Acetonitrile Derivative
Ab	Antibody
ANOVA	Analysis of Variance
BCS	Body Condition Score
BVDV	Bovine Viral Diarrhoea Virus
BZ	Benzimidazole
cm	Centimetre
COWS	Control of Worms Sustainably
EGR	Estimated Liveweight gain /Growth Rate
ELISA	Enzyme-Linked ImmunoSorbent Assay
EL4	Early larval stage four within host
EnL4	Encysted larval stage four within host
EPG/epg	Eggs per Gram
EWt	Estimated Liveweight
FEC/fec	Faecal Egg Count (s)
FSG	First Season Grazing/Grazer (s)
GI	Gastrointestinal
GIN	Gastrointestinal Nematodes
GR	Liveweight gain /Growth Rate
iu	International Units
kg	Kilogram
L/l	Litres
L4	Fourth Stage Trichostrongyle Larvae
LEV	Levamisole
ml	Millilitre
µl	Microlitre
ML	Macrocyclic Lactone
MWt	Measured Liveweight (weigh scales)
OD	Optical Density
ODR	Optical Density Ratio
PGE	Parasitic Gastroenteritis
RCF	Relative Centrifugal Force
ROC	Reporter Operator Curve
SAC	Scottish Agricultural College
SCC	Somatic Cell Count
SCOPS	Sustainable Control of Parasites in Sheep
SD	Standard Deviation
SOPA	Scottish Organic Producers Association
SSG	Second Season Grazing/Grazer(s)
TST	Targeted Selective anthelmintic Treatment
UK	United Kingdom
USA	United States of America
Wt	Liveweight
δWt	Difference in liveweight
xg	Times gravity

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ABSTRACT

The work described in this thesis was designed to investigate the current impact of parasitic gastroenteritis on organic and conventional dairy farms in first season grazing youngstock in Scotland, and to elucidate a marker of significant parasite challenge within individual calves, in order to target these calves with an anthelmintic treatment. It was felt particularly that any recommendations should be practical and easily implemented on-farm, and optimise anthelmintic usage, with regard to animal health, welfare and performance on both organic and conventional farms.

There is world-wide recognition that nematode parasite infections are one of the greatest causes of lost productivity of grazing livestock. In the UK, the single most important cause of parasitic gastroenteritis in cattle is infection with the abomasal nematode, *Ostertagia ostertagi*, although concomitant infection with the less pathogenic intestinal nematode, *Cooperia oncophora* is common. Often, non-organic (conventional) producers use anthelmintic treatment programmes that prevent disease or treat all animals in a group without necessarily considering the basic epidemiological information needed for an optimal strategic control. Organic producers are encouraged to avoid this approach, thus it may be hypothesised that organic livestock harbour higher parasite burdens compared to livestock in conventional systems. However, little information is available on current UK organic dairy anthelmintic use and subsequent parasite challenge to youngstock. This thesis aimed to investigate current management practices on three Scottish organic farms compared to three Scottish conventional farms and examine different ways of assessing parasite challenge (including novel markers) with a view to the implementation of a targeted selective treatment (TST) programme. Liveweight gain assessment by means of weigh-bands as a tool to investigate the effect of parasitism on the host was also examined. In year one of the study, the six farms were visited on four occasions throughout the grazing season where fifteen first season grazers on each farm had their liveweight measured (weigh-band or weigh-scale), a faecal egg count (FEC) recorded and plasma pepsinogen, plasma fructosamine and *Ostertagia ostertagi* antibody concentrations measured.

Knowledge of the epidemiology and pathophysiology of gastrointestinal nematode infestation has led to the identification of parasitic biomarkers for use either as a diagnostic tool or for providing a threshold for anthelmintic treatment. Faecal egg counts (FEC) are the most widely used parameter, both clinically and in studies on gastrointestinal nematode infections of ruminants, because of their relative convenience and low cost. Organic producers are encouraged to use faecal egg counts in order to direct anthelmintic treatment to calves, or groups of calves, that have counts of 200 eggs per gram or more (Soil Association, 2010). The recent launch of COWS (Control of Worms Sustainably in Cattle) in May 2010 - an initiative to prevent widespread anthelmintic resistance and to use anthelmintics appropriately in cattle in the UK - has also seen conventional farmers encouraged to use faecal egg counts in the same manner as their organic counterparts (Taylor, 2010i). None of the biomarkers, including FEC, investigated in the study reflected liveweight gain adequately to use in a targeted selective anthelmintic treatment programme. An ideal biomarker would give indication of calves that would most benefit from anthelmintic treatment before liveweight gain was affected. The biomarkers in this study indicated presence of gastrointestinal parasitism but could not target the animals that had poor liveweight gains. The emphasis on FEC in advice to farmers regarding the need for anthelmintic treatment requires re-evaluation.

The data from year one showed that the conventionally farmed first season grazers (FSG) had significantly higher liveweight gains than the organically farmed calves. Anthelmintic treatment was applied to the organic calves in the study when the calves were known to be harbouring gastrointestinal parasite infection from positive faecal egg counts. The organically farmed first season grazers in this study had high gastrointestinal parasite challenge, indicated by parasite-based markers such as FEC and plasma pepsinogen concentration. The conventional producers in this study exposed FSG to 652% more days of anthelmintic than the organic producers and gained superior liveweight gains over the grazing season. Essentially, the organic producers fulfilled the ethos of organic production, reducing anthelmintic usage and showing necessity of anthelmintic treatment. However, subclinical and clinical parasitic gastroenteritis reduces animal welfare, the essence of the organic ethos. The organic industry needs to investigate whether there is a superior alternative to

FEC that still promotes the organic ethos and reduces subclinical and clinical parasitic gastroenteritis.

The possibility of using liveweight gain as a marker for anthelmintic treatment was investigated. An accurate assessment of liveweight is necessary if calf liveweight gain is to be calculated accurately and used as a threshold for anthelmintic treatment. Cattle weigh-scales are expensive and often not available on farm, particularly where youngstock may be grazing at pasture and gathered in the field for handling. With this in mind, cattle weigh-bands, which measure heart girth and relate this to liveweight, have been devised and used in practice in order to estimate cattle liveweight. Realistically, if a liveweight gain threshold were to be recommended for use on farms in the UK, the weigh-band must estimate liveweight and hence liveweight gain accurately. Given that many farmers do not possess weigh-scales on farm, use of heart-girth measurements to estimate liveweight gain is the best option available to farmers currently.

Year two involved the implementation of a targeted selective anthelmintic treatment (TST) programme on two organic farms and one conventional farm; all were previously involved in the year one study. Anthelmintic treatment was applied only to FSG calves growing at $<0.75\text{kg/day}$ at two points in the grazing season. Organic Farm 1 (O1) and Organic Farm 2 (O2) increased the liveweight gain of the FSG in year two by 50% and 44% respectively. Farm O2 exposed the FSG to 1160% more days of anthelmintic than in 2009; however, approximately 10% of the group were left untreated. Conventional FSG showed reduced liveweight gain from the previous year by 19%. However, respiratory disease was present on-farm also and may have confounded findings. Applying a performance-based targeted anthelmintic regime treatment in the field is possible and using it on farms where anthelmintic treatment was already minimal, such as organic farms, increased liveweight gain in first season grazers without significantly increasing anthelmintic treatment. Applying a TST regime to a conventional farm where previously a suppressive anthelmintic treatment had been applied may have reduced liveweight gain in the first season grazers (FSG) but maintained it at an acceptable level. The acceptance by farmers of TST strategies, and their implementation, may require a high level of input and education to the farming community.

CHAPTER ONE

CHAPTER ONE: A REVIEW OF THE PUBLISHED LITERATURE

1.1 INTRODUCTION

Parasitic gastroenteritis (PGE) is ubiquitous in grazing livestock of all ages. It can manifest itself in many ways causing clinical disease with ill health, production losses and, in exceptional cases, death. More commonly, subclinical disease is encountered, primarily causing production and financial losses and to the detriment of animal welfare. There is world-wide recognition that nematode parasite infections are one of the greatest causes of lost productivity of grazing livestock (Perry and Randolph, 1999). This has been recognised for many years but still persists today despite significant advances in our knowledge of these parasites. Anthelmintics were developed and used to control gastrointestinal nematodes (GIN) over one hundred years ago (Gordon, 1935). They have succeeded in reducing GIN challenge but none can reduce challenge to a level where re-infestation becomes negligible (Sutherland and Scott, 2010). In order for an anthelmintic strategy to be successful, in-depth knowledge of pathophysiology and epidemiology of the parasite, in the context of immunity and management of the host, is required. Due to the many factors affecting gastrointestinal parasite challenge, a successful anthelmintic strategy has to target the GIN species causing harm to the host, have minimal environmental impact, maximise refugia and be dynamic to account for climate variation and farm management practices.

1.1.1 History of Nematodes

It is hypothesised that nematodes evolved one hundred million years ago (Hedges, 2002; Meldal *et al.*, 2007; Vanfleteren *et al.*, 1994) and most likely originated in the sediments at the bottom of all major bodies of water (Bryant, 1994). Nematodes eventually spread to the land where they have become one of the most abundant organisms in the soil (Sutherland and Scott, 2010). At this stage, it is

thought that nematodes made the leap from a free-living existence to a parasitic one (Dorris *et al.*, 1999). On land, nematodes have to cope with extremes of temperature, desiccation, shortage of nutrients and predation from nematophagous organisms, whereas within a host, temperatures are more stable, nutrients are presented readily, either in the form of digesta or host tissues and, presumably, there is protection from nematophagous organisms. The move from a free-living to parasitic existence now appears an easier concept to grasp. The spread of grasses on land fifteen million years ago, the gradual replacement of browsing herbivores by grazing animals (Janis *et al.*, 2000) and the more recent domestication of livestock was a major contributor to the success of parasitic nematodes. Man then completed the ubiquitous dominance of gastrointestinal parasites with the movement of livestock to different continents and the world-wide parasite versus livestock production contest was born. Since then numerous battles have been fought against GIN. Man has been brandishing the following with varying success: plant materials since the 2nd century AD (Waller *et al.*, 2001), nematophagous fungi (Grønvold, 1989; Larsen and Nansen, 1990) and more recently and effectively, anthelmintics in the 20th century. However, the parasites brandished their ability to mutate, to dodge the bullet so to speak, to persist in the environment and within the host and to evade or moderate the host's defences. The battle still rages.

1.2 GASTROINTESTINAL NEMATODES OF THE BOVINE

The vast majority of gastrointestinal parasites of cattle belong to the superfamily Trichostrongyloidea, order Strongylida, phyla Nematoda and comprise some twenty species (Table 1.1). Generally, species are site specific and are located either in the abomasum, the small intestine or large intestine. It is widely accepted that most species are host specific and parasitise cattle alone, although cross infection between sheep and other ruminants can occur, for example with *Nematodirus battus* and *Haemonchus* spp. The most notable exception to this generalisation is *Trichostrongylus axei*, which can parasitise a variety of ungulate hosts.

Site	Nematode genus/species
Abomasum	<i>Ostertagia ostertagi</i> <i>Haemonchus</i> spp. <i>Trichostrongylus axei</i>
Small Intestine	<i>Cooperia oncophora</i> <i>Cooperia</i> spp. <i>Trichostrongylus</i> spp. <i>Nematodirus</i> spp.
Large Intestine	<i>Oesophagostomum radiatum</i> <i>Trichuris</i> spp.

Table 1.1. Gastrointestinal parasites of cattle. Parasites in bold are the most common and important in the UK.

In the UK, the single most important cause of parasitic gastroenteritis in cattle is infection with the abomasal nematode, *Ostertagia ostertagi*, although concomitant infection with the less pathogenic intestinal nematode, *Cooperia oncophora* is common (Parkins *et al.*, 1990). Gastrointestinal nematodes remain ever-present within the cattle population in the UK. At significant cost to the farmer, these parasites are controlled by regular anthelmintic treatment and/or pasture rotation. It is estimated that in 2007, over £44 million was spent in Great Britain on endoparasiticides and over £15 million on endectocides (NOAH, 2008). The following review of the literature and thesis will focus on parasitic gastroenteritis resulting from infection with *Ostertagia ostertagi* and *Cooperia oncophora*.

1.2.1 *Ostertagia ostertagi*

Ostertagia ostertagi is considered to be the most important parasite of cattle in temperate climates. The parasite was first described in 1890 by Ostertag and named *Strongylus convolutes* and was later renamed by Stiles in 1892 as *Strongylus ostertagi* the present name was assigned by Ransom in 1907. Published reports on the significance of the parasite date back to the beginning of the 20th century (Armour, 1967). The parasite is well adapted to cooler conditions and survives well over winter on pasture, in soil or in an encysted larval state (EnL₄) within the host. The adults are slender, brownish-red worms reaching approximately 1cm in length and can be observed by eye on the mucosal surface of the abomasum at post mortem. *Ostertagia* spp. females are considered to have a low fecundity, laying approximately fifty eggs

per day (Sutherland and Scott, 2010). Infections can be acquired from turnout by the ingestion of overwintered larvae, but generally *O. ostertagi* infections do not predominate until the latter part of the grazing season (Nansen *et al.*, 1990). The clinical signs of Type I ostertagiosis, which is seen in groups of young grazing cattle, usually three to four weeks after exposure to large numbers of infective larvae, include inappetence, profuse watery diarrhoea, dehydration and marked weight loss. Infection in the latter part of the grazing season can lead to the establishment of an arrested larval burden (EnL₄) which, if high enough, may be followed by a Type II ostertagiosis, which occurs as the inhibited larvae emerge the following spring (Armour, 1970; White and Fisher, 1994). Synchronous emergence of larvae can cause severe clinical disease and death in youngstock. The most economically important effect of GIN is the loss of appetite and therefore reduced growth rate in the affected host (Stromberg and Gasbarre, 2006).

1.2.2 *Cooperia oncophora*

Cooperia oncophora is considered to be mildly pathogenic and is particularly common in young cattle in their first grazing season. The parasite was described in the literature by Railliet in 1898 and Ransom in 1907. The adult worms live in the small intestine causing mainly diarrhoea and weight loss. It has been shown that *C. oncophora* can lead to a decrease of fourteen kilograms in liveweight gain in beef cattle in their first twelve months (Sutherland and Leathwick, 2011). *C. oncophora* is the main contributor to faecal egg counts at least until the middle of the grazing season (Hertzberg *et al.*, 1992). Cattle mount a rapid immune response to this parasite and, consequently, both intestinal burdens and faecal egg counts decline towards the end of the grazing season (Armour, 1989).

1.3 EPIDEMIOLOGY

Understanding the epidemiology of cattle nematodes is the foundation on which strategic parasite control programmes are designed. Without this information, one is not able to use anthelmintics to provide the optimal benefits for controlling both the adult worm and the pasture larval populations. It is important to know if larvae are available when animals are turned out onto pasture, when larval populations reach their maximal numbers and when they are induced to become hypobiotic (Stromberg and Averbeck, 1999).

1.3.1 Free-living Phase

Few nematode parasites complete their life cycles entirely within the host. A defined stage of the life cycle usually exits the definitive host and develops further in the environment, sometimes in one or more additional host species, to another defined life cycle stage capable of reinfecting the definitive host. Eggs are excreted in the host's faeces, hatch and undergo further development within the faecal pat to become larvae capable of infecting the definitive host. The dynamics of the free-living phases of *O. ostertagi* and *C. oncophora* are very similar and are considered together (Rose, 1961; Rose, 1962; Rose, 1963). Hatching and larval development are primarily temperature-dependent processes, although moisture is also required as these stages are susceptible to desiccation. The development of egg to larval stage 1 (L₁) can take as little as twenty-four hours and then hatching takes place (Perry, 2002). L₁ and L₂ are both microbivorous within the faecal pat, whereas L₃ retain the cuticle of L₂ and cannot feed. There is no further development from L₃ until a host is encountered. The time taken for development from egg to L₃ under laboratory conditions for *O. ostertagi* and *C. oncophora* at 22-23°C is 3-7 and 3-9 days respectively, 14-16°C is 7-16 and 4-21 days, and 10-11°C is 18-28 and 21-56 days (Rose, 1961; Rose, 1962; Rose, 1963). The presence of water is vital to the free-living nematode for movement through the faecal pat and, once the third larval stage has been achieved, a film of moisture on the surface of the adjacent vegetation is necessary for migration onto pasture (Sutherland and Scott, 2010). The L₃ are motile but have limited capacity for active migration as their energy is finite. Most larvae

move less than 5cm away from the faecal pat (Rose, 1961) and their ability to move vertically up the herbage is also limited, with the majority of larvae being found in the lower 5cm of the sward (Silangwa and Todd, 1964; Williams and Bilkovich, 1973). Rainfall is known to be very important in disseminating infective larvae away from faecal pats onto pasture. There is initial wetting of the crust followed by infective larvae close to the surface of the pats being splashed out in droplets through the kinetic energy of the falling rain. Passive movement of larvae by this means can account for up to ninety per cent of the translocation of larvae from the pat onto the pasture and larvae can be found up to 90 cm from the pat (Rose, 1962; Grønvold, 1984ii; Grønvold, 1987). The trajectory of the droplets carrying infective larvae is normally at a height of 30 cm above ground. When the droplets land on the herbage the larvae are deposited at the top of the swards making it more likely that they will be ingested by a grazing animal (Grønvold and Hogh-Schmidt, 1989). Larvae can also disseminate on pasture through transport hosts such as the earthworm (Grønvold, 1979), insects (Todd *et al.*, 1971), birds (Grønvold, 1984i) and cattle (Hertzberg *et al.*, 1992). Temperatures below 10°C slow the development of the larvae. It is possible that larvae utilise molecules, such as glycerol, as cryoprotectants to survive freezing (Wharton, 2002) and survive for some years on herbage (Rose, 1961; Rose, 1963) and in soil (Al Saqur *et al.*, 1982). Grazing cattle are infected when they ingest infective larvae from the pasture. In the early part of the grazing season these larvae will be the remainder of the previous season's population that have over-wintered on the pasture. Later in the season, the larvae on the grass originate from eggs that have been passed from the hosts themselves.

1.3.2 Parasitic Phase

The parasite phase commences with the exsheathment of the infective L₃ within the proximal gastrointestinal tract (usually the rumen) of the host. Exsheathment is stimulated by factors such as the equilibrium between bicarbonate and carbonic acid, carbon dioxide concentration, digestive secretions and temperature. As diet can affect the rumen environment, exsheathment can be affected by diet. DeRosa *et al.* (2005) showed that over ninety-seven per cent of *O. ostertagi* larvae exsheathed one-hundred and twenty minutes after exposure in the rumen contents of grass-fed cattle and three-hundred and sixty minutes in the rumen

contents of grain-fed cattle. Once exsheathed, the L₃ migrate to their predilection site, which is the gastric glands of the abomasum for *O. ostertagi* (Ritchie *et al.*, 1966) and the crypts of the intestinal mucosa for *C. oncophora* (Armour *et al.*, 1987). Further development through larval stages L₄ and L₅ occur within fourteen days post-ingestion and typically at day eighteen, adult worms emerge onto the mucosal surface. Male and female worms mate and the females commence egg production. The minimum pre-patent period (ingestion of L₃ to egg production) is typically fifteen to eighteen days for *C. oncophora* and eighteen to twenty-one days for *O. ostertagi*. In some circumstances, parasitic development may become arrested at the EnL₄ stage. This phenomenon is referred to as hypobiosis or inhibition and occurs predominantly in *O. ostertagi*. Resumption of development usually occurs after several months of inhibition, typically during spring the following year (Michel *et al.*, 1974) causing Type II ostertagiosis (Armour, 1970). Arrested development is probably a parasite adaption to enhance over-winter survival and to ensure that pastures are seeded with worm eggs the following spring (Eysker, 1979).

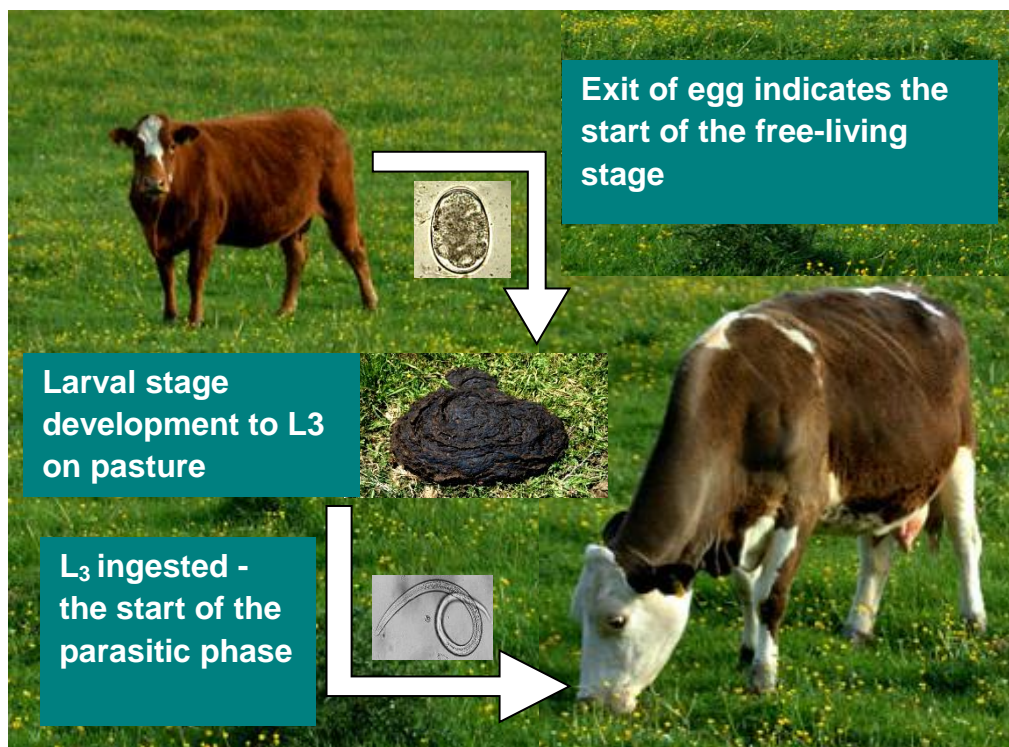


Figure 1.1 Free-living and parasitic stages of *O. ostertagi* and *C. oncophora*.

1.3.3 Host Immunity

Although parasitising a host lends protection from certain environmental elements and nematophagous organisms, there is still the host's defence system to contend with. The immune system response against gastrointestinal nematodes varies with worm species and the exposure of the host to the parasite. This in turn is affected by climate, management, anthelmintic administration and, finally, by a variety of host factors that include genetic make-up, age, sex, as well as hormonal and nutritional status (Vercruysse and Claerebout, 1997). A protective acquired immunity develops against *Cooperia* spp. within one grazing season. Where *Ostertagia ostertagi* is concerned there is no specific age-related immunity and a protective immunity develops more slowly, usually over at least two grazing seasons (Armour, 1980; Armour, 1989). This prolonged susceptibility to infection is a major reason why *O. ostertagi* remains the most economically important gastrointestinal parasite in the UK.

The manifestations of immunity development against the gastrointestinal nematodes of cattle are expressed in a sequence of events: firstly, decreased parasite fecundity; secondly, stunting of worm growth, then retardation and arrested development; this is followed by adult worm expulsion and finally resistance to establishment of new infections (Armour, 1989; Vercruysse and Claerebout, 1997). The reduction in the number of parasite eggs released in the faeces results from several different types of immune responses, ranging from those that alter the parasite physiology or morphology, such as loss of the vulval flap in *O. ostertagi* females, to those that protect the host from reinfection presumably through killing the developing worms or preventing the establishment of the invasive third-stage larvae (Stromberg and Gasbarre, 2006). The reasons for the delayed onset of protective immunity directed against *O. ostertagi* are unclear. In sheep, the abomasal parasite, *Haemonchus contortus* also provokes a weak protective immune response. Interestingly, in both cattle and sheep, the parasites that are most pathogenic and most difficult to mount a protective immune response against, reside in the abomasum. Therefore, the possibility arises that the abomasum is a poor site for the presentation of parasite antigens (Gasbarre, 1997). However, *O. ostertagi* can elicit profound changes in the host immune system, including a rapid and enormous

expansion of lymphocytes and lymphoid cells, increased expression of interleukin and extensive changes in the abomasal tissue and its draining lymph nodes (Gasbarre, 1994; Stromberg and Gasbarre, 2006) and so this theory does not fully explain the weak immune response. It was also postulated that the immune response is inappropriate in eliminating gastrointestinal parasites. Soluble mediators released by immunocompetent lymphocytes are important in determining the overall type of response after exposure to infectious organisms. These lymphokines, such as interleukin and interferon, control the growth and differentiation of cells of the immune system and thus control the make-up of the cell populations responding to the infectious agent. In cattle, *O. ostertagi* infection elicits a very strong response of interleukin which, surprisingly, appears to be appropriate against gastrointestinal nematodes. It may well be, therefore, that protective immunity requires other immune mechanisms or that the parasite has found a way of evading or suppressing the immune response (Gasbarre, 1997). Many potential mechanisms have been proposed to account for the unique weak protective immunity generated, as mentioned above, but immunosuppression has been the most thoroughly investigated. *O. ostertagi* infection can exhibit undefined and antigen-specific suppressive effects on the host immune response and, indeed, suppressive molecules have been shown to exist in extracts of third-stage and fourth-stage larvae (Cross and Klesius, 1989; Gomez-Munoz *et al.*, 2004). Although it remains to be conclusively demonstrated that immunosuppression is a consistent and important feature of *O. ostertagi* infections, it is clear that at least at certain periods of the parasite life cycle, or in very heavy infections, a transient reduction in the immune reactivity of the host takes place (Gasbarre, 1997).

The predominant immunoglobulin involved in the humoral immune response to gastrointestinal nematodes is IgG (Canals and Gasbarre, 1990; Gasbarre *et al.*, 1993; Sanchez *et al.*, 2004). The role of IgA in cattle is still unclear. Total IgG in serum has been related to acquired immunity to *O. ostertagi* in cattle (Kloosterman *et al.*, 1984): animals with a greater IgG titre have fewer and shorter worms, with fewer ova per female, and more females with reduced vulval flaps (Kloosterman *et al.*, 1984). As exposure to *O. ostertagi* over a prolonged period is required to elicit an immune response, it has been suggested that using a suppressive worming regime that is sufficiently effective against parasites may not allow immunity to develop and could

be detrimental to second or third season grazers. It has been demonstrated that chemoprophylaxis can interfere with the development of immunity, and that impairment is greater with treatment systems that are intensive and long lasting (Claerebout *et al.*, 1998i; Claerebout *et al.*, 1998ii). However, productivity in the second grazing season of cattle treated with long lasting anthelmintics in their first grazing system may be unaffected, indicating development of a protective immune response (Vercruyse and Dorny, 1999). A recent study of second season grazing (SSG) cattle showed no correlation between SSG performance and treatment history as FSG cattle (Larsson *et al.*, 2010). Still, cumulative egg counts were significantly higher in SSG that had been treated with anthelmintic as FSG, although they were generally low overall. One recent study examining anthelmintic treatment, its subsequent effect on the immune system and the response to viral challenge showed that anthelmintic treatment had no effect on antibody response to vaccination against, or direct viral challenge of, infectious bovine rhinotracheitis (Shutz *et al.*, 2012).

Production of IgG is under genetic control and the ability to produce *O. ostertagi* antibodies as measured by optical density ratio (ODR) is thought to have a heritability of 0.13 (Hayhurst *et al.*, 2010). Thus, it would be expected that this trait may be of potential interest in genetic selection programs as an aid to reducing the effect of *O. ostertagi* in dairy herds. However, it has been shown that cattle selected for high IgG levels showed inferior performance parameters (Colditz, 2002). Greer *et al.* (2005) then showed that the adverse effects of gastrointestinal parasitism, such as feed intake and liveweight gain in sheep can be abolished by immunosuppressive treatment. This observation could be the manifestation of nutrients being partitioned away from liveweight gain towards immune function in these animals. As the immune response is, to some degree, genetically controlled it can be seen that not all animals within a group behave the same when gastrointestinal parasites are encountered. Within a closed population it has been shown (using faecal egg counts) that approximately twenty-five per cent of calves appear to grow adequately despite a gastrointestinal parasite challenge, fifty per cent generate an acquired immunity during the first grazing season and twenty-five per cent have an inadequate response and fail to show a reduction in faecal egg count consequent to exposure (Leighton *et al.*, 1989). Therefore, at the end of the grazing season, a quarter of the group may still carry heavy worm burdens. Evidence of this phenomenon is shown in the skewed

distribution of worm burden within a group; a high level of worm burden is seen in a small proportion (~20%) of the group (Barger, 1985).

1.4 PATHOLOGY AND PATHOPHYSIOLOGY

Knowledge of the pathological and pathophysiological changes that occur within the host caused by gastrointestinal parasites is pertinent to understanding the production losses encountered. Parasitic gastroenteritis (PGE) in youngstock affects both feed intake and feed utilisation, thus the major impact is on liveweight gain (Goldberg, 1965; Fox *et al.*, 1989; Shaw *et al.*, 1998i). In adult cattle, PGE may cause reduced fertility (Holste *et al.*, 1986; Bohlender, 1988), decreased milk yield (Michel *et al.*, 1982) and inferior feed conversion ratios (Flack *et al.*, 1967). Pathophysiological changes can also provide diagnostic markers for PGE (Eysker and Ploeger, 2000). *Ostertagia ostertagi* is considerably more pathogenic than *Cooperia oncophora* and thus the following will discuss mainly the effects of *O. ostertagi* infection on the host. It must be noted however, that a mixed infection with both parasites is the most likely field occurrence, particularly in youngstock. The dual infections appear to cause greater effects than comparable mono-specific infections and may reflect a reduction in the ability of the host to compensate for dysfunction at various locations in the gastrointestinal tract (Parkins *et al.*, 1990; Forbes, 2008). The pathophysiology of gastrointestinal nematodes has been well documented in the literature (Fox, 1993; Fox, 1997). Mucous cell hyperplasia occurs initially, affecting the pits and glands adjacent to those occupied by the larvae. The hyperplasia subsequently becomes more generalised, leading ostertagiosis to be characterised as a hyperplastic gastritis (Murray *et al.*, 1970). At the same time, some parietal cells are lost and the activity of any remaining cells is severely reduced, leading to a rise in the pH of the abomasal contents. The serum concentrations of pepsinogen and gastrin increase whilst serum albumin and fructosamine (studies in sheep and horses) decrease (McKellar, 1993; Heath and Connan, 1991). In heavy infections, the cellular changes can be observed as gross pathology, with affected glands appearing swollen and pale and the mucosal surface of the abomasum taking on the appearance of “Morocco leather” (Armour, 1970). The major effect on the host is anorexia (Fox, 1993; Forbes, 2008). *C. oncophora* causes a mucoid enteritis and loss of villous structure in the small intestine (Armour *et al.*, 1987). The summation of the

pathophysiological effects of gastrointestinal parasite challenge is a relative protein deficiency in the host (Steel, 1974). This fact is supported by the observation that disease can often be alleviated by feeding supplementary protein (Coop and Kyriazakis, 1999).

1.5 PARASITIC BIOMARKERS

Knowledge of the epidemiology and pathophysiology of the gastrointestinal nematode has led to the identification of parasitic biomarkers for use either as a diagnostic tool or for providing a threshold for anthelmintic treatment. Development of resistance to anthelmintic drugs has also motivated the search for diagnostic methods to identify animals for targeted selective treatments.

1.5.1 Faecal Egg Counts

Michel developed two important hypotheses: 1) pasture contamination by infected calves is not determined by worm burden and 2) that worm burdens of calves cannot be determined using egg output (Michel, 1968). Despite this information being available for years, it is still a commonly held belief within the veterinary community, and of farmers, that faecal egg counts (FEC) relate to worm burdens. FEC are the most widely used parameter, both clinically and in studies on gastrointestinal nematode infections of ruminants, because of their relative convenience and low cost. There are inherent methodology weaknesses with FEC, which mean that they are really only semi-quantitative at best, and should be viewed at the group-level rather than in individual animals. It is also well understood that the counting sensitivity (also known as minimum egg detection threshold) of the method affects the usefulness of the data obtained because this affects the proportion of the homogenised faecal sample observed and therefore the mean number of eggs counted. However, over-dispersion in FEC is almost always reported because of the tendency for nematode eggs to aggregate, resulting in true variability in faecal egg concentrations within faeces. This leads to additional variation in the mean count observation for each sample, which is almost ubiquitously assumed to correspond to a single continuous positive distribution, typically either a gamma or lognormal distribution (Denwood *et al.*, 2012). Eysker and Ploeger (2000) suggested that FEC is

of particular benefit approximately five to ten weeks after turnout in young cattle, when FEC relates best to infective larvae ingestion. Initial infections are low at the beginning of the first grazing season, producing a gradual increase in FEC that is directly related to the level of infective larvae on pasture. This observation may be used for herd health monitoring to assess in the middle of the grazing season whether low or high initial infections have occurred and, subsequently, to decide on further worm control measures for youngstock. This is only possible if a producer has not used anthelmintics in the early grazing season and the animals are set stocked.

FEC in clinical Type I ostertagiosis may exceed one thousand eggs per gram (epg). However, correlation between FEC and worm burden is usually low (Eysker and Ploeger, 2000). If the group mean FEC value in grazing youngstock five to ten weeks after turnout exceeds 200 epg, this is suggested as being indicative of the requirement for treatment (Vercruyse and Claerebout, 2001). Therefore 200 epg is commonly used as a threshold for anthelmintic treatment (Soil Association, 2010). Monitoring FEC at housing of youngstock has been advocated as it is logistically easier if calves are being handled. The results can be used to decide whether anthelmintic treatment is required at housing and to predict whether problems with nematodes would be expected in the second grazing season. However, it should be borne in mind the limitations of FEC and that results at this time may not reflect the true parasite burden status of the calves. After housing, no correlation between FEC and exposure exists anymore and only serum pepsinogen and *O. ostertagi* antibody concentration remain as candidates for monitoring the impact of gastrointestinal parasites on the host (Eysker and Ploeger, 2000).

The findings of a recent study in Argentina may warrant reconsideration of the value of FEC in adult cattle. Mejía *et al.* (2011) compared three methods commonly used to diagnose nematode infections in relation to milk production in a fully grazing dairy herd of one-hundred and fifty cows. Cattle had faeces, blood and milk samples taken during the first postpartum month for FEC, pepsinogen and anti-*Ostertagia* antibody determination, respectively. With the results obtained, the cows were separated into two groups allocated as high or low parasite infection groups, according to each method used and the authors set a threshold for each method. Milk production was then compared between the groups. When cows were separated by

the FEC method (EPG = 0 ($N = 106$) vs. EPG > 0 ($N = 44$)) a difference of nearly eight-hundred litres of milk per cow per lactation was found. On the other hand, milk production between groups separated by pepsinogen concentration or by anti-*Ostertagia* antibody results did not differ. They concluded that FEC during the first postpartum month may be a useful tool for the diagnosis of production impairment induced by high nematode challenge in adult grazing dairy cows. The authors suggested that anthelmintic treatment of only the FEC-positive recently calved cows may improve milk production, while reducing selective pressure on the nematode population for the development of resistance. However, studies that have treated adult cattle with high FEC found no difference in response (Michel, 1968; Gross *et al.*, 1999).

1.5.2 Pepsinogen

The importance of serum pepsinogen concentration linked to ostertagiosis has been known for years (Anderson *et al.*, 1965). Although considerable variation in serum enzyme activity is observed in naturally infected animals (Entrocasso *et al.*, 1986), the value of this parameter in diagnosing gastrointestinal parasitic disease is widely accepted (Ploeger *et al.*, 1990i; Charlier *et al.*, 2011; Berghen *et al.*, 1993; Shaw *et al.*, 1998ii). There is good correlation between individual pepsinogen concentration in peripheral blood at housing and *O. ostertagi* worm burdens (Dorny *et al.*, 1999). Charlier *et al.* (2011) observed that more variation in individual pepsinogen concentration was explained by differences between calf groups than by differences between animals within a calf group. This underlines the value of a group-level diagnosis and suggests it is not worthwhile to go to the effort of making individual diagnoses and individual anthelmintic treatments based on pepsinogen concentrations alone.

An increase in serum pepsinogen concentration reflects mucosal damage as a consequence of *O. ostertagi* infection. There is hypoplasia and metaplasia of the parietal cells resulting in a decrease in acid production and a subsequent reduction of the pepsinogen transformation into pepsin. The accumulated pepsinogen may escape into the blood between the broken cell junctional complexes. Some controversy still exists over the concentration of pepsinogen, which may be considered indicative of

ostertagiosis (Berghen *et al.*, 1993). Pepsinogen assay methods are mainly based on the immediate conversion of the proenzyme into the proteolytic enzyme in acid conditions. Several modifications of the basic methods are used, which make the interpretation of results from different laboratories confusing and not strictly comparable (Michel *et al.*, 1978; Charlier *et al.*, 2011).

1.5.3 *Ostertagia ostertagi* Antibodies

In contrast to pepsinogen determination in cattle, a commercial assay for quantifying antibodies to gastrointestinal nematodes is currently available (Svanovir[®] *O. ostertagi*-Ab, Uppsala, Sweden). Antibody concentration against gastrointestinal nematodes correlate reasonably well with pepsinogen concentration and have been proposed as an alternative for monitoring purposes (Berghen *et al.*, 1993; Eysker and Ploeger, 2000). An enzyme-linked immunoabsorbent assay (ELISA) using a crude adult *Ostertagia ostertagi* antigen has been available during the last 20 years (Keus *et al.*, 1981), although it is only recently that advances in herd-health monitoring have involved the ELISA-based milk *O. ostertagi* (MOO) test that detects the concentration of antibody against *O. ostertagi* in a milk sample (Svanovir[®] *O. ostertagi*-Ab, Uppsala, Sweden). Moreover, the Svanovir[®] assay is considered to be highly reproducible (Charlier *et al.*, 2009ii). Therefore, using antibody measurement instead of pepsinogen for monitoring gastrointestinal nematode infections is appealing. However, the Svanovir[®] assay has been primarily designed for use in adult cows using milk samples (Charlier *et al.*, 2009iii) and an extensive evaluation for use in calves has not yet been performed. Furthermore, the pepsinogen concentration provides a more direct assessment of the clinical effects of gastrointestinal worm infection (abomasal damage) than specific antibodies. Evidently, a positive antibody response does not indicate either clinical disease or functional immunity; it simply indicates prior or current exposure to *O. ostertagi* antigen. Adult animals may, therefore, demonstrate *O. ostertagi* antibodies with no clinical signs of parasitic gastroenteritis, with insignificant FEC and pepsinogen concentrations, if they are challenged with *O. ostertagi* larvae.

It is generally recognised that wide within-host variation exists in the ability to develop an antibody response against gastrointestinal nematode antigens, implying

that an antibody ELISA may not be very appropriate for diagnosis in individual animals (Eysker and Ploeger, 2000). Nevertheless, several studies have shown quantitative relationships between individual and herd-level *O. ostertagi* milk ELISAs and production traits such as milk production and fertility, and to responses to anthelmintic treatment (Sanchez *et al.*, 2002; Sanchez *et al.*, 2004; Sanchez *et al.*, 2005). On a practical scale, bulk milk ELISAs can be very useful in herd monitoring. The major disadvantage of using milk as a substrate is that it can only be used to monitor adult, lactating cattle. In youngstock, serum or plasma *O. ostertagi* antibody concentrations can be measured (Keus *et al.*, 1981) and have been shown to increase within three weeks (Forbes *et al.*, 2009) to two months (Gasbarre *et al.*, 1993) after exposure to infective larvae.

1.5.4 Fructosamine

Fructosamine is a stable ketoamine compound, formed when a glucose molecule reacts non-enzymatically with a protein molecule which then undergoes spontaneous transformation into a stable ketoamine. The fructosamine concentration in peripheral blood is a measure of the glycation of all serum proteins. Consequently, the principal factors which influence fructosamine formation are the half-life of serum albumin and the average glucose concentration to which the albumin is exposed while in circulation. Therefore, it has been proposed that fructosamine measurement will reflect alterations in the rate of protein turnover. Measurement of fructosamine in equine plasma has been reported to be a potentially useful adjunct to assessment of cyathostome larval challenge (Murphy *et al.*, 1997). Murphy *et al.* (1997) showed that infected ponies failed to gain weight at the same rate as control animals and a decrease in plasma fructosamine concentration was detected in infected groups, becoming apparent in all animals four to six weeks post-infection. Hypoalbuminaemia is a frequent, but not consistent, feature of naturally-occurring cyathostome infections and can also be seen in cattle with sub-clinical parasitic gastroenteritis (Ellis *et al.*, 2011). Fructosamine estimation may, therefore, be a more effective and sensitive means, compared with albumin measurement, for monitoring relatively subtle effects of parasite infections that might occur after low level infection in ruminants. Stear *et al.* (2001) investigated fructosamine concentration and resistance to natural infection of *Teladorsagia circumcincta* infection in sheep.

Animals with low fructosamine concentrations were associated with lower liveweights, and lambs with lower fructosamine concentrations subsequently acquired more nematodes of all species. The authors concluded that fructosamine concentrations were associated with current levels of nematode infection and also appeared to predict future levels of infection. If this is the case in cattle, fructosamine concentration as a biomarker for use in a selective targeted anthelmintic programme requires further study.

1.5.5 Pasture Larval Count

Pasture larval counts are extremely laborious and are therefore rarely used as routine methods of assessment of risk of nematode infections. However, they are particularly useful in longitudinal studies on the population dynamics of nematodes (Eysker and Ploeger, 2000).

1.5.6 Gastrin

Gastrin is a hormone produced by the G cells present mainly in the glands of the antral pyloric region of the abomasum. The release of gastrin is mediated by vagal stimulation, mechanically by stomach distension and chemically by peptides, amino acids or calcium. Through a negative feedback mechanism its release can be triggered by high pH levels as happens in ostertagiosis (Purewal *et al.*, 1997). The use of gastrin as a diagnostic marker for parasitic gastroenteritis has been examined in both sheep and cattle (Bell, 1979; Anderson *et al.*, 1981; Entrocasso *et al.*, 1986). Measurement of gastrin concentration is expensive and yields no more useful further information than the cheaper pepsinogen concentration assay.

1.6 MANAGEMENT SYSTEMS

1.6.1 Organic vs. Non-organic

The word 'organic' was coined by JI Rodale in the USA in 1942 when he started publishing the magazine *Organic Gardening*. At about the same time in the UK, Lady Balfour wrote *The Living Soil*, the book that stimulated the founding of the Soil

Association in 1946. The Soil Association was the first to define organic standards in its magazine *Mother Earth* in the 1960s. The EU published its first organic regulation (EEC regulation no. 2092/91) in 1991, which was most recently updated in 2009 and provides a standard that involves the right to label food as organic. It covers all aspects of food animal production including disease prevention and veterinary treatment. Producers registered with organic farming bodies have to abide by specific standards in order to sell their produce with the 'organic' label. In relation to gastrointestinal parasitism organic farmers are advised to:

- Implement good livestock management practices;
- Optimise stocking rates;
- Use rotational, clean and mixed grazing systems;
- Have breeds with higher resistance to infection;
- Breed for resistance.

There is acceptance that, despite these efforts, anthelmintics are often necessary in controlling parasitic gastroenteritis. In this circumstance, producers may treat individual animals with anthelmintics after they have checked that they are infected (for example, through faecal egg counts) and may only use treatments previously agreed in a health plan. With the permission of the organic body, producers may use anthelmintics on a whole herd, flock or group of animals but only as part of a disease control programme, and this must have been agreed in the health plan. Whenever producers treat their animals with anthelmintics, they must:

- Inform their organic body as to how they intend to improve control in the future without using these treatments;
 - Monitor how effective their control programme is, for example, by faecal egg counts;
 - Where possible, target anthelmintic treatments at the breeding females rather than their offspring (sheep); and,
 - Preferentially use benzimidazoles or levamisoles rather than other drenches.

Often, non-organic (conventional) producers use anthelmintic treatment programmes that prevent disease or treat all animals in a group without necessarily considering the basic epidemiological information needed for an optimal strategic control (Vercruyse and Claerebout, 2001). Organic producers tend to avoid this approach, thus it may be hypothesised that organic livestock harbour higher parasite burdens compared with livestock in conventional systems. The literature gives conflicting evidence of this hypothesis. Diarrhoea and reduced weight gains during the grazing season were reported more frequently from organic herds compared with conventional herds in Sweden (Svensson *et al.*, 2000). Depending on pasture management, an average thirty kilogram reduction in liveweight was found between organically managed and bolus-treated young cattle in Sweden (Dimander *et al.*, 2000). However, a further study to substantiate these findings indicated that acceptable parasite control was, in fact, achieved on organic dairy farms in Sweden (Höglund *et al.*, 2001), since Swedish farmers use 'parasite-safe' pastures and supplementary feeding (Svensson *et al.*, 2000). This is in agreement with the results obtained in Denmark, although Danish farmers also promote alternate grazing with other species (Thamsborg *et al.*, 1999). Sato *et al.* (2005) suggested that a higher parasitic burden could be explained because organic farms have increased access to pasture during a grazing season, as organic cattle tend to graze for longer periods. More recently, Maggs *et al.* (2008) in a small study on Scottish farms, reported that the organic and non-organic dairy farms they compared had similar levels of parasitism, based on the assessment of faecal egg counts from youngstock. However, the faecal egg counts in both systems were generally very low in this study, which could imply that the control strategies used on both types of farm were effective. The chosen year of study had dry meteorological conditions that may have resulted in lower parasite challenge. Additionally, it is recognised that single time-point assessment of parasite status by faecal egg counting is not accurate. A longitudinal study of one organic farm on the west coast of Scotland indicated high gastrointestinal parasite challenge causing significant subclinical disease in the youngstock (Ellis *et al.*, 2011). If organic producers are, in fact, adequately controlling gastrointestinal parasites without the use of long-acting anthelmintics, how are they succeeding?

An investigation by Svensson *et al.* (2000) showed that organic producers in Sweden practised a range of grazing management procedures, which they claimed were designed to limit the effects of internal parasites in their young replacement stock. One frequently reported procedure was the turnout in spring of young animals onto pasture that had not been grazed during the previous late summer and autumn by similar classes of animals. However, Dimander *et al.* (2000) showed that when they moved cattle onto grass that had only been grazed early in the previous year by young cattle that there was still sufficient larvae on the pasture to cause significant disease in the grazing cattle that were moved to that field. The dose and move strategy in which cattle are treated with an anthelmintic and then moved to a safe pasture was the first strategy employed to utilise parasite epidemiology knowledge and pasture management (Stromberg and Gasbarre, 2006); but it is now widely accepted that this is associated with an increased risk of encouraging anthelmintic resistance (Michel, 1985; Waghorn *et al.*, 2006).

Rotational grazing, where animals are moved at defined intervals to fresh grazing leaving pasture ungrazed for periods of time, is also commonly used by organic producers. In the case of intensive rotational grazing, where pasture growth and productivity are maximised, animals may return to pasture when larvae, resulting from the eggs shed in the previous grazing, are infective. This practice also forces cattle to eat all of the forage available including the grass closest to the faecal pat where most of the infective larvae are available (Stromberg and Averbeck, 1999). The stocking rate is usually increased with rotational grazing systems and, therefore, it would be assumed that parasite challenge would increase in animals managed in this way. However, there are conflicting views on whether parasite challenge is increased (Ciordia *et al.*, 1964; Bransby, 1993, Hammond *et al.*, 1997) but most importantly there is general agreement that there does not appear to be any decrease in the parasite population with any of the rotational grazing systems (Stromberg and Averbeck, 1999). Alternate, or integrated grazing has been used to control gastrointestinal parasites. It is common practice to graze calves followed by older cattle, taking advantage of increased resistance in these older immunocompetent animals. The most common mixed or alternate species grazing is the combination of sheep and cattle. This relies on the differing susceptibility of the different host species as *O. ostertagi* show little cross-infectivity between cattle and sheep

(Stromberg and Averbek, 1999) and is a practice commonly used in Scotland (Gettinby *et al.*, 1987).

Organic producers are encouraged to use faecal egg counts in order to direct anthelmintic treatment to calves, or groups of calves, that have counts of 200 eggs per gram or more (Soil Association, 2010). The recent launch of COWS (Control of Worms Sustainably in Cattle) in May 2010 - an initiative to prevent widespread anthelmintic resistance and to use anthelmintics appropriately in cattle in the UK - has also seen conventional farmers encouraged to use faecal egg counts in the same manner as their organic counterparts (Taylor, 2010i).

Many recommendations on how to control levels of parasitism through management practices have been made. The main weakness of many grazing management strategies is the fact that producers consider them as too complex, time and effort consuming and they are often not adapted to local grass availability and climatic conditions and usually do not fit with farm management (Vercruyse and Dorny, 1999). Acceptance of a certain degree of production loss without compromising welfare may be an option in organic production systems in which a central ethos is not to maximise production (Thamsborg *et al.*, 1999). However, assessing risk factors for disease and the impact on health and production must be given high priority in organic farming, as this is a system that emphasises animal welfare considerations (Thamsborg *et al.*, 1999).

1.6.2 UK Dairy Industry

The European Union (EU) produces over one-hundred and thirty million tonnes of milk a year (Dairy Co., 2012). The United Kingdom is the third largest milk producer in the EU and the ninth largest in the world. Milk production in the UK follows a seasonal trend with traditional peak production in May, after the calving season, and a trough in October/November. The UK produces around thirteen billion litres of milk each year, which is then processed into a wide range of dairy products. Almost fifty per cent of the milk produced on farms in the UK is processed into liquid milk. Other key dairy products are cheese, milk powders and butter (Dairy Co., 2012).

Despite a reduction in milk production on the farm over the last three years, the processing of liquid milk and cheese has grown over the same period. This has had a corresponding negative effect on the production of milk powders and condensed milk, which have both declined in recent years. A report (The White Paper 2012) in June 2012 by Dairy UK, a dairy trade association, revealed that:

- 99% of people regularly consumed milk and dairy products (up from 96% in 2010);
- 96% regularly drank fresh milk (up from 94% in 2010);
- 95% regularly ate cheese (up from 90% in 2010).

Liquid milk, cheese and fresh product markets continue to grow. Kantar Worldpanel estimates the UK dairy market to be worth £10.12 billion annually, accounting for twelve per cent of food and drink sales.

Both organic and the majority of conventional dairy farms aim to utilise production from grazed grass during the spring, summer and autumn months. Conventional farms that choose to confine adult milking cows will still often graze youngstock and dry cows. The primary aim of dairy farms in the UK is to produce large volumes of milk so the dam's calf is removed and reared artificially, usually within twenty-four hours of calving. Typically, at six weeks, or twelve on organic farms, calves are weaned from milk onto conserved forage or grass. For practical reasons, youngstock are generally considered as first season grazers (FSG) or second season grazers (SSG). The age that a calf encounters grazing will be dependent on the time of year it was born. Calves born late in the summer or autumn may not graze until the following year. A youngstock group on dairy farms will consist of heifer calves destined for milk production (replacements). Calves with a beef breed sire and male dairy breed calves may also be included in this group, dependent upon farm management practices.

1.6.3 Youngstock Rearing

To achieve the optimal target of calving at two years of age, dairy heifers need to be served by fifteen months of age at eighty-five per cent of their expected adult liveweight (Losinger and Heinrichs, 1997). Good parasite control is important in order

to achieve liveweight gains of between 0.7-0.8 kg/day at pasture (Van Amburgh *et al.*, 1998). This subject is explored in detail in Chapter Four.

1.7 ANTHELMINTICS

Until recently there had been three classes of broad-spectrum anthelmintics. Class I anthelmintics include both the benzimidazoles (BZ) and the pro-benzimidazoles (PRO-BZ), which bind to β -tubulin and inhibit microtubule formation. Class II includes the imidazothiazoles, known commonly as levamisoles (LEV), which bind to nicotinic acetylcholine receptors and cause spastic paralysis through depolarisation of nematode muscle. Class III includes the avermectins and milbemycins and are known collectively as the macrocyclic lactones (ML). They are known to interact with ligand gated chloride channels causing hyper-polarisation of membranes leading to flaccid paralysis of the worm. The latest class was introduced to the market in 2008: the amino-acetonitrile derivative, monepantel (AAD 1566) acts at specific nematode acetylcholine receptors and causes spastic paralysis through depolarisation of nematode muscle (Kaminsky *et al.*, 2008). This class is commercially available for use in sheep in the UK. Since their introduction in the 1980s (Chabala *et al.*, 1980), the ML class of anthelmintics has become the dominant anthelmintic in both sheep and cattle. Originally found in a soil sample from a Japanese golf course (Campbell *et al.*, 1983), MLs were effective at a low dose rates against a wider range of parasites than existing anthelmintics; they also had activity against certain arthropods (Benz and Ernst, 1979). Depending on the specific product formulation, they can be administered as either injectable, pour-on or intra-ruminal bolus format and have a prolonged activity of variable duration against parasites. Anthelmintics licensed for cattle with their administration strategies and length of persistence against *O. ostertagi* and *C. oncophora* are shown in Table 1.2. Anthelmintics are categorised as POM-VPS, which means that they can be supplied by veterinary surgeons, pharmacists or suitably qualified persons. Unfortunately, producers often obtain anthelmintics with little advice regarding the optimal treatment strategy for their management system (Barton *et al.*, 2006).

Anthelmintic Class	Active Ingredient	Product Name	Pharmaceutical Company	Parasites	Application Method	Duration of Action	Meat Withdrawal	Milk Withdrawal
Benzimidazole	Oxfendazole	Autoworm First Grazer	Pfizer	GIN, lungworm	Pulse release bolus	21 weeks	8 months	n/a
	Oxfendazole	Autoworm Finisher	Pfizer	GIN, lungworm	Pulse release bolus	15 weeks	6 months	n/a
	Fenbendazole	Panacur	MSD	GIN, lungworm	Bolus	n/a	200 days	n/a
	Fenbendazole	Panacur Oral Suspension (2.5% / 10%)	MSD	GIN, lungworm	Oral drench	n/a	12 days	5 days
Levamisole	Levamisole	Levacur SC 3%	MSD	GIN, lungworm	Oral drench	n/a	20 days	n/a
Macrocyclic Lactone	Moxidectin	Cydectin 10% LA	Pfizer	GIN, lungworm, arthropods	Subcutaneous injection	120 days	108 days	80* days
	Doramectin	Dectomax Pour-on	Pfizer	GIN, lungworm, arthropods	Pour-on	35 days ¹ 28 days ²	35 days	60* days
	Eprinomectin	Eprinex Pour-on	Merial	GIN, lungworm, arthropods	Pour-on	28 days ¹ 21 days ²	15 days	Zero
	Ivermectin	Ivomec Classic	Merial	GIN, lungworm, arthropods	Injection	21 days ¹ 14 days ²	15 days	60* days

Table 1.2. Examples of each anthelmintic class and commonly† used anthelmintics in the UK dairy industry. * Not permitted for use in cattle producing milk for human consumption or industrial purposes, or in dry cows and pregnant heifers within stated days before calving. † Levamisoles are not used commonly in the UK. ¹ *Ostertagia ostertagi*, ² *Cooperia oncophora*.

1.7.1 Anthelmintic Strategies

A survey conducted over thirty years ago on anthelmintic usage by cattle and sheep farmers in England and Wales suggested that producers did not use anthelmintics at the correct time of year to obtain maximum returns from their investment (Michel *et al.*, 1981). With more products available to farmers nowadays it is likely that this statement still holds true. Indeed similar surveys in Western Europe indicated that parasitic nematode control was not thoroughly understood by farmers (Ploeger *et al.*, 1990iii; Ploeger *et al.*, 2000; Borgesteede *et al.*, 1998; Schnieder *et al.*, 1999). In recent years, a variety of different anthelmintics have been introduced to the market. As there are many more products with different pharmacological and pharmacodynamic properties to choose from, and with changes in geographic spread of parasites - perhaps reflecting changes in climate - selection of the appropriate control strategy is not easy (Ward, 2006). Control strategies depend on both farming system aims and host-parasite interactions and as such: the identification of crucial points of interaction between the environment provided by the host (including genetics and the immune response), and critical periods in the physical environment in which the eggs and larval stages must develop (Stromberg and Gasbarre, 2006).

The simplest way of dealing with parasitic infections is to use anthelmintics when clinical disease appears. This approach can be accompanied by heavy production losses in the herd and it is highly questionable from a welfare point of view (Vercruysse and Claerebout, 2001). Prophylactic regimes during the early part of the grazing season are designed to prevent recycling of the infection acquired from over-wintered larvae on pasture. First season grazers are targeted primarily, but some producers will also treat second season grazers and adult cattle. It is important to note that all recommended control strategies are based on set-stocked groups of animals, and do not account for pasture rotation or potential mixing of groups (i.e. adding in-calf heifers to a management group). Some of the ML class of anthelmintics are inherently persistent in their activity against gastrointestinal parasites and lungworm, for example moxidectin. The treatment regimens recommended by the pharmaceutical companies for these products take into account each product's persistency and the pre-patent period of the parasite. For example, it is recommended that doramectin (Dectomax™, Elanco) is administered at turnout, then

eight weeks later as its persistency of action is as follows: *O. ostertagi* 35 days; *Cooperia oncophora* 28 days and *Dictyocaulus viviparus* 42 days. The longest acting anthelmintic injection commercially available contains moxidectin (Cydectin LA™, Pfizer) and protects against *O. ostertagi* and *D. viviparus* for 120 days. These products have long meat withdrawal periods, for example for moxidectin it is 65 days. Eprinomectin (Eprinex™, Merial), is available as a pour-on preparation and protects cattle against reinfection with both *O. ostertagi* and *D. viviparus* for 28 days. The advantage of this product is that it has a zero milk withdrawal period, which may be advantageous in some circumstances.

Controlled anthelmintic release devices, first developed in the 1980s, involve placing the device, as a bolus, into the rumen of the grazing animal. Pulse and continuous release anthelmintic boluses are available commercially. The major advantage of the bolus is the reduced need for collecting and handling youngstock at grass.

1.7.2 Anthelmintic Resistance

When anthelmintic products are used repeatedly, a resistant population of worms can be created. Underestimating weight and under-dosing, exposing all worms to a subtherapeutic dose (Stromberg and Gasbarre, 2006), increase selection for resistance. Programmes relying strictly on drug administration without good regard for pasture management will also lead to the selection of drug resistance in the parasite population. This selection has been well documented in small ruminant species where gastrointestinal nematodes are now resistant to all classes of anthelmintics, excluding the newly included AADs. Anthelmintic resistance in cattle in the UK is limited to a few isolated cases and well-documented reports of drug resistance in cattle nematodes are scarce (Stafford and Coles, 1999). However, the possibility of widespread resistance to the major classes of anthelmintic compounds requires consideration of new approaches and less reliance on anthelmintics (Stromberg and Gasbarre, 2006). Targeted selective anthelmintic treatment has been advocated and used successfully in small ruminants to increase the in-refugia population and hence reduce drug resistance in parasite populations (Van Wyk and Bath, 2002; Leathwick *et al.*, 2006i; Leathwick *et al.*, 2006ii; Busin *et al.*, 2013). Both

anthelmintic resistance and targeted selective treatment are addressed and the relevant literature reviewed in Chapter Four.

1.8 SUMMARY AND SUBSEQUENT WORK

There is evidence that anthelmintic use in cattle is suboptimal and, against a background of the potential to develop anthelmintic resistance, new approaches to the use of anthelmintics were investigated. The critical aspects were to ascertain which animals within a group of grazing cattle required treatment, using markers that are accurate and timely in their indication of parasite challenge and effect on the host.

This thesis aims to address these issues by means of two studies. The first is a study of organic and non-organic farms in Scotland to ascertain the current anthelmintic use strategies actually employed on-farm under field conditions. This was designed to give information on animal performance and also allow for the evaluation of a number of biomarkers of parasite challenge to investigate the potential for their use in a targeted selected treatment approach. The second study describes the approaches towards a targeted selective treatment strategy and the outcomes when a performance-based approach was employed on commercial farms.

CHAPTER TWO

CHAPTER TWO: A COMPARISON BETWEEN ORGANIC AND CONVENTIONAL FARMS OF THE EPIDEMIOLOGY AND IMPACT OF PARASITIC GASTROENTERITIS IN DAIRY CALVES AND AN ASSESSMENT OF POTENTIAL BIOMARKERS FOR PRODUCTION LOSSES

2.1 INTRODUCTION

The preceding literature review highlighted the fact that studies on gastrointestinal parasitism in the United Kingdom on both organic and non-organic (conventional) farms are scarce. There are still questions as to whether organic farms control gastrointestinal parasitism adequately in youngstock (Maggs *et al.*, 2008; Ellis *et al.*, 2011).

Given the emphasis of the organic production standards on reducing anthelmintic usage (Soil Association, 2010), it could be hypothesised that UK organic dairy youngstock have higher parasite challenge compared with non-organic youngstock. However, little information is available on current UK organic dairy anthelmintic use and subsequent parasite challenge to youngstock. Accurate assessment of parasite burden status is difficult, due to differences in the host-parasite relationship between different animals and changes in this relationship over time. The current study was designed to examine current management practices on organic farms compared with conventional farms and to investigate different ways of assessing parasite challenge (including novel markers) and its effect on the host. Liveweight gain assessment by means of weigh-bands was used as a tool to investigate the possible effect of parasitism on the host.

2.1.1 Objectives

The objectives of the 2009 study were to observe first season grazing cattle in three organic and three conventionally farmed herds in the UK and to: 1) Describe the

patterns of helminth infestation over time; 2) To describe the production performance of animals over time; 3) To assess biomarkers of gastrointestinal parasite infection (faecal egg count, plasma fructosamine, plasma pepsinogen, plasma *Ostertagia ostertagi* antibody, liveweight gain) with a view to identifying opportunities for the application of targeted selective treatment for gastrointestinal parasitism in cattle.

2.2 MATERIALS AND METHODS

2.2.1 Farm Selection

A project licence was issued by the Home Office for the study, of which Professor Mike Stear was the holder. Personal licences were approved for Professor Mike Stear, Dr. Kathryn Ellis and the author to carry out the necessary procedures on all six designated farms. The University of Glasgow Veterinary School ethics committee approved the study.

Farm	Method of Recruitment into Study
C1	Contact through local veterinarian
C2	Contact through local veterinarian
C3	University of Glasgow Dairy Farm. Deemed a 'designated area' under the Animals (Scientific Procedures) Act 1986
O1	University of Glasgow provides a veterinary service and routine fertility visits
O2	Personal contact following previous related work
O3	Personal contact following previous related work

Table 2.1. Method of recruitment of the six farms to the 2009 study.

Six dairy farms in Scotland were recruited to the study: three organic and three conventional. The farms were identified by number from Conventional (C) 1 to 3 and Organic (O) 1 to 3. The method of recruitment for each farm is shown in Table 2.1. The University of Glasgow Veterinary School provides a first opinion veterinary service to two of the dairy farms (C3 and O1). The remaining conventional dairy farms (C1 and C2) were recruited through contact with a local farm animal veterinary surgeon. The remaining organic farms (O2 and O3) were recruited through personal contact

with The University of Glasgow following previous work with the farmers. An attempt was made to match the organic and conventional farms with regards to herd size, location, breed and milk yield. However, due to the necessity of handling calves at grass regularly throughout the summer months with this study, recruitment choice of farmers willing to enter into the study was limited. Summary production information about each farm is detailed in the following sections and summarised in Table 2.2. Production information was obtained from farmer interview and questionnaire.

Farm	Location	Enterprises	No. milking cows	No. FSG (dairy replacements)
C1	West Central Scotland	Dairy and Beef	187	150 (50)
C2	West Central Scotland	Dairy	180	150 (50)
C3	West Central Scotland	Dairy, Beef and Sheep	78	80 (20)
O1	West Central Scotland	Dairy	66	16 (9)
O2	South West Scotland	Dairy, Beef and Sheep	105	92 (30)
O3	South West Scotland	Dairy and Beef	220	68 (68)

Table 2.2. Overview of the six farms involved in the 2009 study. FSG: First Season Grazers.

2.2.2 Conventional Farm 1

Conventional Dairy Farm 1 is situated in Renfrewshire, central Scotland on four hundred and fifty acres of land. It has a total of two hundred and twenty pedigree Holstein Friesian dairy cows. The farm also has suckler cows and an Aberdeen Angus bull. There are approximately two hundred and fifty calves and heifers on farm. All cattle graze from May to September/October and calve all year round.

2.2.3 Conventional Farm 2

Conventional Dairy Farm 2 is situated in Lanarkshire, central Scotland on four hundred and fifty acres of owned and sixty acres of rented land. The dairy herd consists of two hundred and ten Holstein Friesian dairy cows. The enterprise is solely dairy, although some dairy-cross calves are reared for beef. There are approximately two hundred and ninety calves and heifers on the farm and five Holstein-Friesian bulls. All cattle graze from May to September/October and calve all year round.

2.2.4 Conventional Farm 3

Conventional dairy farm 3 is situated in Dunbartonshire, central Scotland on eight hundred and fifty acres of land. There are eighty five Holstein-Friesian milking cows. Beef and sheep enterprises are also run on the farm. Two bulls are kept on the farm. Calving is year round. Dairy replacements and beef x dairy calves are grazed together.

2.2.5 Organic Farm 1

Organic dairy farm 1 is situated in Dunbartonshire, central Scotland on two hundred and thirty acres of land. The milking herd consists of seventy British Friesians, Ayrshires, Brown Swiss crosses and Jersey crosses. The farm has held organic status since 2001 and is registered with the Scottish Organic Producers Association (SOPA). Calving is year round.

2.2.6 Organic Farm 2

Organic dairy farm 2 is situated in Dumfries and Galloway, south west Scotland and covers eight hundred and fifty acres. The farm has held organic status for twelve years and is registered with The Soil Association. The farm milks one-hundred and five Ayrshire and Ayrshire crosses with thirty dry cows at any given time. There are five Aberdeen Angus suckler cows and five-hundred sheep on the farm. Approximately forty per cent of the herd calve November to December, the rest calve year-round. Heifers calve down between February and April.

2.2.7 Organic Farm 3

Organic dairy farm 3 is situated in Dumfries and Galloway, south west Scotland on four hundred and fifty acres of owned and thirty acres of seasonally rented land. The herd consists of two hundred and twenty milking pedigree Holsteins and one hundred and fifty youngstock. The farm has held organic status since 2003 and is registered with the Scottish Organic Producers Association (SOPA). Heifers calve down from September to January. Adult cattle calve year-round apart from in June and July.

Dairy production and fertility data is given in Table 2.3. An overview of the general health of the herd from a farmer questionnaire (Appendix) and interview is shown in Table 2.4

Farm	Average Yield (Litres/cow/ annum)	Mean SCC (‘000/ml)	Calving Interval (days)	Age at First Calving (months)
C1	8,600	300	400	27
C2	8,400	95	384	30
C3	7,200	232	460	26
O1	6,132	250	405	30
O2	7,500	n/a*	435	30
O3	8,200	165	420	28

Table 2.3. Production and fertility data from the six farms involved in the 2009 study. SCC is a rolling three month geometric mean. Information gained is from farmer interview and are therefore approximations. SCC: Somatic Cell Count. *Farm O2 does not milk record.

Farm	Vaccination used on Farm	Herd Health Monitoring	Do you have any concerns regarding gastrointestinal parasitism on farm?	What are your main health concerns regarding your whole herd?	Do you have any health concerns in the first season grazing youngstock?	Other comments
C1	Blue-tongue, blackleg, BVD, leptospirosis, IBR and calf respiratory disease	Annual bulk milk samples analysed for Johne's disease, which have been negative	The farmer did not feel that gastrointestinal parasites were a problem on farm	Liver fluke	Pneumonia in young calves	Calves were bolused with multi-trace element bolus (Cosecure™ Telsol, Copper: 13.4g Cobalt: 0.5g Selenium: 0.3g as sodium selenite) before turnout
C2	Blue-tongue and blackleg	Quarterly bulk milk samples analysed for leptospirosis, BVD and Johne's disease, which have been negative	In 2008 the farm had a problem with gastrointestinal parasitism in the first season grazing calves and this is of major concern. The same anthelmintic regime and grazing field for FSG was used in 2008 and 2009	Lameness and mastitis	Gastrointestinal parasitism in replacement heifers	The farm sometimes buys in milking cows and bulls
C3	Blue tongue, BVD, leptospirosis and calf respiratory disease	Quarterly bulk milk samples analysed for Johne's disease, IBR and liver fluke, which have been negative	The farmer felt that gastrointestinal parasites were adequately controlled on-farm	Liver fluke is present on the farm and the farmer felt that this was one of the primary health concerns in the milking herd	None	Young calves including the calves in the study were affected by ringworm
O1	Blue-tongue, lungworm and leptospirosis	No regular monitoring	The farmer felt that gastrointestinal parasites and liver fluke were a primary health concern in the youngstock on the farm and liver fluke a concern in the adult milking cows	Liver fluke and mastitis	The farmer felt that gastrointestinal parasites and liver fluke were a primary health concern in the youngstock on the farm	Liver fluke is present on farm Young calves including the calves in the study were affected by ringworm
O2	Blue-tongue	No regular monitoring	Gastrointestinal parasites and liver fluke are a major concern to the farmer	The farmer felt that liver fluke is the main health concern on the farm in adult cattle	The farmer felt that young stock had a high challenge with gastrointestinal parasites	Liver fluke is present on the farm and adult cattle are treated at drying-off. The farm bought-in nine heifers in 2008
O3	Blue-tongue, BVD and lungworm	Quarterly bulk milk are analysed for leptospirosis, IBR and Johne's disease which have been negative	The farmer felt that gastrointestinal parasitism was not a major concern on farm	Heat detection and fertility	None	

Table 2.4. Perceived cattle health by the farmer on each farm in the study, from farmer questionnaire. BVD, Bovine Viral Diarrhoea; IBR, Infectious Bovine Rhinotracheitis.

2.2.8 Experimental Animals

Fifteen calves with no previous experience of grazing (first season grazers - FSG), were randomly selected on each farm by choosing the first fifteen calves from a larger management group that were caught and put through a crush. The same fifteen calves were sampled on each repeat visit. Prior to the start of sampling, the calves identified for involvement in the project were tested for Bovine Virus Diarrhoea Virus (BVDV) antibody. It is known that animals persistently infected with BVDV are more likely to have reduced immune function and are often ill-thriven as a result. To reduce this potential confounding effect the recruited calves were all screened. If the calves were antibody negative, they were tested for BVDV antigen. Calves that then tested positive for BVDV antigen (persistently infected with the virus) were excluded from the study. Organic Farm 1 had two calves that tested positive for BVD antigen and were excluded from the study.

2.2.9 Experimental Design

Each animal was sampled on four occasions during 2009, with exact timing dependent on farmer compliance and convenience to handle the cattle: Visit 1 (pre-turnout); Visit 2 (5-10 weeks post-turnout); Visit 3 (13-16 weeks post-turnout); Visit 4 (housing). Dates of each visit are shown in Table 2.5. At each sampling occasion, the calves' liveweights were estimated by weigh band (Coburn[®] tape for estimating liveweights of beef cattle, The Coburn[®] Company, Inc.). As FSG animals in the UK are often dairy and dairy x beef it was decided that a beef weigh tape would be used. The beef tape provides four different estimates of liveweight using body condition scores (BCS). The dairy tape provides three different estimates of liveweight using the breeds, Holstein, Guernsey and Jersey and was calibrated in the USA. It was felt that BCS rather than breed would give better estimates of liveweight in the majority of cattle in the study. The animals on Farm C3 were also weighed on weigh scales (Ritchie[®] mechanical weigh scales, spring balance model 327G) and liveweights recorded to the nearest ten kilograms. The weigh crush was tared and checked before each use. The shoulder height and BCS on a 1-5 scale were recorded. Each calf had a blood sample taken from jugular or coccygeal venepuncture into an EDTA tube (all visits) and a faecal sample taken *per rectum* obtained at visits 2, 3 and 4. A bulk milk

sample obtained every month during the grazing season on each farm was frozen at -20°C for *O. ostertagi* antibody ELISA analysis.

Farm	Visit 1	Visit 2	Visit 3	Visit 4
C1	29 th April	22 nd July	14 th October	1 st December
C2	30 th April	15 th July	10 th September	19 th November
C3	9 th April	28 th August	25 th September* 28 th October [^]	
O1	7 th April	24 th June	10 th September	8 th December
O2	10 th April	29 th June	30 th September	2 nd December
O3	10 th April	29 th June	30 th September	2 nd December

Table 2.5. Date of sampling visits on each farm in 2009. * 6 calves sampled earlier as beef-cross and were to be sold at market. [^]Housed at visit 3.

2.2.10 First Grazing Season Calf Management

The following section details the management of the FSG in the study on each farm and is summarised in Table 2.6.

2.2.10.1 Conventional Farm 1

The first season grazers (FSG) were turned out in late April in a group of nineteen on seven acres of land. Sheep had been overwintered and FSG had previously grazed the land. An oxfendazole bolus (AutowormTM, Pfizer) was administered to each calf on turnout on 29/04/2009. This method of gastrointestinal parasite control has been used on the farm for over three years. The farm also administers anthelmintic to the second season grazing calves using an oxfendazole bolus (AutowormTM second season grazer, Pfizer). *Fasciola hepatica* is a problem on the farm and calves were treated with nitroxynil (TrodaxTM, Merial) in December 2009. No supplementary feed was given when calves were at pasture.

2.2.10.2 Conventional Farm 2

First season grazers were turned out late April in a group of thirty grazing eighteen acres of land. The land is used every year for FSG. During the previous year, the FSG on this land had signs of clinical disease from gastrointestinal parasitism, although doramectin (Dectomax™, Elanco) had been administered in pour-on form during that year. Doramectin pour-on solution (Dectomax™, Elanco) was administered at turnout on 29/04/2009, on 15/07/2009 and again on 10/09/2009. Trematode eggs were not present on faecal samples throughout the grazing season. No supplementary feed was given when calves were at pasture.

2.2.10.3 Conventional Farm 3

The first season grazers were not turned out until mid-July due to sheep grazing the pasture earlier in the season. They were kept in a group of nineteen on eight acres of land for ease of collecting for this study. The group were treated with moxidectin injection (Cydectin™ 10%, Pfizer) at turnout. There is known to be *Fasciola hepatica* present on farm and a few trematode eggs were present in the faeces of the FSG in the study over the grazing season. No flukicides were administered during the study. Supplemental feeding of calf pellets and round bale silage occurred until the calves were turned out onto pasture.

Farm	Turnout Date	Housing Date	Grazing Management	Prior use of Paddock (last season)	Management of Paddocks	Anthelmintic and / or Flukicide Administration		Other Information
						Date	Product	
C1	Late April 2009	Early Dec 2009	Set-stocked group of 19	First-season grazers. Sheep over-wintered	Topped and fertilizer applied	29/04/2009	Oxfendazole (Autoworm First Season Grazer, Pfizer.)	Suspicion of IBR infection through the group during the grazing season (no tests to confirm this) Pneumonia problem in young calves. 14/10/09: 1 calf with >25 <i>F.hepatica</i> eggs, 4 others with <5 eggs No lungworm larvae seen in faeces
						01/12/2009	Nitroxylin (Trodx, Merial)	
C2	Late April 2009	Mid Nov 2009	Set-stocked in group of 30 calves on 18 acres. Moved early September to 45 acre field with 13 more calves	First season grazers	The second field had 2 cuts of silage and slurry applied	30/04/2009	Doramectin (Dectomax Pour-on, Elanco)	No fluke eggs seen in faeces No lungworm larvae seen in faeces
						15/07/2009	Doramectin (Dectomax Pour-on, Elanco)	
						10/09/2009	Doramectin (Dectomax Pour-on, Elanco)	
C3	Mid July 2009	Late Oct 2009	Set-stocked in group of 19 on 8 acres	Sheep overwintered		Mid July 2009	Moxidectin (Cydectin 10% LA injection, Pfizer)	25/09/09: 1 calf with 1 <i>F.hepatica</i> egg 28/10/09: 4 calves with <5 <i>F.hepatica</i> eggs No lungworm larvae seen in faeces 28/10/09 : Most of group had purulent nasal discharge
O1	Late April 2009	Late Dec 2009	All 14 FSG rotated around 7 fields. Calves moved every 2 weeks. Each field is rested for at least 3 weeks	First season grazers Some fields had adult cattle and second season grazers on the year before	Slurry applied to 1 field	17/09/2009	Fenbendazole (Panacur Oral suspension 10%, MSD) administered to 3 calves with FECs \geq 200 epg. Eprinomectin (Eprinex Pour-on, Merial) all young stock.	01/12/09: 1 calf with 8 and 3 calves with < 5 <i>F.hepatica</i> eggs No lungworm larvae seen in faeces (Oct 2009) PM on 1 calf in study severe <i>O. ostertagi</i> and <i>D. viviparus</i> burden
						30/10/2009	Triclabendazole (Fasinex 10%, Novartis)	
						08/12/2009		
O2	Early May 2009	Late Nov 2009	One large group of 60 FSG grazed on 3 separate fields of 45 acres each in rotation	2 of the 3 fields had first season grazers on them the previous grazing season. Sheep over-wintered		Mid July	Fenbendazole (Panacur Oral suspension 10%, MSD)	FECs performed by own vet mid July- 300epg. recommended anthelmintic treatment No fluke eggs seen in faeces No lungworm larvae seen in faeces
						Housing	Fenbendazole (Panacur Oral suspension 10%, MSD)	
O3	Early May 2009	Late Dec 2009	Set-stocked in group of 20 animals on 8 acres	First season grazers Sheep-over-wintered		30/09/2009	Fenbendazole (Panacur Oral suspension 10%, MSD)	Own vet performed test for <i>F.hepatica</i> eggs which indicated low level of infection No lungworm larvae seen in faeces

Table 2.6. Summary of grazing management and anthelmintic treatment of FSG on each farm in 2009.

2.2.10.4 Organic Farm 1

All FSG were vaccinated for lungworm (Huskvac™, MSD) prior to turnout. The first season grazers were turned out in late April onto a small paddock near the farm and given supplemental calf pellet feed for this time. Two weeks later the calves were rotated and subsequently were moved every two weeks around seven different fields and were extensively grazed. First season grazers, second season grazers and adult dairy cattle grazed the land the previous year. Faecal egg counts (FEC) were taken on 24/06/09 and 10/09/09 and only calves with a FEC of two hundred and above eggs per gram were treated with fenbendazole orally (Panacur™, MSD). The farmer has used this method of anthelmintic treatment over the previous two grazing seasons. Two calves had FEC of 200 epg or over on 24/06/2009 but as the farmer felt they were looking well, they were not treated. Three calves had a FEC of 200 epg or over on the 10th September and only these calves were treated. In October a calf was euthanased due to poor liveweight gain. The calf had not been treated with an anthelmintic as the FEC for the animal had been less than 200 epg. A post-mortem was performed at The University of Glasgow Veterinary School on the calf the major findings being a gastrointestinal worm burden with *Ostertagia ostertagi*, illustrated in Figure 2.1 and a lungworm burden with *Dictyocaulus viviparus*. Due to the post-mortem findings, the rest of the first season grazers were treated with eprinomectin pour-on (Eprinex™, Merial) on 30/10/2009 to prevent problems with Type II ostertagiosis the subsequent spring and lungworm infection as there were questions over adequate transport and storage of the Huskvac™ (MSD) vaccine. *Fasciola hepatica* was present on the farm. Trematode eggs were found in faecal samples taken during the grazing season from the first season grazers. The FSG were treated with triclabendazole (Fasinex™ 10%, Novartis) on 08/12/2009. Supplementary calf pellets were offered to the group for two weeks following turnout.



Figure 2.1. Abomasum of calf euthanased for poor growth on Farm O1 in October 2009.

2.2.10.5 Organic Farm 2

The first season grazers were turned out in early May in a large group of sixty calves. Three fields of between forty to fifty acres were grazed in rotation. Visit 2 to the farm was on the 24/06/09 when calves were growing well. However, in mid-July it was felt by the farmer that the calves were not growing as well as expected and faecal samples were given to the local veterinary practice for faecal egg count analysis. The pooled FEC was 300 epg and all FSG were treated with fenbendazole orally (Panacur™, MSD) mid-July. The treatment was repeated again at housing in late November. No supplementary feed was given when calves were at pasture.

2.2.10.6 Organic Farm 3

The first season grazers were turned out in early May in a group of twenty, set-stocked, on eight acres. The field was overwintered with sheep and grazed by FSG the previous year. Pooled faecal egg counts were monitored regularly through the summer with the farm's local veterinary practice. In September the mean pooled faecal egg count was 250 epg. All FSG were treated with fenbendazole orally (Panacur™, MSD) on

30/09/2009. The local veterinary practice monitored trematode eggs and reported a low level of infection in the FSG calves. No supplementary feed was given when calves were at pasture.

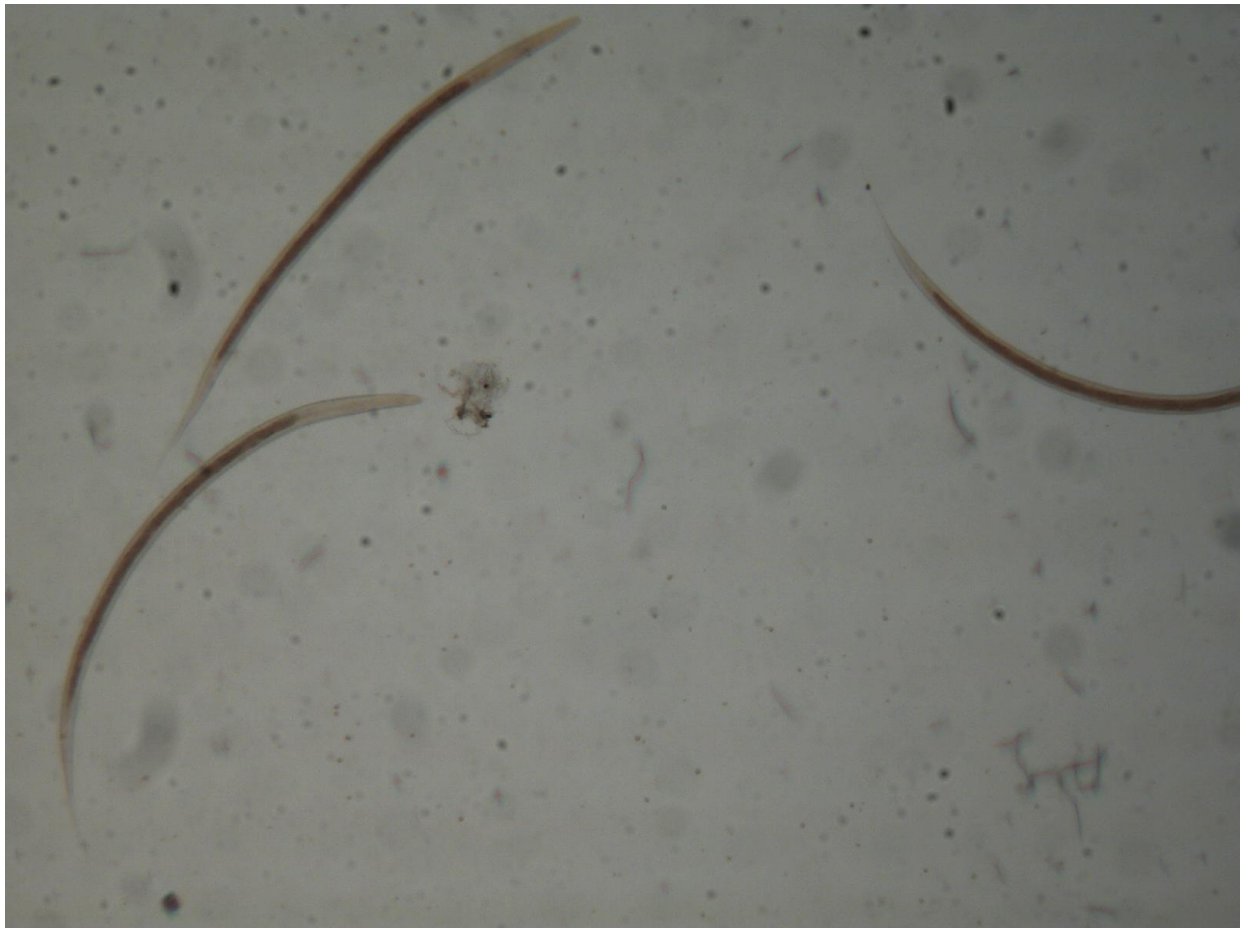


Figure 2.2. *Cooperia oncophora* larvae, cultured from Farm C2, shown under a microscope (10x10 magnification).

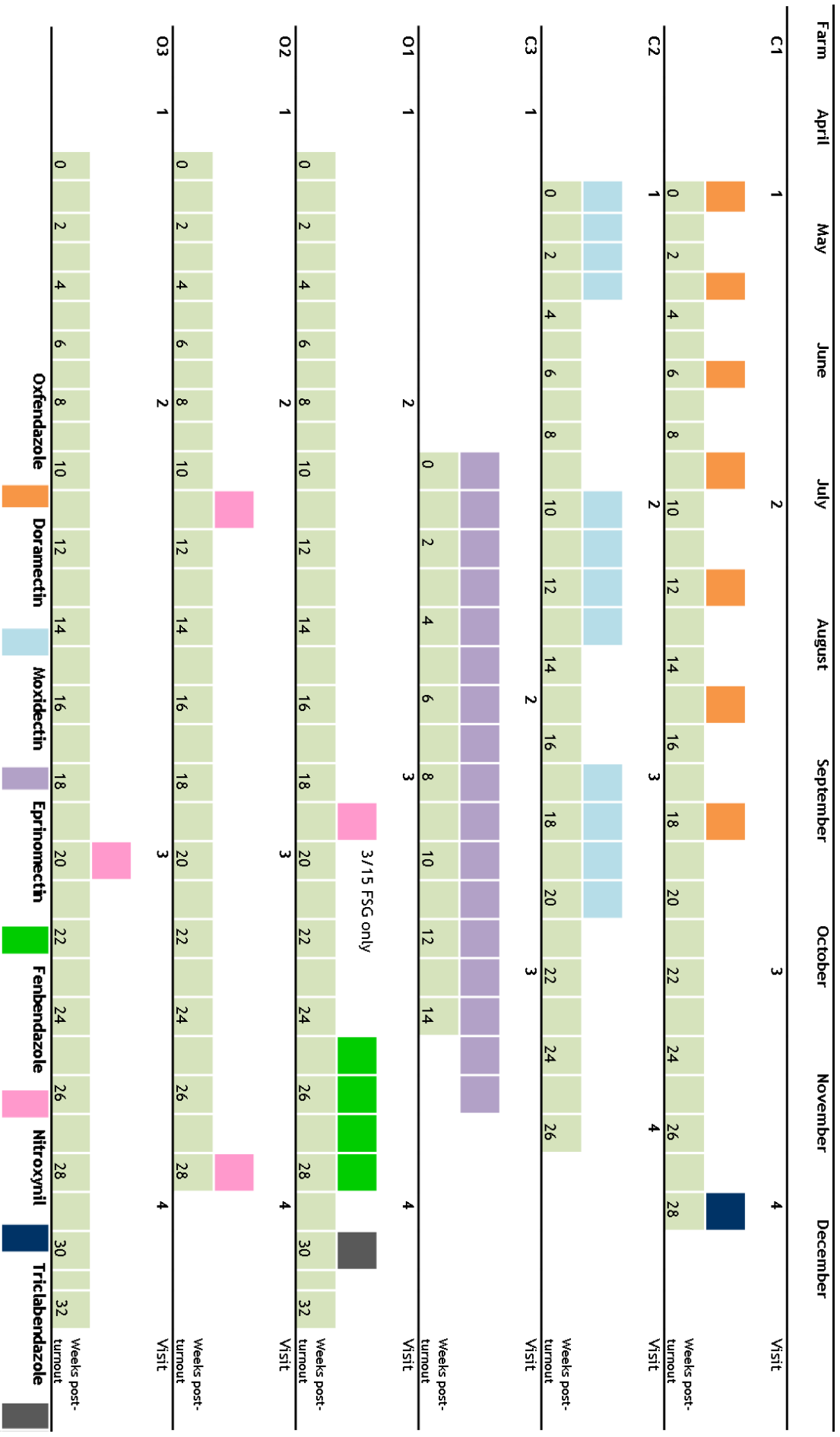


Figure 2.3. Timeline for each farm from April 2009 to December 2009. Illustrates time of grazing period in light green and turnout is represented by 0, timings of visits (numbers 1-4) and anthelmintic treatment (coloured bars). Length of coloured bar depicts duration of activity of anthelmintic.

2.2.11 Laboratory Analysis

2.2.11.1 Faecal Egg Counts

Faecal egg counts were obtained at visits 2, 3 and 4 by using a modified McMaster salt flotation technique based on 3g of faeces and with a sensitivity of 50 eggs per gram (epg). The samples were stored at 4°C for a maximum of two days prior to analysis. If it was necessary to freeze the samples, due to time constraints in performing the FEC, the frozen samples were thawed slowly at 4°C over 24 hours before analysis. A modified McMaster egg counting method (Gordon and Whitlock, 1939; MAFF, 1986) was used to count trichostrongyle eggs in faeces. A handful of faeces were taken directly from the rectum of each animal. Three grams of the faeces were homogenised with 42 ml of tap water then sieved through a 250 micron aperture sieve and the filtrate collected. After thorough mixing, 15 ml of the filtrate was transferred to a centrifuge tube and centrifuged for 5 minutes at a relative centrifugal force (rcf) of 1006 xg. The supernatant was discarded and the remaining faecal pellet broken up using a whirl mixer. The tube was then filled with saturated sodium chloride solution to its former level and inverted six times. A Pasteur pipette was used to transfer 0.15 ml to fill two chambers of a McMaster slide. The preparation was then examined using the x 25 objective of a stereo microscope. The total number of eggs within the grids of each chamber were counted and the result multiplied by 50 to give the estimated number of eggs per gram. Whenever possible each sample was duplicated.

2.2.11.2 Culture of Third-Stage Larvae

Culture of third-stage larvae for speciation took place in November and December 2009. Faeces were taken directly from the rectum of each animal. The faeces were pooled into a pot on each farm and mixed together. Three x 3g of faeces were rolled into faecal balls for each farm. To enable soft wet faeces to form a faecal ball, vermiculite V4 (Silvaperl), an insulating material, was added to the faeces (Armour, 1967). Faecal balls were placed in plastic culture trays (400 x 200 x 75 mm). The trays were loosely sealed and placed inside a polythene bag, which was punctured to enable airflow. The trays were incubated at room temperature, which

was recorded daily (19°C - 21°C day-time) for 10 - 14 days. The trays were then flooded with tepid water (22°C) and allowed to soak for between 2 - 4 hours. The material was mixed and sieved through a 1.0 mm sieve. The filtrate was then collected and allowed to sediment for 2 hours at 4°C. The volume was reduced using a vacuum line and baermannised (See section 2.2.11.3). After a minimum of six hours, 10ml of fluid was withdrawn and the *O. ostertagi* and *C. oncophora* larvae (Figure 2.2) in 1 ml were identified using a stereo microscope and numbers of each were counted. The ratio of *O. ostertagi* and *C. oncophora* larvae was calculated for each farm.

2.2.11.3 *Dictyocaulus viviparus*

In order to detect *D. viviparus* larvae in the faeces, a modified Baermann technique was used (Parfitt, 1955). Three grams of pooled faeces per farm at visit 3 were soaked for three hours in tap water before sieving the material through a flour sieve placed on top of a 30 micron aperture Endecott sieve. The larval suspension was drawn through a coarse filter paper (Whatmans Grade 113, 18.5cms) using a Buchner funnel and vacuum pump. A single milk filter (Maxa Milk filters, A. McCaskie, Stirling) was put on top of the retained material, the combination inverted and placed on a Baermann filter funnel. After a minimum of six hours, 10ml of fluid was withdrawn and the larvae in 1 ml differentiated and counted.

2.2.11.4 *Fasciola hepatica*

Trematode egg counts were performed using the Boray method (Boray, 1985) on Farm C1 at visit 3 and 4, Farms O1 and C2 at visit 4 and Farm C3 at visit 3. Farm O3 used their own veterinary surgeon to test for trematode eggs late in the grazing season. A handful of faeces were taken directly from the rectum of each animal. Three grams of the faeces were homogenised with a sufficient volume of water to produce a fluid suspension, which was then passed through a coarse 250 micron aperture sieve into a suitable container. The material retained by the sieve was thoroughly washed with a fine water jet and the washings also collected. The resultant suspension was transferred to a conical measure and allowed to stand for two minutes, after which the supernatant was drawn off using a water vacuum pump. The remaining material was transferred to a 15 ml glass tube, the conical measure

rinsed and the washings added. The glass tube was allowed to stand for a further two minutes. The supernatant was again withdrawn using a water vacuum pump and two drops of 5% Methylene blue was added to the remaining material. Portions of this material were then examined in a petri dish for *F. hepatica* eggs using the x 12 magnification of a stereo microscope. All material was examined and the total number of eggs recorded. After twenty-five eggs had been counted the result of >25 eggs was recorded and the sample discarded.

2.2.11.5 Pepsinogen

At each visit, blood was taken from all animals (jugular or coccygeal venepuncture) and collected into a tube containing the anticoagulant ethylenediaminetetraacetate (EDTA). The EDTA tubes were kept at 4°C until they were centrifuged at a relative centrifugal force (rcf) of 1789 xg for 10 minutes. The plasma was collected into an eppendorf tube with a pipette and frozen at -20°C. Pepsinogen assays were performed by the Scottish Agricultural College (SAC) laboratory using a modification of the colorimetric method (Mylrea and Hotson, 1969). The laboratory used a Hache Lange DR2800 spectrophotometer. The method used converts pepsinogen to pepsin by dilute hydrochloric acid (1M). The bovine albumin substrate is split into peptide units with tyrosine ends, which remain in solution after precipitation of any remaining proteins by trichloroacetic acid (10%). The tyrosine reacts with Folin and Ciocalteu's reagent to form a blue compound that is proportional under given conditions, to the plasma pepsinogen concentration. Pepsinogen concentrations were calculated as units of tyrosine (iu), where 1 unit = 1µM tyrosine released per litre of plasma per minute at 37°C and expressed as iu/l.

2.2.11.6 Fructosamine

At each visit blood was taken from all animals (jugular or coccygeal venepuncture) and collected into a tube containing the anticoagulant ethylenediaminetetraacetate (EDTA). The EDTA tubes were kept at 4°C until they were centrifuged a relative centrifugal force (rcf) of 1789 xg for 10 minutes. The plasma was collected into an eppendorf tube with a pipette and frozen at -20°C. Plasma fructosamine levels were measured using a commercial test kit (Horiba ABX) based on

the ability of ketoamine to reduce nitrotetrazolium blue (NBT) in an alkaline buffer solution. The formation of formazine is directly proportional to the concentration of Fructosamine at 540 nm (600 aux) and was measured photometrically using an analyser (Olympus AU640 clinical chemical analyser, Beckman Coulter).

2.2.11.7 *Ostertagia ostertagi* Antibodies

Each month, a bulk tank milk sample was collected by the farmer from each farm and stored at -20°C . At each visit blood was taken from all animals (jugular or coccygeal) and collected into a tube containing the anticoagulant ethylenediaminetetraacetate (EDTA). The EDTA tubes were kept at 4°C until they were centrifuged at a relative centrifugal force (rcf) of 1789 xg for 10 minutes. The plasma was collected into an eppendorf tube with a pipette and frozen at -20°C . The concentration of *O. ostertagi* antibodies in plasma was analysed using a SVANOVIR[®] *O. ostertagi*-Ab ELISA Kit. The kit is designed to detect bovine *O. ostertagi*-specific antibodies in milk commercially. The kit procedure is based on a solid-phase indirect Enzyme Linked Immunosorbent Assay (ELISA). In this procedure milk or plasma samples are exposed to non-infectious *O. ostertagi* antigen coated onto wells of microtitre strips. *O. ostertagi* antibodies bind parasite antigen in the wells. The HRP (horseradish peroxidase) conjugate subsequently added forms a complex with these *O. ostertagi* antibodies. Unbound material is removed by rinsing before the addition of substrate solution. Subsequently a blue-green colour develops, which is due to conversion of the substrate by the conjugate. A positive result is indicated by the development of a blue-green colour. The result is read by a microplate photometer where the optical density (OD) is measured at 405 nm. *O. ostertagi*-specific antibody levels have been determined in serum at a dilution of 1/140 (Charlier *et al.*, 2009i) using the SVANOVIR[®] *O. ostertagi*-Ab ELISA Kit. Plasma/milk was thawed at room temperature for 12-16 hours before analysis. For plasma analysis, each eppendorf tube was agitated and 2 μl removed and added to 278 μl of distilled water in order to obtain a 1/140 dilution. For milk analysis, milk was centrifuged at a relative centrifugal force (rcf) of 447 xg for 15 minutes to form a lipid layer on top of the milk sample. Milk was then pipetted from underneath the lipid layer. The PBS-Tween buffer was prepared by adding 25 ml of PBST solution (SVANOVIR[®] *O. ostertagi*-Ab ELISA Kit) to 475 ml of distilled water, which was mixed thoroughly. 100 μl of positive

and negative control serum (0.05% Merthiolate) were added to two wells each on *O. ostertagi* antigen coated microtitre strips. For plasma analysis, 100 µl of 1/140 diluted plasma was added to the remaining wells. For milk analysis, 100 µl of milk was added to the remaining wells. The plates were sealed with cling-film, shaken thoroughly on a shaker and incubated for 1 hour at room temperature. The plate was rinsed with the PBS-Tween buffer solution three times. At each rinse cycle the wells were filled to the top with buffer solution and the plates tapped hard to remove all remains of fluid. Lyophilized HRP conjugate (horseradish peroxidase conjugated anti-bovine IgG monoclonal antibodies) was prepared just before use. 11.5 ml of PBS-Tween buffer was added to the conjugate carefully. The solution was left for one minute then thoroughly shaken on a shaker. 100 µl of conjugate was added to each well. The plates were sealed with cling-film, shaken thoroughly on a shaker and incubated for one hour at room temperature. The plate was rinsed with the PBS-Tween buffer solution three times. At each rinse cycle the wells were filled to the top with buffer solution and the plates tapped hard to remove all remains of fluid. 100 µl of substrate solution ABTS was added to each well. The plates were sealed with cling-film, shaken thoroughly on a shaker and incubated for 30 minutes at room temperature in the dark. The reaction was halted by adding 50 µl of stop solution (1% SDS) to each well using the same well order as used when adding the substrate solution. The plates were shaken thoroughly on a shaker before the optical density (OD) was measured at 405 nm in a spectrophotometer.

Calculation of the optical density ratio (ODR) value is shown below.

$$\text{ODR} = \frac{\text{OD}_{\text{sample or control}} - \text{OD}_{\text{negative control}}}{\text{OD}_{\text{Positive control}} - \text{OD}_{\text{negative control}}}$$

Every sample was duplicated and some quadrupled to reduce laboratory error. If the positive or negative controls differed in OD value by more than twenty-five per cent, the results from that plate were discarded.

2.2.12 Experimental Animal Description

An effort was made to match each conventional farm to a similar organic farm in regards to location, herd size, breed and milk yield. As previously mentioned, due to the nature of the study involving the regular handling of FSG through the grazing season, compromises had to be made in this regard. Farm C1 and farm O3 had similar milk yields, numbers of cows and the Holstein-Friesian breed of cattle and matched well. However, the FSG on Farm O3 were significantly older than the calves on the rest of the farms in the study.

2.2.12.1 Breed

Figure 2.4 illustrates the different cattle breeds present within the fifteen FSG on each farm. The predominant breed on farms C1, C2 and O3 was the Holstein-Friesian. Farm C3 was the only farm to have only dairy x beef calves in the group. Farms O1 and O2 had predominantly dairy calves in the FSG group.

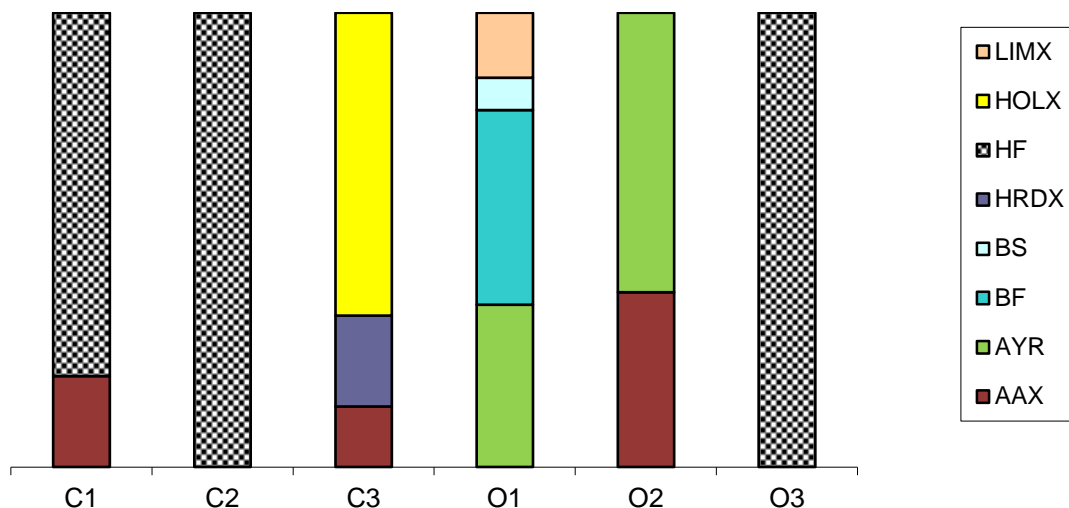


Figure 2.4. Histogram showing the percentage of the different cattle breeds within the fifteen FSG calves in the study on each farm. LIMX, Limousin cross; HOLX, Holstein-Friesian cross; HF, Holstein-Friesian; HRDX, Hereford cross; BS, Brown Swiss; BF, British Friesian; AYR, Ayrshire; AAX, Aberdeen Angus cross.

2.2.12.2 Sex

The male to female ratio in each group is shown in Figure 2.5. Farms C2 and O3 had only dairy replacements in their group of FSG. The other farms included male calves and dairy x beef heifers.

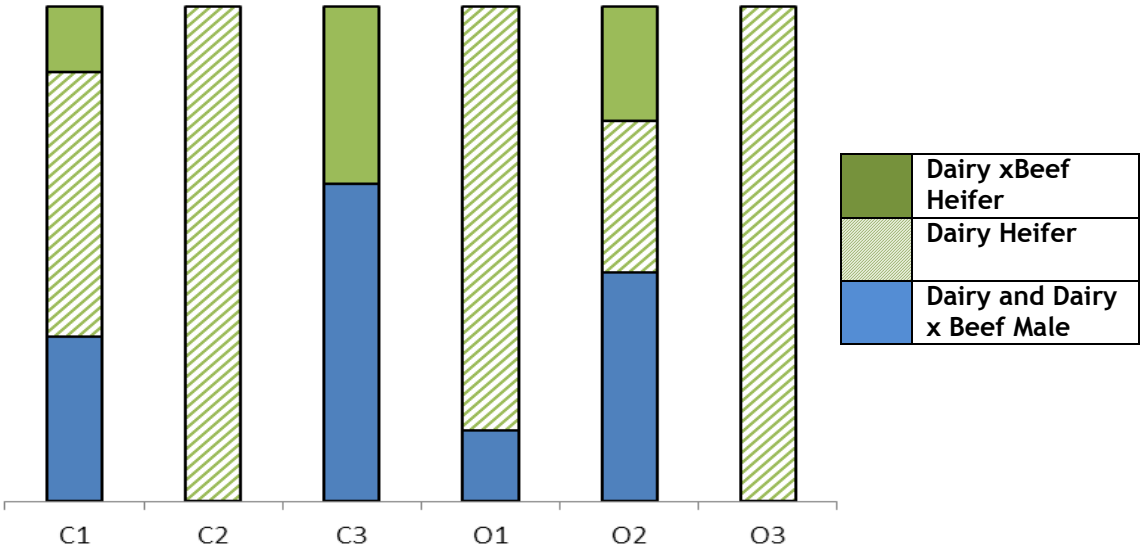


Figure 2.5. Histogram showing the proportion of male to female calves from the fifteen FSG on each farm. The female calves are further divided into heifer replacements and dairy x beef heifers.

2.2.12.3 Age

The average age in days of the FSG group on each farm on visit 1 and on the 30th April 2009, to allow comparison of ages on the same date, is shown in Table 2.7. At over thirteen months of age on average, the calves on Farm O3 were significantly older than the calves on the rest of the farms, illustrated in Figure 2.6.

Farm	Visit 1 (Date)	Age \pm SD (days)	Age on 30 th April (days)
C1	29 th April	191 \pm 29	192
C2	30 th April	217 \pm 16	217
C3	9 th April	127 \pm 28	148
O1	7 th April	218 \pm 76	241
O2	10 th April	129 \pm 17	149
O3	10 th April	405 \pm 38	425

Table 2.7. Mean age \pm SD of the fifteen first season grazers on visit 1 and on the 30th April 2009 on each farm.

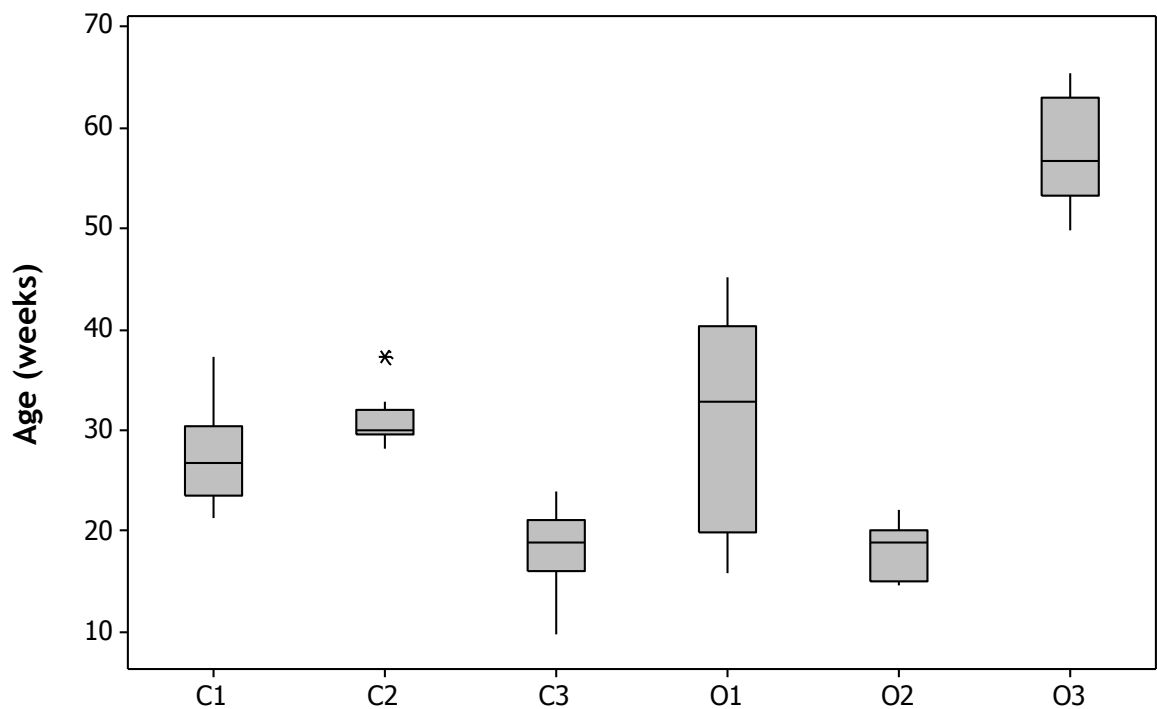


Figure 2.6. Box and whisker plot showing the age in weeks of the FSG at visit 1 on each farm.

Farms C3 and O2 had the youngest FSG groups with the average age of calves in both groups being approximately five months old. Farm O1 has the largest group variance in age. The box in the box and whisker plot extends from the upper quartile to the lower quartile. The horizontal line within the box represents the median value of the data set. The vertical lines, or whiskers, show the maximum and minimum values in the data set. A star represent a result outlying the data set.

2.2.13 Statistical Analysis

The null hypotheses were that plasma pepsinogen, *Ostertagia ostertagi* antibodies, plasma fructosamine and FEC showed no correlation with liveweight gain. The Pearson's correlation test was used on normally distributed data and the Spearman's rank correlation test was used on non-normally distributed data to investigate these hypotheses. A one-way analysis of variance (ANOVA) was used to calculate statistical significance between fructosamine concentration and liveweight gain in the conventional and organically reared calves over the grazing season. Statistical analysis of the data was performed using Microsoft Excel and Minitab 16 for Windows.

2.2.14 Meteorology

The study was conducted during the grazing season of 2009. The temperature and precipitation during this grazing season are shown in Figure 2.7 (Data from Met Office, Paisley Weather Station). Weather conditions in 2009 were warmer than usual early in the grazing season for the UK (April recorded its third highest maximum temperature since 1917). Temperatures then remained warm through spring and early summer. Overall, 2009 was a year of high rainfall during summer and autumn (August and November recorded the highest rainfall in the west of Scotland for those months since 1917).

Type I ostertagiosis risk was low in early 2009 as the previous summer had not been particularly dry. Although the temperature in April reached a higher than average maximum temperature it was only by 0.1°C, which has little significance on trichostrongyle larvae development on pasture. Hatching of infective larvae from the overwintered larvae on the pasture on the west coast of Scotland would have taken approximately between 7-16 (*O. ostertagi*) and 4-21 days (*C. oncophora*) at 14-16°C and 18-28 (*O. ostertagi*) and 21-56 (*C. oncophora*) days at 10-11°C in April and May (Rose, 1961; Rose, 1963). High rainfall during summer and autumn will have broken up the faecal pats disseminating infective larvae over the pasture (Rose, 1962; Grønvold, 1984ii; Grønvold, 1987) making mid-summer 2009 high risk for gastrointestinal parasitism.

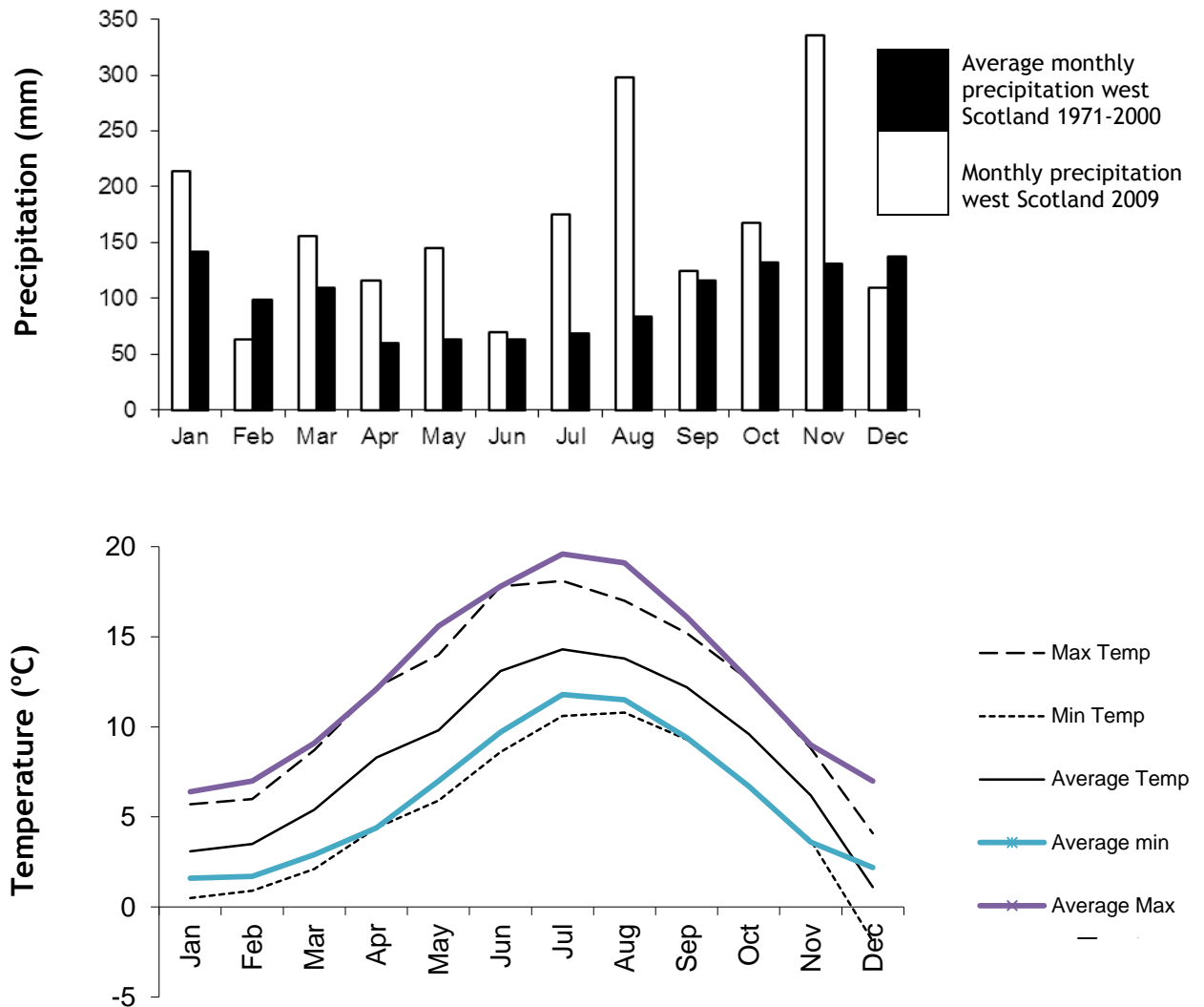


Figure 2.7. Meteorological data from the Paisley weather station in 2009. Top figure shows the comparison between total monthly precipitation for the west of Scotland (open bars) and the average of the total monthly precipitation for the west of Scotland between 1971 and 2000 (closed bars). Bottom figure shows maximum (large dashed), minimum (small dashed) and average (smooth line) temperature monthly in the west of Scotland. The purple line shows maximum, and blue line minimum, average monthly temperature for the west of Scotland between 1971 and 2000.

2.3 RESULTS

An overall summary of the results and all analyses and animal data is tabulated in Table 2.10 and summarised further graphically below. Overall, the conventional farms had higher liveweight gains compared to the organic farms over the grazing season. On the organic farms there was high gastrointestinal parasitism challenge that

appeared to infer poorer liveweight gains suggested by FEC, plasma pepsinogen and fructosamine analysis.

2.3.1 Liveweight Gain

The growth curves shown in Figure 2.8 illustrate that Farms C1, O2 and O3 had similar growth curves Farms C2 and O1 had similar growth curves and did not produce a steeper line mid-grazing season seen in Farms C1, O2 and O3. Farm C3 produced an almost straight growth curve. The animals on Farm C3 were housed earlier than the animals on the other farms, thus the shorter growth curve. Figure 2.9 shows box and whisker plots of liveweight gain over the grazing season for each farm.

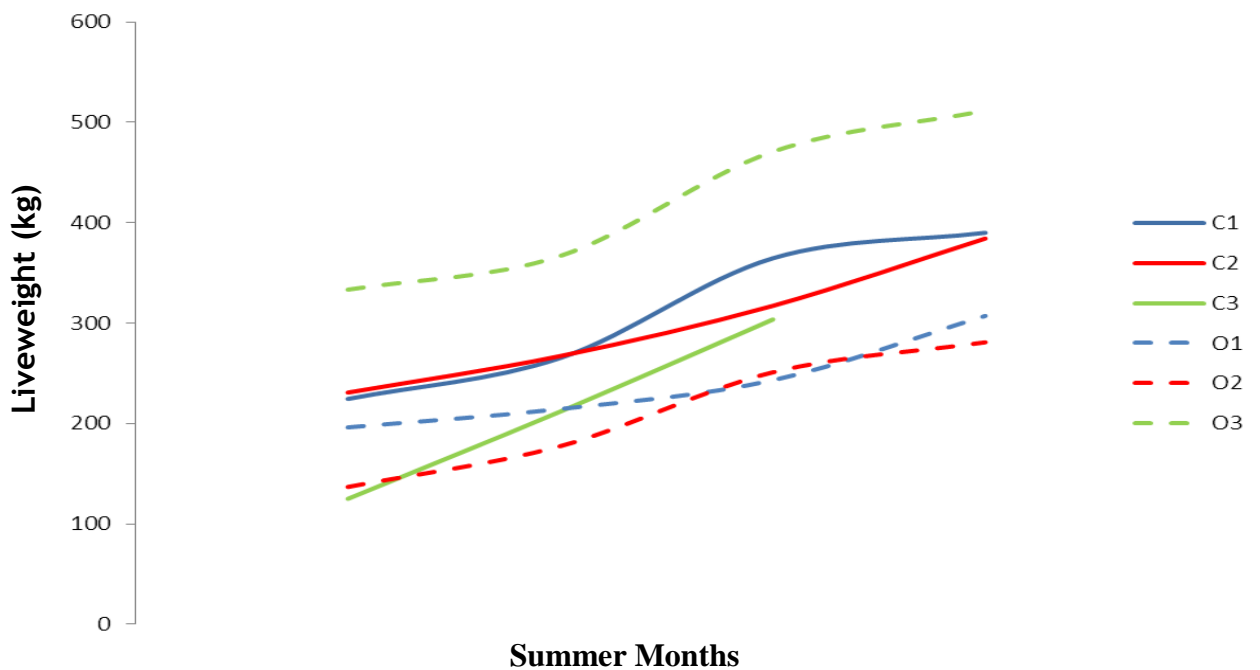


Figure 2.8. Growth curve of mean liveweight on each farm from visit 1 to housing. Turnout and housing were at different times for each farm.

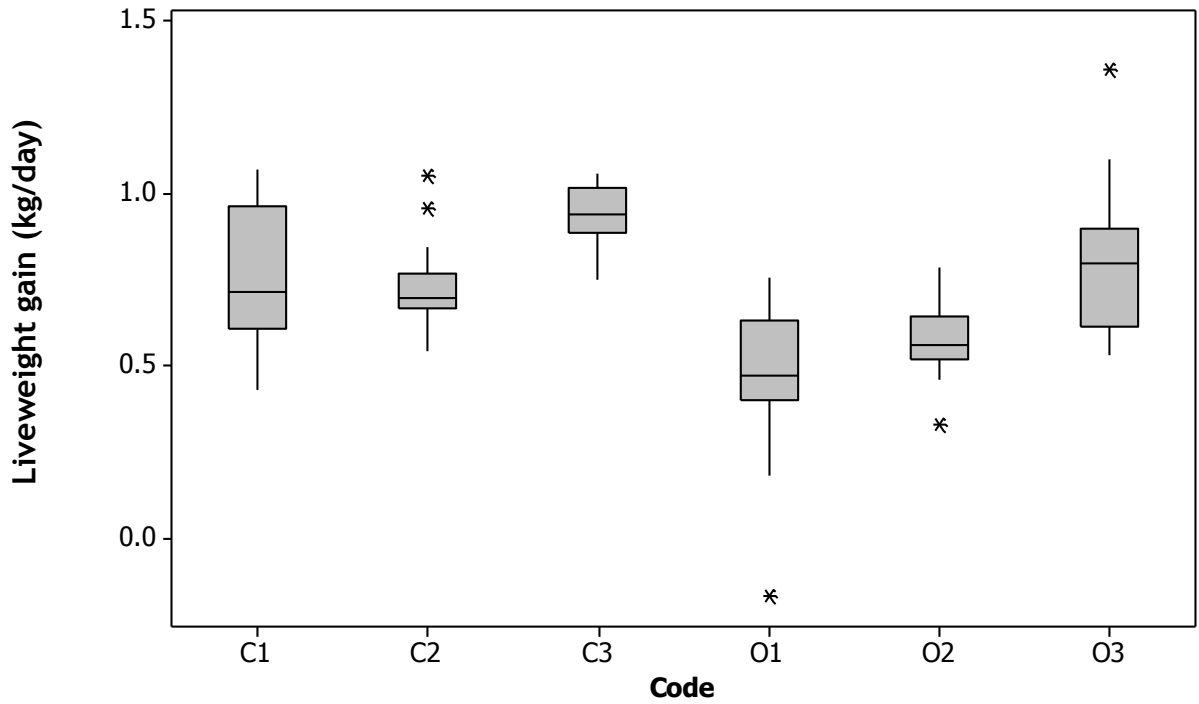


Figure 2.9. Box and whisker plot showing the estimated liveweight gain of FSG calves using a weigh band on all farms (C1-3, O1-3) between visit 1 and housing at visit 4. The time between each visit on each farm was different.

Farm C3 has the highest mean liveweight gain over the grazing season (0.93 ± 0.10 kg/day) but the shortest grazing season of only fourteen weeks (six calves only ten weeks). Farms O1 and O2 produced the poorest liveweight gains.

2.3.2 Plasma Pepsinogen

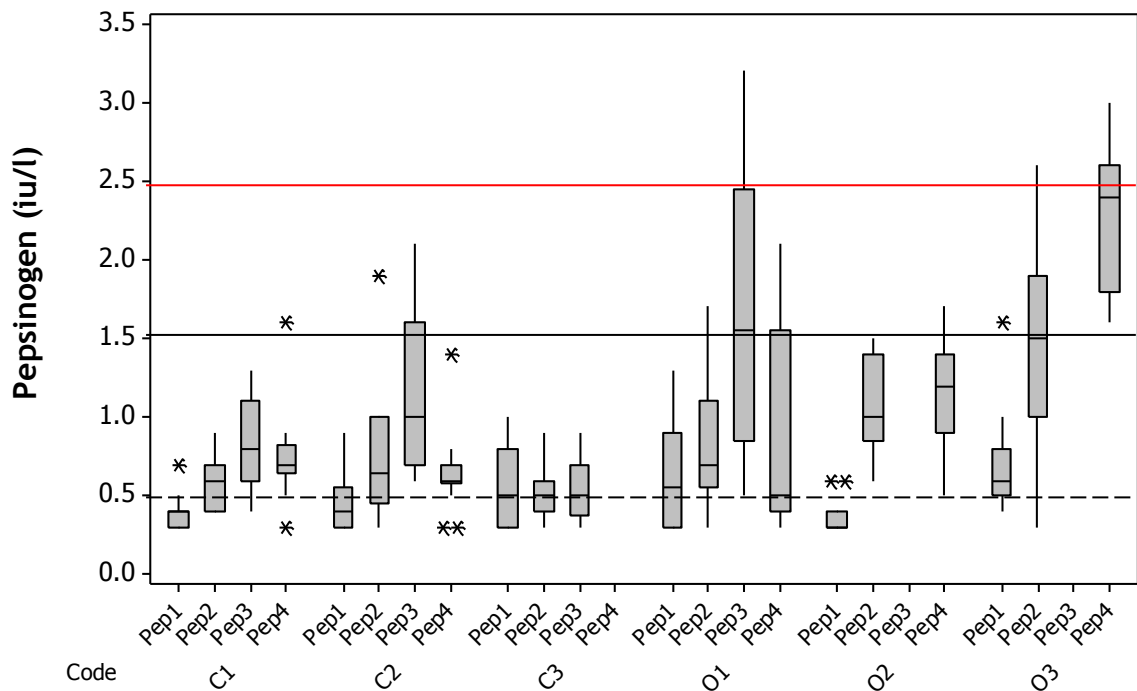


Figure 2.10. Box and whisker plot showing the plasma pepsinogen values on all farms (C1-3, O1-3) on visits 1 to 4 (Pep1-Pep4). Values above: top red line indicates abomasal parasitism likely to be associated with clinical disease; middle line indicates abomasal parasitism likely to cause impaired liveweight gain; bottom dashed line indicates exposure to abomasal parasitism (SAC Laboratory). On visit 3 for both farms O2 and O3 the blood tubes were spoiled and could not be analysed. The time between each visit on each farm was different.

Figure 2.10 illustrates that plasma pepsinogen concentration increased from visit 1 to 3 on all farms but Farm C3, which had no increase in plasma pepsinogen levels during the study. By visit 4, plasma pepsinogen concentrations had fallen on Farms C1, C2 and O1 from their highest value at visit 3. The pepsinogen concentration at housing on Farm O3 was higher than the other farms. Plasma pepsinogen was not normally distributed (probability plot P-value < 0.005) so histograms were produced (Figure 2.11) in order to illustrate any outliers skewing the distribution. The histograms illustrate no significant outliers markedly skewing the distributions at any of the visits on any of the farms.

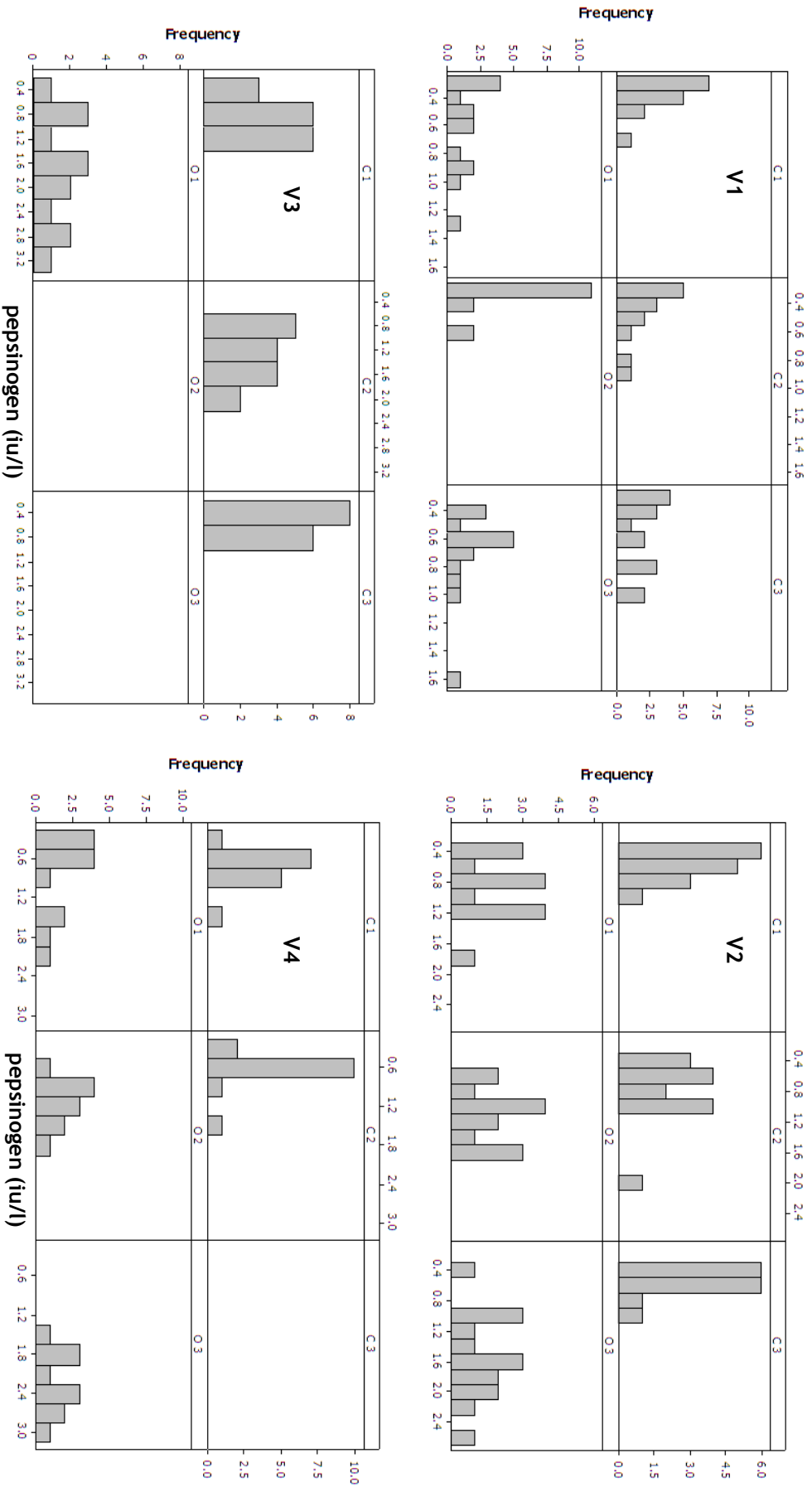


Figure 2. 11. Histograms of plasma pepsinogen concentration for each calf at each visit (V1-V4)

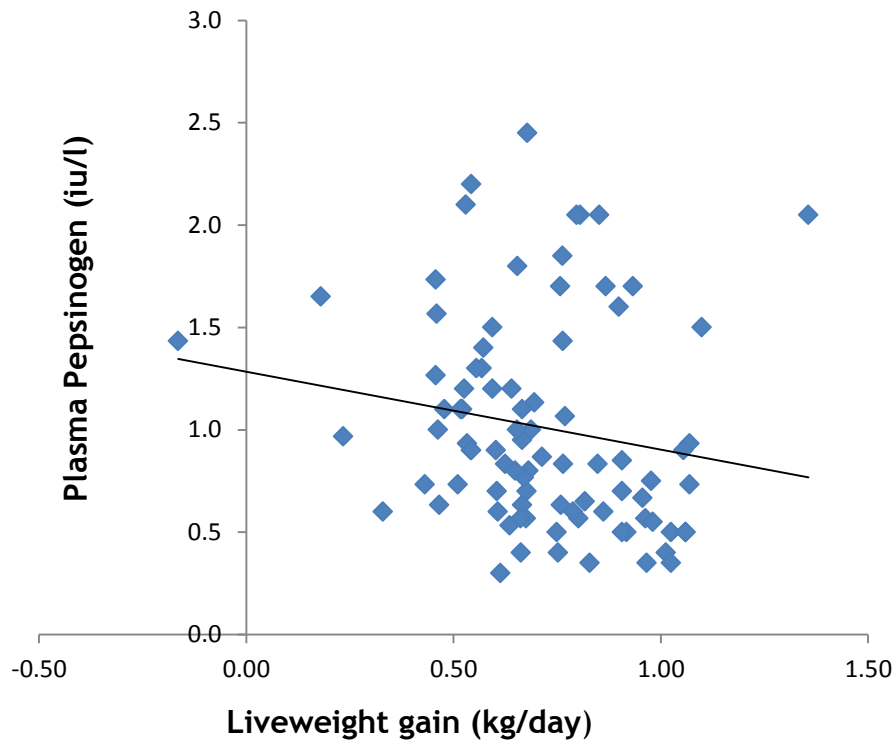


Figure 2.12. Correlation between liveweight gain and mean plasma pepsinogen level over the grazing season.

The study found a weak negative correlation between liveweight gain over the grazing season and mean plasma pepsinogen concentration. The Spearman's rank correlation showed a weak negative correlation of -0.23.

2.3.3 Plasma Fructosamine

Plasma fructosamine was normally distributed (probability plot P-value = 0.75). Fructosamine values on all farms fell between visits 1 and 2. Fructosamine concentrations were lowest and plasma pepsinogen concentrations highest at visit 3 on all farms (no values for Farms O2 and O3 at visit 3). Figure 2.13 illustrates fructosamine concentration in peripheral blood on each farm at each visit. At visit 2 (F2) Farms O1, O2 and O3 have lower fructosamine concentrations than the other farms. At visit 3 there is more individual calf variation within the groups of FSG (F3) but the only organic farm represented is O1.

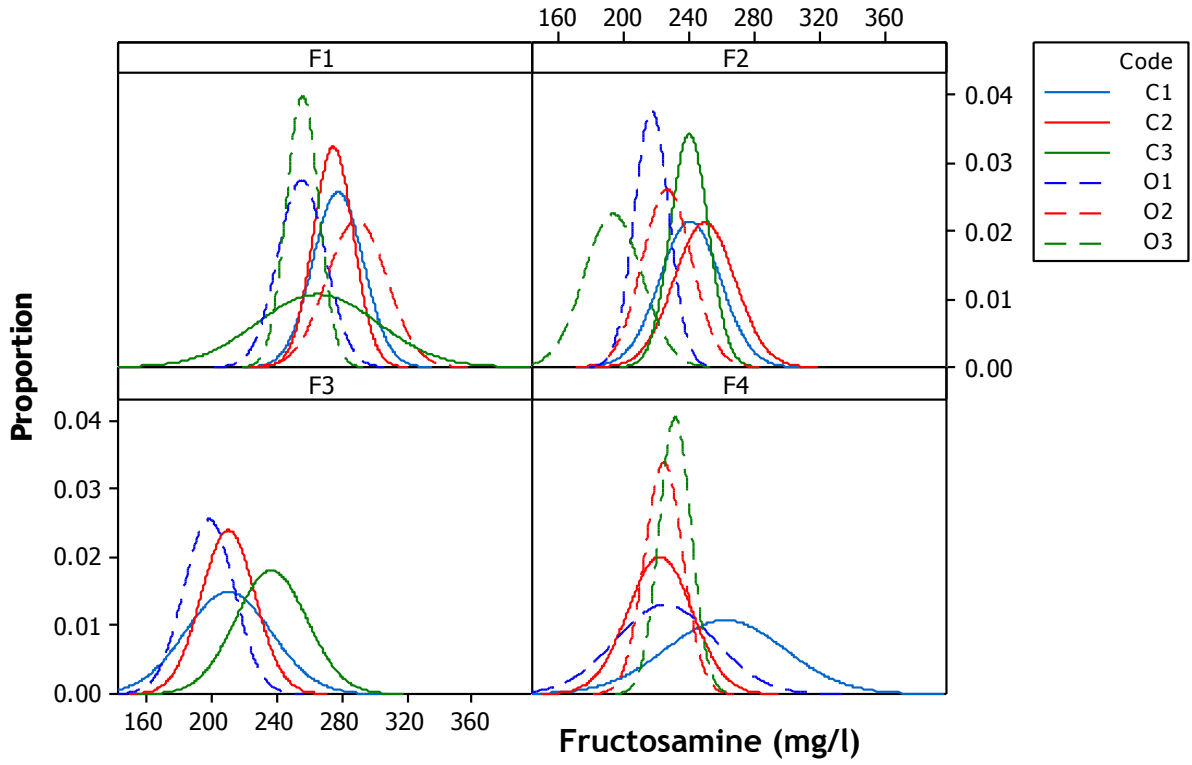


Figure 2.13. Distribution curve for plasma fructosamine at visits 1; F1, 2; F2, 3; F3 and 4; F4 for each farm (C1-3, O1-3).

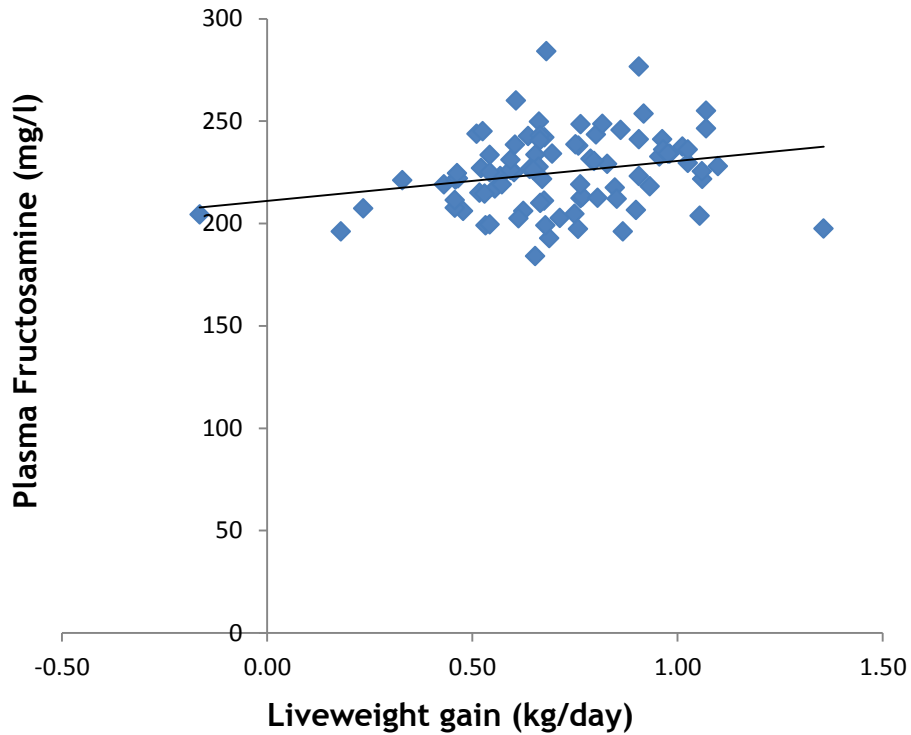


Figure 2.14. Correlation between liveweight gain and plasma fructosamine levels.

The study found no correlation between plasma fructosamine levels and liveweight gain. Pearson’s correlation was 0.13.

2.3.4 *Ostertagia ostertagi* Antibody

2.3.4.1 Plasma

The optical density ratio (ODR) of *O. ostertagi*-Ab ELISA in plasma was normally distributed (probability plot P-value = 0.75). There was large variation within the calves on each farm but more variation on the organic farms at turnout, illustrated in Figure 2.15. As the grazing season progresses the ODR values increase and have a wider distribution between calves in the group.

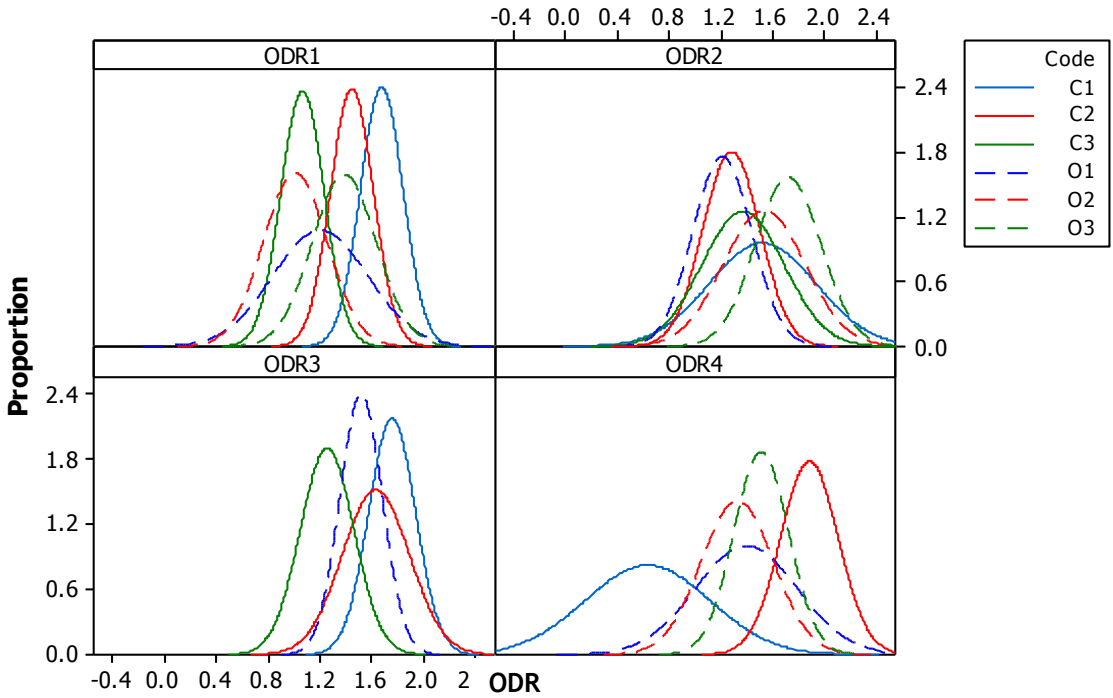


Figure 2.15. Distribution curve for *O. ostertagi* antibody (ODR) at visits 1; ODR1, 2; ODR2, 3; ODR3 and 4; ODR4 for each farm (C1-3, O1-3).

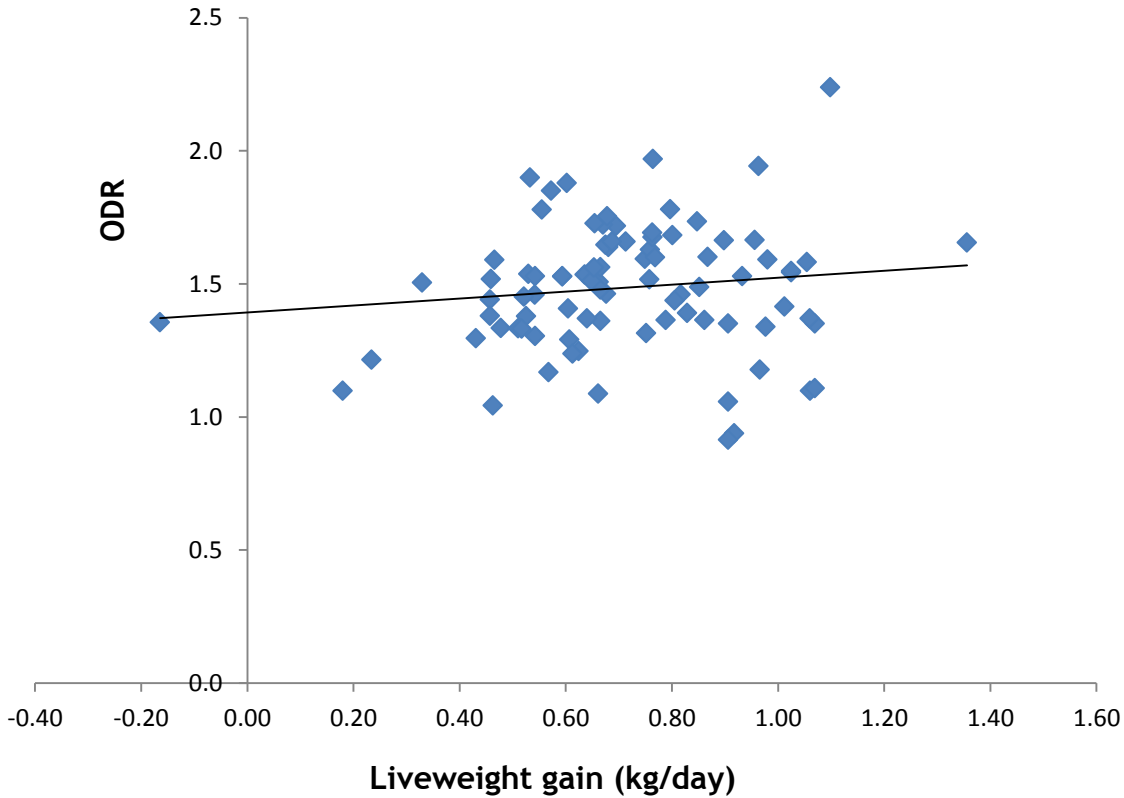


Figure 2.16. Correlation between liveweight gain and plasma *O. ostertagi*-Ab levels.

There was no correlation between *O. ostertagi*-Ab ODR and liveweight gain (Figure 2.16). Pearson’s correlation was 0.12.

2.3.4.2 Bulk Milk

All farms were requested to keep bulk milk samples from each month during the grazing season frozen at -20°C for analysis of *O. ostertagi*-Ab. The results of this analysis are shown in Table 2.8. Some samples were spoiled or not taken. All farms showed high antibody concentration in bulk milk during the grazing season.

Farm	Month	ODR
C1	June	0.77
	August	0.83
C2	August	0.67
	September	0.80
C3	June	1.16
	Aug	1.17
O1	June	0.96
	July	0.94
	Aug	1.10
O2	July	0.80
	August	0.98
	September	1.10
O3	August	0.94

Table 2.8. Bulk milk *O. ostertagi*-Ab ELISA for farm and month.

2.3.5 Faecal Egg Count

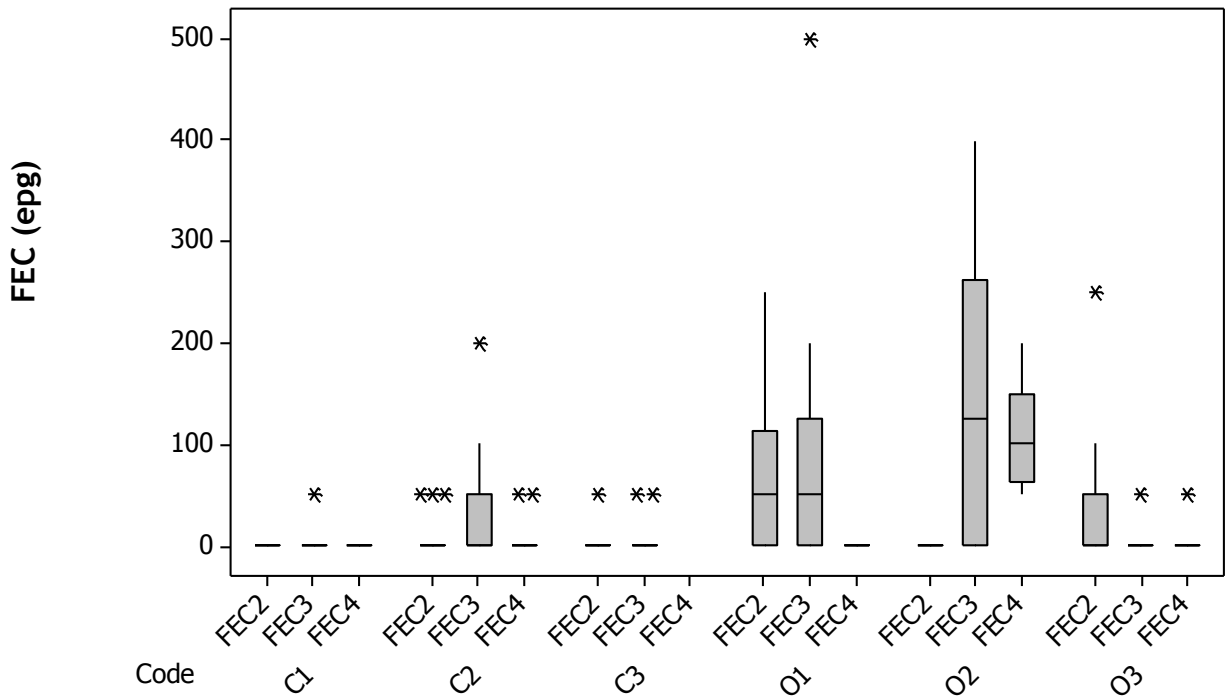


Figure 2.17. Box and whisker plot showing the FEC values on all farms (C1-3, O1-3) on visits 1 to 4. The time difference between each visit on each farm is different.

Faecal egg count was not normally distributed within this study group (probability plot P-value < 0.005). No farm in the study at any visit had a mean FEC of 250 epg and over, shown in Figure 2.17. Figure 2.18 illustrates histograms of faecal egg counts on each farm at visits 2, 3 and 4. No outliers skewed the mean results.

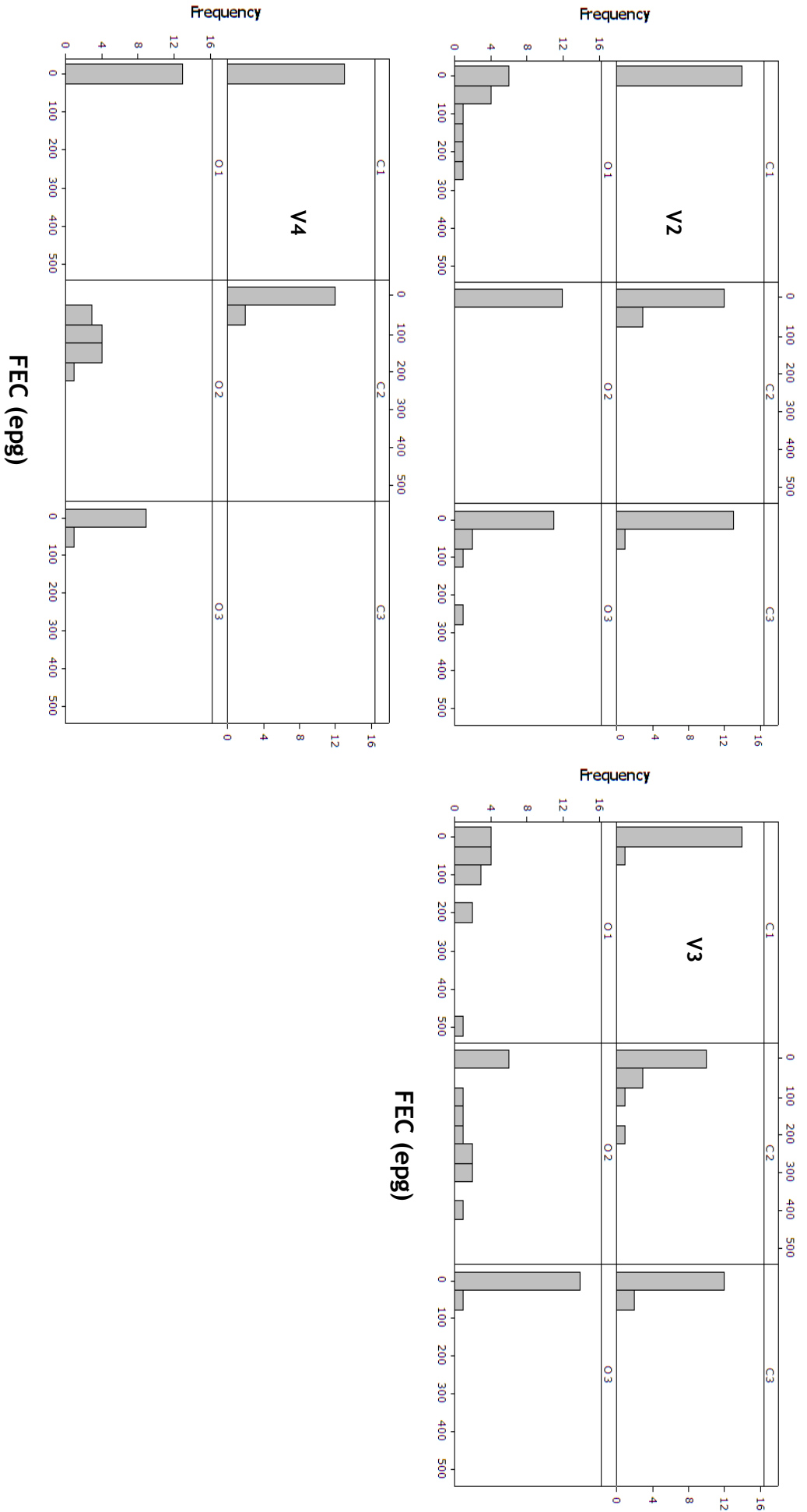


Figure 2. 18. Histograms of FEC on each farm at visits 2, 3 and 4.

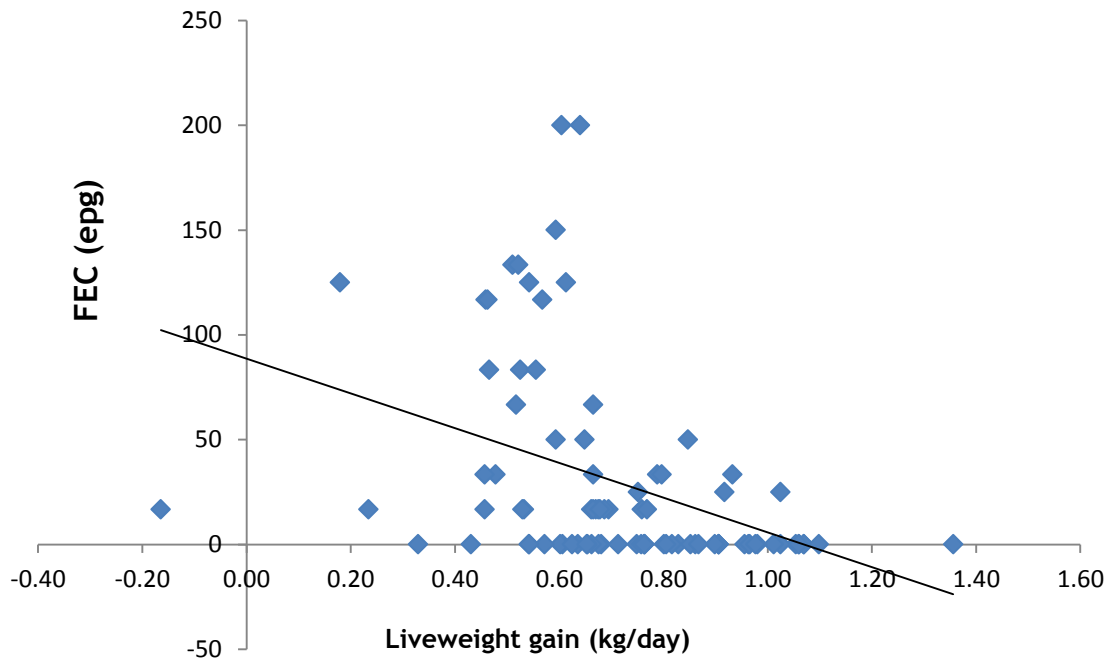


Figure 2.19. Correlation between liveweight gain and FEC.

Figure 2.19 shows a negative correlation between liveweight gain and FEC in the study. The Spearman's rank correlation showed a negative correlation of -0.52.

2.3.5.1 Larval Culture

Larval cultures were carried out using faeces from visit 4. FEC were low at visit 4 and only Farms C1, C2 and O2 had larvae cultured and counted. The results are shown in Table 2.9 below.

Farm	<i>O. ostertagi</i>	<i>C. oncophora</i>
C1	0	7
	0%	100%
C2	0	12
	0%	100%
O2	81	61
	58%	42%

Table 2.9. Numbers and percentage of *O. ostertagi* and *C. oncophora* larvae at visit 4.

2.3.5.2 Lungworm and Liver Fluke

No *D. viviparus* larvae were found on any of the farms during the study. On Farm C1 at visit 3, one calf was found to have > 25 *F. hepatica* eggs. Four other calves were also found to have *F. hepatica* eggs in their faeces. However, at visit 3 (housing) no *F. hepatica* eggs were found on farm. At visit 3, *F. hepatica* eggs were found in faecal samples on Farm C3. At visit 4, *F. hepatica* eggs were found on Farm O3. *Nematodirus* spp. eggs were found in the faeces of the following: one calf at visit 3 on Farm C2; two calves at visit 2 and one calf (the same calf as visit 2) at visit 3 on Farm O1; one calf at visit 2 on Farm O3.

Farm	Visit	Date	Age \pm SD (days)	EWt \pm SD (kg)	BCS (1-5)	EGR \pm SD (kg/d)	Pep \pm SD (iu/l)	Fruct \pm SD (mg/l)	ODR \pm SD	FEC (epg)
C1	V1	29/04/2009	191 \pm 29	225 \pm 32	3.0	(V1-4) 0.77 \pm 0.26	0.39 \pm 0.11	279 \pm 15	1.6 \pm 0.17	
	V2	22/07/2009		265 \pm 38	3.0	(V1-2) 0.49 \pm 0.36	0.56 \pm 0.16	240 \pm 19	1.5 \pm 0.42	0
	V3	14/10/2009		365 \pm 44	3.0	(V2-3) 1.18 \pm 0.44	0.84 \pm 0.30	211 \pm 27	1.8 \pm 0.18	0
	V4	01/12/2009		390 \pm 41	3.0	(V3-4) 0.53 \pm 0.68	0.76 \pm 0.29	262 \pm 37	0.6 \pm 0.48	0
C2	V1	30/04/2009	217 \pm 16	231 \pm 22	3.0	(V1-4) 0.72 \pm 0.16	0.46 \pm 0.20	276 \pm 12	1.5 \pm 0.17	
	V2	15/07/2009		267 \pm 25	2.0	(V1-2) 0.48 \pm 0.36	0.74 \pm 0.42	249 \pm 19	1.3 \pm 0.22	0
	V3	10/09/2009		317 \pm 20	2.5	(V2-3) 0.88 \pm 0.28	1.17 \pm 0.47	211 \pm 17	1.6 \pm 0.26	0
	V4	19/11/2009		384 \pm 28	2.5	(V3-4) 0.93 \pm 0.32	0.64 \pm 0.26	222 \pm 20	1.9 \pm 0.48	0
C3	V1	09/04/2009	127 \pm 28	125 \pm 26	2.5	(V1-3) 0.93 \pm 0.10	0.57 \pm 0.25	266 \pm 37	1.1 \pm 0.17	
	V2	28/08/2009		212 \pm 30	2.5	(V1-2) 0.60 \pm 0.17	0.51 \pm 0.17	240 \pm 12	1.4 \pm 0.32	0
	V3	28/10/2009		303 \pm 39	2.5	(V2-3) 2.1 \pm 0.84	0.54 \pm 0.20	238 \pm 22	1.3 \pm 0.21	0
O1	V1	07/04/2009	218 \pm 76	196 \pm 66	3.0	(V1-4) 0.46 \pm 0.23	0.62 \pm 0.31	256 \pm 14	1.2 \pm 0.37	
	V2	24/06/2009		214 \pm 82	3.0	(V1-2) 0.25 \pm 0.48	0.81 \pm 0.38	217 \pm 11	1.2 \pm 0.23	50
	V3	10/09/2009		243 \pm 68	2.5	(V2-3) 0.37 \pm 0.30	1.66 \pm 0.84	199 \pm 16	1.5 \pm 0.17	50
	V4	08/12/2009		307 \pm 61	2.5	(V3-4) 0.77 \pm 0.55	0.87 \pm 0.64	225 \pm 30	1.4 \pm 0.40	0
O2	V1	10/04/2009	129 \pm 17	137 \pm 23	2.5	(V1-4) 0.57 \pm 0.11	0.35 \pm 0.11	290 \pm 18	1.0 \pm 0.25	
	V2	29/06/2009		178 \pm 18	2.5	(V1-2) 0.28 \pm 0.65	1.06 \pm 0.32	226 \pm 15	1.5 \pm 0.31	0
	V3	30/09/2009		251 \pm 35	3.0	(V2-3) 0.86 \pm 0.37				125
	V4	02/12/2009		281 \pm 29	3.0	(V3-4) 0.36 \pm 0.59	1.15 \pm 0.34	224 \pm 12	1.3 \pm 0.28	100
O3	V1	10/04/2009	405 \pm 38	333 \pm 47	2.5	(V1-4) 0.80 \pm 0.22	0.69 \pm 0.31	257 \pm 10	1.4 \pm 0.25	
	V2	29/06/2009		367 \pm 34	2.5	(V1-2) 0.42 \pm 0.43	1.5 \pm 0.57	193 \pm 18	1.7 \pm 0.25	0
	V3	30/09/2009		471 \pm 44	2.5	(V2-3) 1.11 \pm 0.27				0
	V4	02/12/2009		512 \pm 38	2.5	(V3-4) 0.80 \pm 0.38	2.27 \pm 0.47	231 \pm 10	1.5 \pm 0.21	0

Table 2.10. Summary of observations: mean Age, mean Estimated Weight (EWt), median Body Condition Score (BCS), mean liveweight gain (EGR), mean Plasma Pepsinogen (Pep), mean Plasma Fructosamine (Fruct), mean Plasma *O. ostertagi*-Ab ELISA (ODR) and median Faecal Egg Count (FEC), from calves by farm (C1-3, O1-3) and visit (V1-4).

2.3.6. Conventional vs. Organic

The following section looks closely at some differences and similarities between the conventional and organic farms in the study. The time in days of exposure of each calf to anthelmintic is shown in Table 2.11. Calves on the conventional farms were exposed to anthelmintics for 652% days longer than the organic calves.

Farm	Anthelmintic	Persistency (days)	Number of Treatments	Total Number of Days / Calf of Anthelmintic Exposure
C1	Moxidectin	120	1	120
C2	Doramectin	35	3	105
C3	Oxfendazole*	1	7	7
O1	Fenbendazole*	1	1	1/5 (3/15 calves)
	Eprinomectin	28	1	28
O2	Fenbendazole*	1	2	2
O3	Fenbendazole*	1	1	1

Table 2.11. Number of days per calf of anthelmintic exposure throughout the grazing season for each farm. * Benzimidazoles have no persistency of activity and so a figure of one day was used for the calculation.

Liveweight gains on the organic farms were lower than their conventional counterparts, likely attributable to gastrointestinal parasitism. Table 2.12 shows higher plasma pepsinogen and FEC and lower plasma fructosamine concentrations in the organically farmed calves.

Farm Type	EGR \pm SD (kg/d)	Pep \pm SD (iu/l)	Fruct \pm SD (mg/l)	ODR \pm SD	FEC \pm SD (epg)
Conventional	0.81 \pm 0.17	0.72 \pm 0.29	234 \pm 18	1.49 \pm 0.25	7 \pm 15
Organic	0.61 \pm 0.24	1.32 \pm 0.52	216 \pm 14	1.48 \pm 0.22	52 \pm 59
p-value*	<0.001	n/a	<0.01	Not signif	n/a

Table 2.12. Summary of observations: Estimated liveweight gain from visit 1 to visit 4 (EGR), average plasma pepsinogen (Pep), average plasma fructosamine (fruct), average plasma *O. ostertagi* antibodies (ODR), and average faecal egg count (FEC), from visits 2, 3 and 4 for the conventional and organic dairy farms in the study. *One-way ANOVA.

2.3.6.1 Liveweight Gain

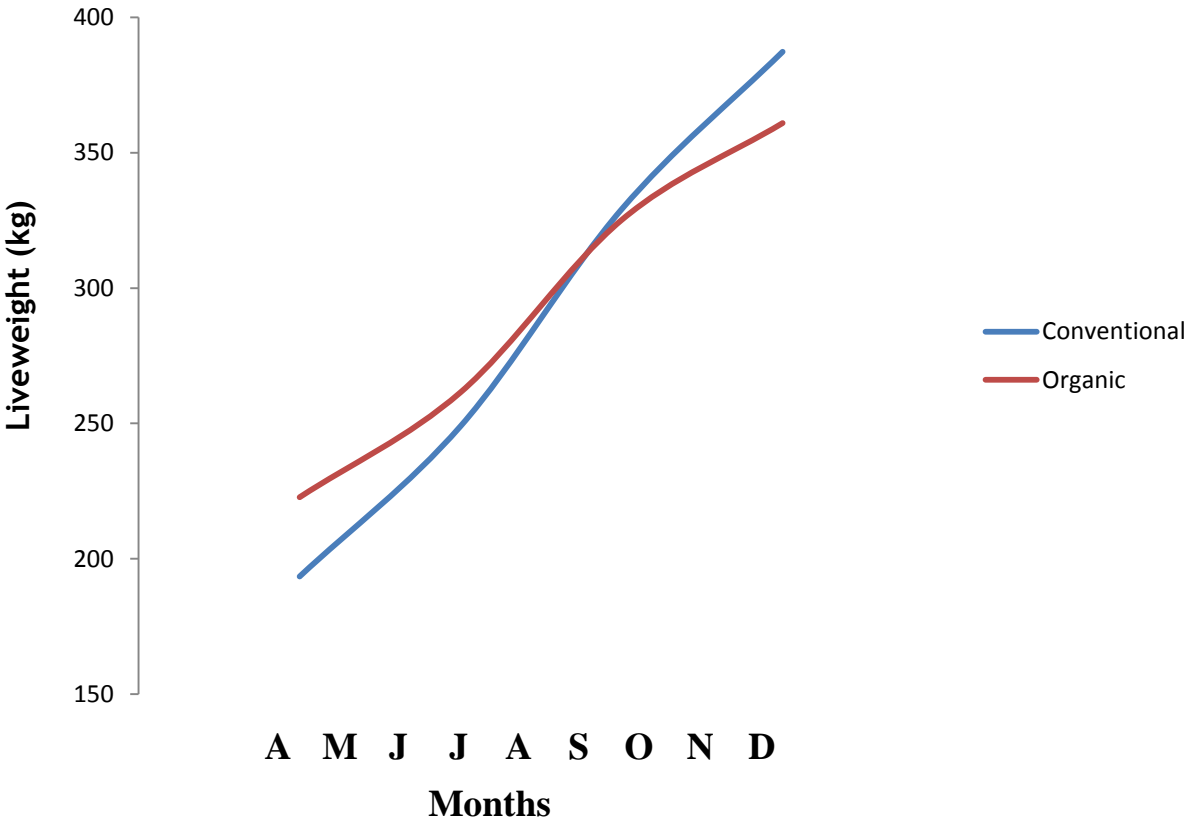


Figure 2.20. Growth curves for the organic and conventionally farmed calves over the grazing season.

Figure 2.20 shows that although the conventionally farmed calves started at a lighter liveweight, they surpassed their organic counterparts mid-grazing season to go on to higher liveweights at housing. Liveweight gain over the grazing season was, on average, 0.61 kg/day for the organically farmed calves and 0.81 kg/day for the conventionally farmed calves ($p < 0.001$ ANOVA).

2.3.6.2 Pepsinogen

Figure 2.21 shows that more calves on the organic farms had higher plasma pepsinogen concentrations than on the conventional farms during the grazing season.

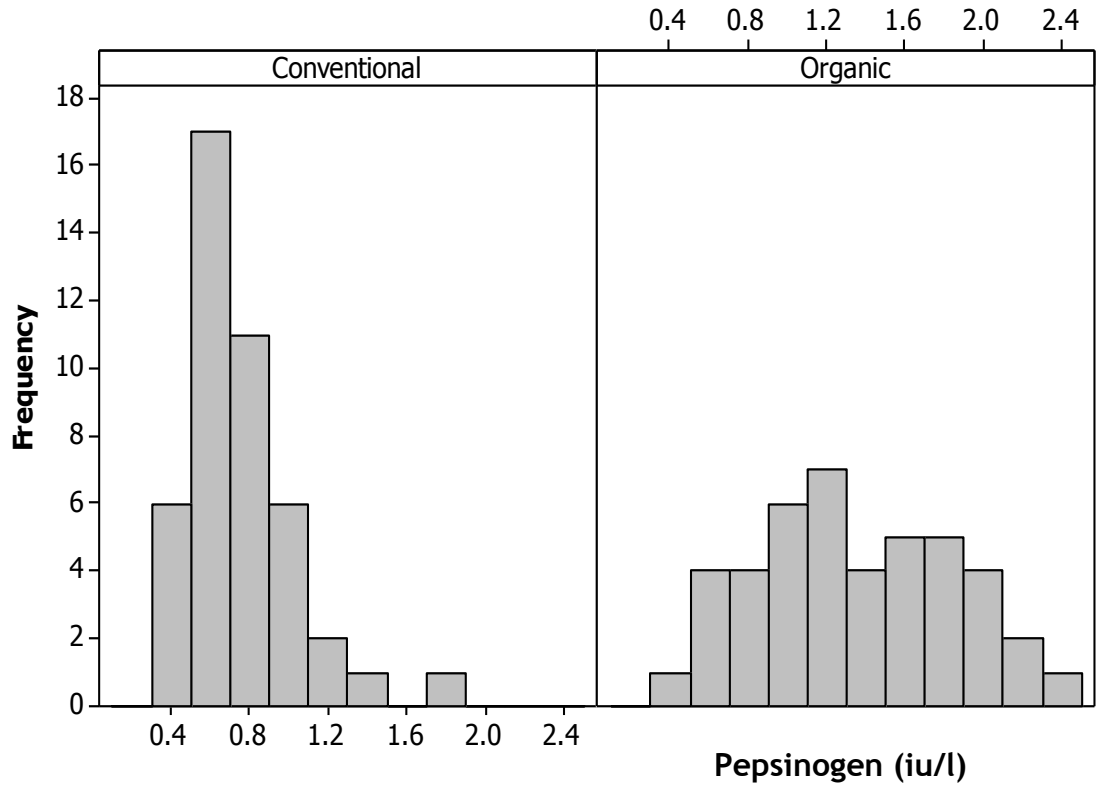


Figure 2.21. Histogram of plasma pepsinogen concentrations on conventional and organic farms on visits 2, 3 and 4, during exposure to gastrointestinal parasites.

2.3.6.3 Fructosamine

Figure 2.22 illustrates that plasma fructosamine concentrations were lower in the organic calves (216 ± 14 mg/l) in comparison to the conventionally farmed calves (234 ± 18 mg/l) as an average between visits 2, 3 and 4 ($p < 0.01$ ANOVA).

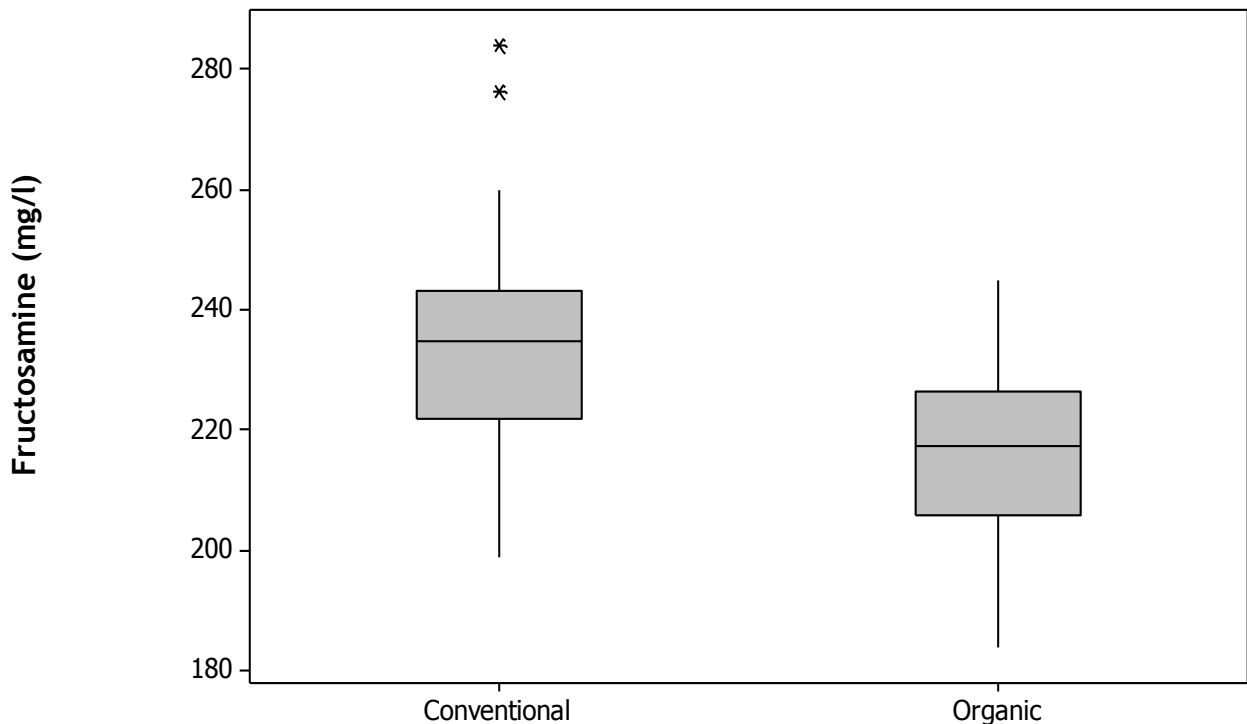


Figure 2.22. Plasma fructosamine on conventional and organic farms on visits 2, 3 and 4, during exposure to gastrointestinal parasites.

At visit 2 the difference between the fructosamine concentration in the conventional and organic calves was statistically significant ($p < 0.001$ ANOVA). The conventional calves had a mean value of 243.30 ± 16.93 mg/ml and the organic calves had a mean value of 211.19 ± 20.32 mg/ml. The liveweight gains between visits 1-2 were not significantly different however, between visits 2-3 they had become statistically significant ($p < 0.001$ ANOVA); conventional calves 1.37 ± 0.75 kg/day, organic calves 0.78 ± 0.45 kg/day. The Pearson's correlation of fructosamine concentration at visit 2 and liveweight gain between visits 2-3 was 0.17.

2.3.6.4 *Ostertagia ostertagi* Antibody

As illustrated in Figure 2.23, the *O. ostertagi* antibody ELISA ODR was not different in organic and conventionally farmed calves.

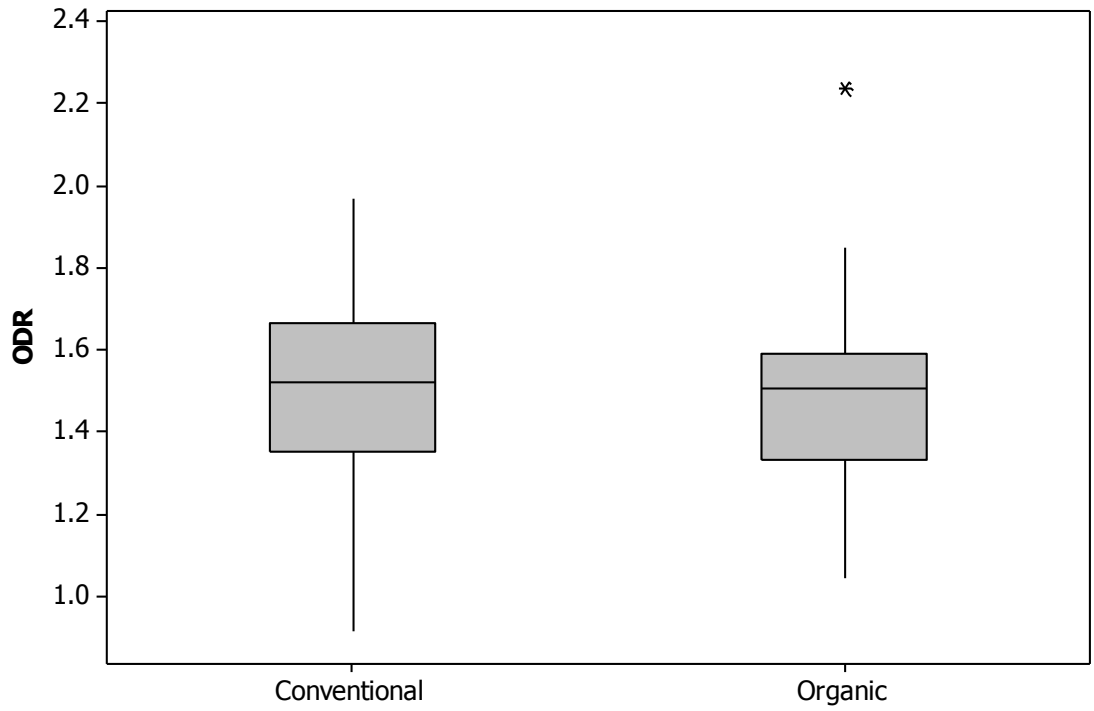


Figure 2.23. Plasma *O. ostertagi* antibody on conventional and organic farms on visits 2, 3 and 4 during exposure to gastrointestinal parasites.

2.3.6.5 Faecal Egg Counts

The organic farms had more calves with higher faecal egg counts in comparison with the conventionally farmed calves (Figure 2.24).

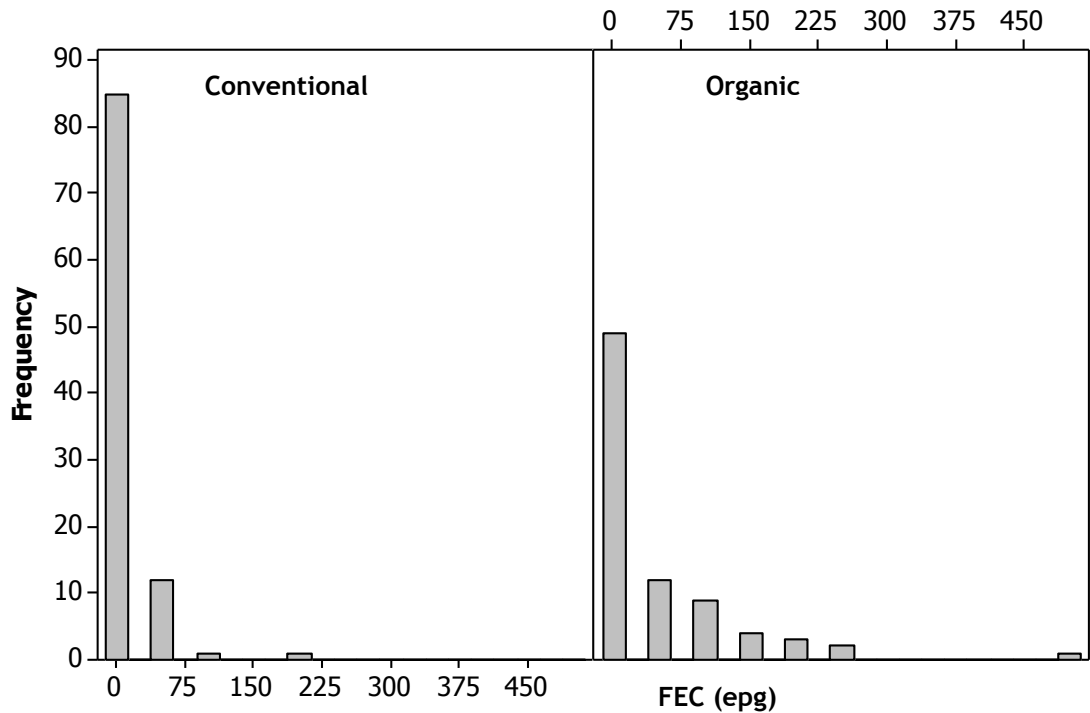


Figure 2.24. Histogram of FEC on conventional and organic farms on visits 2, 3 and 4 during exposure to gastrointestinal parasites.

2.4. DISCUSSION

The most notable difference between the FSG groups on each farm was that Farm O3 calves were significantly older and hence much larger in size than the FSG on the other farms. In fact you would expect these calves to have been second season grazers (SSG) as they were old enough to have grazed the previous year. Assurances were sought from the farmer that these animals had not grazed previously.

A clinical case of parasitic gastroenteritis and lungworm was presented on Farm O1, which had a calf euthanased due to poor liveweight gain and poor condition. On post-mortem, the major findings were gastrointestinal parasitism and lungworm. All calves on farm had been vaccinated with Huskvac™ (MSD) but as it is a live vaccination and adequate storage of vaccine is important, it was felt that all calves

should be treated for lungworm in case of vaccine failure. As the anthelmintic protocol on this farm was to treat calves with FEC of 250 epg and over, this calf had not been treated: the calf's FEC had always been less than 250 epg.

2.4.1 Liveweight Gain

Farm C3 showed the largest liveweight gain from turnout to housing in the FSG of 0.93 kg/day. It should be noted that Farm C3 was the only farm that did not include dairy replacement heifers in the FSG group. As beef breeds are selected for high liveweight gain it would be expected that these calves would grow well. Also, the calves on Farm C3 were not turned out onto pasture until the middle of July and had daily supplemental calf pellet feed prior to this. On the other farms in the study, turnout to pasture was on, or just after, visit 1 but turnout on Farm C3 was over twelve weeks after visit 1. Ideally, liveweight gains would have been calculated from as close to turnout as possible but this was not the case with Farm C3. Three out of the six farms achieved or surpassed the 0.75 kg/day target growth for heifers (Van Amburgh *et al.*, 1998; Wathes *et al.*, 2008); Farm C1, 0.77 ± 0.26 kg/day, Farm C3, 0.93 ± 0.10 kg/day, and Farm O3, 0.80 ± 0.22 kg/day. Farm C2 was close to this target; 0.72 ± 0.16 kg/day but Farms O1 and O2 produced much lower liveweight gains; 0.46 ± 0.23 kg/day and 0.57 ± 0.11 kg/day respectively. Farm O1 contained some smaller cattle breeds, such as Jersey crosses and therefore this farm's target growth rate to serve at 55-60% of body weight at fifteen months would be slightly lower than 0.75 kg/day.

When looking at organic and conventional FSG growth rates, the conventionally farmed FSG had significantly higher growth rates than the organically farmed calves. Farm O3 was included in this calculation and, with a liveweight gain of 0.80 kg/day on average over the grazing season, it increased the average liveweight gain for the organic farms. As mentioned previously, the animals on this farm were significantly older than the animals in the other groups and there was concern that these calves could have grazed the previous year. Excluding Farm O3 for the moment, the average liveweight gain between Farm O1 and Farm O2 was 0.52 kg/day, which is also significantly different from the conventional farms. Even with the inclusion of Farm O3, the organically farmed calves did not reach the targeted liveweight gain of 0.75

kg/day in order to calve at the optimum age of twenty-three to twenty-four months of age (Wathes *et al.*, 2008). Although all of the organic FSG were treated with anthelmintic mid-grazing season their liveweight gains did not recover to what they should have been. There is evidence that a milking animals' longevity within a herd may be longer if she can calve at two years of age (Wathes *et al.*, 2008). It is concerning for organic dairy farmers if they cannot reach this target as longevity within the milking herd is surely part of the organic ethos.

2.4.2 Plasma Pepsinogen

The Scottish Agricultural College (SAC) laboratory give guidelines to veterinary surgeons in practice for interpretation of the plasma pepsinogen concentration as follows: over 0.4 iu/l indicates the presence of abomasal parasitism but is unlikely to impact on liveweight gain; over 1.5 iu/l indicates abomasal parasitism likely to impact on liveweight gain; over 2.5 iu/l indicates abomasal parasitism likely to be associated with clinical disease. Plasma pepsinogen in grazing youngstock is inferred to indicate ingestion of infective *Ostertagia ostertagi* larvae from pasture and the emergence of L₄ in the abomasum of the calves, causing an immune reaction. At visit 1 all animals had not previously grazed pasture. From the SAC guidelines, a plasma pepsinogen concentration below 0.4 iu/l would be expected for all animals at this stage. However, only Farms C1 and O2 have average pepsinogen concentrations under this at visit 1; 0.39 ± 0.11 iu/l and 0.35 ± 0.11 iu/l respectively. Farm C2 has a value slightly higher than expected at 0.46 ± 0.20 iu/l. Farms O1, O3 and C3 have much higher than expected concentration at 0.62 ± 0.25 iu/l, 0.69 ± 0.31 iu/l and 0.57 ± 0.25 iu/l respectively. As a mean concentration is taken, an explanation for this could be an outlier result skewing the average on these farms or the possibility that the calves had been previously exposed to *O. ostertagi* and re-emergence of EL₄ was occurring. Figure 2.11 illustrates histograms of plasma pepsinogen values on each farm. On visit 1, farms O1 and O3 both have outliers but Farm O1 only had three calves with a value under 0.4 iu/l and Farm O3 had no calves under 0.4 iu/l and so an outlier result skewing the mean calculation cannot adequately explain the results. The calves on Farm O1 were born between 01/06/2008 and 17/12/2008. The calves born over the summer months in 2008 would have been born at pasture but removed from the dam within twenty-four hours and brought indoors to be reared. The farmer was certain

that these calves did not graze in 2008. The calves on Farm O3 were born between 08/01/2008 and 26/04/2008. These calves were born indoors and again the farmer was certain that these calves did not graze during 2008, although it would be normal practice to graze animals at this age, particularly on organic farms. The calves on Farm C3 were born between 23/10/2008 and 31/01/2009. It is not possible that these calves grazed before the 2009 grazing season. Laboratory variation is a valid explanation for the results at visit 1: the author has to assume all animals had not grazed previously, and these results are used as the baseline. Blood sample tubes were spoiled at visit 3 on farms O2 and O3; however, with extrapolation of the results from farms C1, C2 and O1 it would be expected that on visit 3, plasma pepsinogen concentrations on these farms were also higher than the levels at visit 2 and visit 4.

The general trendline on the correlation between plasma pepsinogen concentration and liveweight gain (Figure 2.12), weakly suggests that as plasma pepsinogen concentration in peripheral blood increases, liveweight gain decreases. However, there is too much individual animal variation and too weak an association to be able to use plasma pepsinogen in individual animals in order to target anthelmintic treatment. Furthermore, different laboratories can vary widely in pepsinogen concentrations making it more difficult to adopt a general recommendation on concentration and treatment (Charlier *et al.*, 2011).

Anthelmintic treatment was applied to the organic calves in the study when the calves were known to have a gastrointestinal parasite infection from positive faecal egg counts. Farm O2 treated calves in July (only 3/15) and Farms O1 and O3 did not treat until September. At this point in time, pasture would be expected to be heavily infected with trichostrongyle larvae. Calves continued to graze and ingest large amounts of larvae that emerge in the abomasum causing an immune reaction and high plasma pepsinogen concentrations in blood. The conventional FSG would not have experienced the same contamination of pasture due to prophylactic treatment, hence the lower plasma pepsinogen concentrations.

2.4.3 Fructosamine

As fructosamine concentration in peripheral blood varies according to long-term glycaemic status and/or protein metabolism, parasitic gastroenteritis, causing a relative protein deficiency, is expected to reduce fructosamine concentration in peripheral blood. Fructosamine values on all farms fell between visits 1 and 2. Diet change from conserved forage and concentrates to grass is likely to have had some effect on this fall. Plasma fructosamine levels fell again between visits 2 and 3 while animals were on the same pasture, which may indicate that the FSG were experiencing a higher protein turnover and relative protein deficiency due to gastrointestinal parasitism. It would also be expected, however, that grass quality would decrease at this time of the grazing season. By housing, fructosamine concentrations are beginning to recover, even as grass quality would have been poorer than mid-grazing season, suggesting further that gastrointestinal parasites were the main cause of the fructosamine fluctuations. Fructosamine concentrations were lowest and plasma pepsinogen concentrations highest at visit 3 on all farms (no values for Farms O2 and O3 at visit 3).

When fructosamine concentration and liveweight gain were correlated, the trendline showed a weak trend towards higher liveweight gains in animals with higher fructosamine values. The variation between individual animals and liveweight gain is too large and the correlation too weak to use as an individual animal marker to target anthelmintic treatment. Previous studies have indicated that fructosamine concentrations may predict worm burdens in lambs (Stear *et al.*, 2001). A significant difference between the mean fructosamine concentration between the organic and conventional calves at visit 2, before significant differences in liveweight gains were seen, is noteworthy. However, the Pearson's correlation between fructosamine concentration at visit 2 and liveweight gain between visits 2-3 showed only a weak positive association (0.17). There was no correlation between fructosamine concentration early in the grazing season and subsequent overall liveweight gain in this study. It should be noted although that worm burdens were not counted.

The lower fructosamine concentrations seen in the organic calves are likely caused by gastrointestinal parasites producing a relative protein deficiency and

increasing protein turnover given the plasma pepsinogen, liveweight gain and faecal egg count results.

2.4.4 *Ostertagia ostertagi* Antibody

All samples were duplicated and some quadrupled to account for inter-assay variation and the average of the results recorded. Different spectrophotometers were used between assay 1 and 2, 3, and 4 due to mechanical failure of spectrophotometer 1. The organic calves appeared to have a greater variation of ODR values between individual animals at turnout. There is no obvious explanation for this, and due to the small sample size, no conclusions can be drawn. The ODR increased in all of the animals at pasture showing exposure and antibody response to gastrointestinal larvae. The variation between individual animals and liveweight gain is too large to use as an individual animal marker to target anthelmintic treatment. Furthermore, the ELISA used is based on a crude antigen from adult *O. ostertagi* and can cross-react with antibodies against other helminths, and this is particularly true when serum and plasma are used (Charlier, personal communication).

All samples on all farms recorded optical density ratios (ODR) high enough to impact on milk yield in adult cattle (Charlier *et al.*, 2005ii). Farm C3 recorded the highest ODRs. Farm C3 subjected FSG calves to the greatest amount of days of anthelmintic treatment (Table 2.10) and grazed their FSG for the least amount of time. Larson *et al.* (2010) noted higher cumulative FEC in SSG that had been subjected to high levels of anthelmintic in the first grazing season. It is possible that as the SSG are not treated with anthelmintic, they contaminate pasture subsequently grazed by milking cows. The ODR increased at visit 2, indicating that the FSG produced antibodies to gastrointestinal nematodes over the grazing season despite the moxidectin injection. Immunity to *O. ostertagi* takes two grazing seasons to become effective (Armour, 1980; Armour, 1989) and so it's possible that exposure of the FSG and possibly SSG to gastrointestinal parasites may be inadequate, placing heifers in the milking herd that still require some immune development. It is possible that this level of antibody response on Farm C3 could be reducing milk production by 2 kg/cow/day (Charlier *et al.*, 2005ii; Charlier *et al.*, 2007).

2.4.5 Faecal Egg Count

The variation in FEC distribution between animals in a group has been described in Chapter One. As expected from the literature, the majority of calves within a study group shed a small number of eggs and a small number of calves within the group shed a larger proportion of eggs (Leighton *et al.*, 1989). Veterinary surgeons in practice are advised by laboratories to advise treatment of animals with a pooled FEC of 250 epg and over (Taylor, 2010i).

No farm in the study at any visit had a mean FEC of 250 epg and over (Figure 2.17). Visit 2 was planned to fall between five and ten weeks after turnout. This time point gives the best prediction, from FEC, of larval ingestion over the previous five to ten weeks and thus the likelihood of parasitic gastroenteritis when no anthelmintics have been applied and animals are set-stocked (Eysker and Ploeger, 2000). Farm O2 recorded no trichostrongyle eggs in faeces at visit 2. The calves on this farm were extensively grazed on three large fields and rotated from one field to another two weeks before visit 2. It is possible that the first field grazed early in the grazing season had small numbers of overwintered larvae and the subsequent field contained higher numbers as the farmer felt that the FSG were not growing as well as expected two to three weeks after visit 2. A pooled faecal sample analysed for trichostrongyle eggs at this point produced a mean FEC of 350 epg and all calves were subsequently treated with fenbendazole. Farm O3 had a surprisingly low FEC throughout the grazing season for FSG animals given no prophylactic anthelmintic treatment. Age-related immunity to *Cooperia oncophora* could be the reason for this as the FSG were much older than one would normally expect and, as *Cooperia oncophora* is more fecund than *Ostertagia ostertagi* (Armour *et al.*, 1987), lower FEC would be expected with only *O. ostertagi* infection. Plasma pepsinogen levels in this group were high throughout the grazing season implying ingestion of *O. ostertagi* larvae and an immune response in the abomasum. It is postulated that the immune system in these animals was effective in preventing the next steps in the parasite life-cycle of the production of eggs by adult worms. As animals are not considered to be functionally immune to *O. ostertagi* until two grazing seasons of exposure (Armour, 1980; Armour, 1989) it is surprising that these animals produced such small numbers of trichostrongyle eggs in their first grazing season. It is possible that by random

selection the calves in this group happened to grow well despite gastrointestinal nematode challenge (Leighton *et al.*, 1989) or that this group had, in fact, grazed last season. This group showed similar parasitological parameters to SSG calves (Ellis *et al.*, 2011).

When FEC were correlated with liveweight gain, a weak trendline indicated that higher liveweight gains are achieved at lower FEC, but the variation within individuals is too large and the correlation too weak to use FEC as an individual animal marker to target anthelmintic treatment. The Spearman's rank correlation showed a negative correlation (-0.52); however, there were many FECs of zero which would skew the ranking used in the calculation. Recently, McNulty and Greer (2011, unpublished data) also reported the lack of correlation between FEC and liveweight gain. The results suggest that the emphasis on using FEC in targeted and targeted selective anthelmintic treatment (Taylor, 2010i; Soil Association, 2010) may need to be addressed.

2.4.6 Larval Culture

Laboratory access meant that larval cultures were only performed late in the grazing season, which meant that only three farms (C1, C2 and O2) had larval culture performed. Late in the grazing season it would be expected that *O. ostertagi* would contribute highly to a larval count (Hertzberg *et al.*, 1992) as shown on Farm O2. Farms C1 and C2 however, recorded only *C. oncophora* larvae but in small numbers. Farm C2 had reported a problem with gastrointestinal parasitism in the first season grazers the previous year using the same anthelmintic regime (DectomaxTM pour-on, Elanco). It could be that the pour-on was stored or administered inadequately or that the dose was underestimated. It may be that the anthelmintic was subject to environmental conditions in 2008, which produced subtherapeutic plasma concentrations (Gokbulut *et al.*, 2012). It is possible that the presence of *Cooperia* spp. at the end of the grazing season may indicate underexposure of female nematodes within the host, and this situation has been shown experimentally to select for anthelmintic resistance (Van Zeveren *et al.*, 2007). However, as *C. oncophora* is more fecund than *O. ostertagi* (Armour *et al.*, 1987), and at this time of year *O. ostertagi* encysts in the abomasum rather than continuing the life cycle to

egg-producing adult, (Armour, 1970) and as similar results were shown on Farm C1, which does not use a pour-on anthelmintic preparation, it is likely that these are the reasons that only *C. oncophora* larvae were seen at this time of year.

2.5. CONCLUSION

The organically farmed first season grazers in this study had high gastrointestinal parasite challenge, indicated by parasite-based markers such as FEC and plasma pepsinogen concentration. The conventional producers in this study exposed FSG to 652% more days of anthelmintic than the organic producers and gained superior liveweight gains over the grazing season. Essentially, the organic producers fulfilled the ethos of organic production, reducing anthelmintic usage and showing necessity of anthelmintic treatment. However, if these animals grow less well over the grazing season due to gastrointestinal parasitism, they are unproductive for longer as they start to produce milk at an older age. An age at first calving over 25 - 26 months appears to affect subsequent fertility and longevity within a milking herd (Wathes *et al.*, 2008). Furthermore, subclinical and clinical parasitic gastroenteritis impacts on animal welfare, the essence of the organic ethos. The organic industry needs to investigate whether there is a superior alternative to FEC that still promotes the organic ethos and reduces subclinical and clinical parasitic gastroenteritis.

None of the biomarkers investigated in the study reflected liveweight gain adequately to use in a targeted selective anthelmintic treatment programme. An ideal biomarker would give indication of calves that would most benefit from anthelmintic treatment before liveweight gain was affected. The biomarkers in this study indicated presence of gastrointestinal parasitism but could not target the animals that had poor liveweight gains. Liveweight gain has been used successfully in performance-based targeted selective treatment (TST) programmes in small ruminants (Leathwick *et al.*, 2006i; Leathwick *et al.*, 2006ii; Gaba *et al.*, 2010; Stafford *et al.*, 2009; Greer *et al.*, 2009; Busin *et al.*, 2013). This approach works well in the sheep industry for the numerous reasons summarised in Table 2.13. However, is this approach appropriate in the cattle industry? For many reasons, also listed in Table 2.13, performance-based TST in first season grazers (FSG) may be challenging to implement and minimal published literature is available on the use of

performance-based TST in first season grazing calves (Greer *et al.*, 2010; McNulty *et al.*, 2011; Höglund *et al.*, 2011).

An instant crush-side decision regarding anthelmintic treatment using liveweight gain is an advantage over faecal egg counts, which take time - even when done on farm - to produce a result. Furthermore, this thesis and others (McNulty and Greer, 2011) have challenged the value of using faecal egg counts in targeted treatment approaches and have demonstrated that faecal egg counts bear no relationship to liveweight gain (McNulty and Greer, 2011b) thus, using solely faecal egg counts to target individual animals is likely to miss animals that require treatment. A major disadvantage of the performance-based TST strategy in cattle is the need for handling animals during the grazing season, which can be labour intensive and on some farms impractical. However, many dairy farmers are now realising the true value of their dairy replacements and recognise that dairy replacements need to be healthy, well-grown individuals with good fertility that are well-equipped to join the herd at first calving, and therefore some farmers may be willing to handle animals more during the grazing season.

Education of farmers, industry advisors, organic associations and veterinarians of anthelmintic resistance and treatment failure in cattle gastrointestinal parasites by COWS should make the industry look for alternative anthelmintic programmes in FSG, and appropriate research needs to have been done in order to make good recommendations. As most farmers do not have weigh-scales, if liveweight gain is to be used as a threshold for anthelmintic treatment then an alternative, cheap, accurate, repeatable and easy to use method of measuring liveweight must be available. Five of the six farms in this study did not have weigh-scales and so a weigh-band was used to estimate liveweight. This method of measuring liveweight gain is discussed in Chapter Three.

Sheep	Cattle
<p>Many farmers have weigh-scales and electronic ear-tag identification.</p>	<p>Most farmers do not have weigh-scales and electronic ear-tag identification.</p>
<p>Farmers are used to gathering animals for anthelmintic drenching over the grazing period.</p>	<p>Farmers opt for minimal handling and ease of anthelmintic administration in FSG anthelmintic choice.</p>
<p>Lambs are generally born at the same time of year and are generally of similar size to one another.</p>	<p>Calving is seasonal on some farms but many farms calve year round. FSG animals tend to be varied in age and size.</p>
<p>Resistance to anthelmintic classes is already widespread (Jackson and Coop, 2000; Wolstenholme <i>et al.</i>, 2004; Kaplan, 2004).</p>	<p>Resistance to anthelmintic classes is largely thought not to be a problem within the UK cattle industry.</p>
<p>The Sustainable Control of Parasites in Sheep” (SCOPS) provides guidelines for sheep producers on best practice for anthelmintic use on farm and encourage performance-based TST programmes (Abbot <i>et al.</i>, 2009).</p>	<p>The Control of Worms Sustainably in Cattle (COWS) promotes best practice with regards to anthelmintic usage on farm. Currently, faecal egg counts are recommended to use in targeted treatment programmes (Taylor, 2010i; Taylor, 2010ii).</p>
<p>Instant, crush-side anthelmintic decision possible. No need to wait for laboratory results and gather animals again for treatment.</p>	<p>Instant, crush-side anthelmintic decision possible. No need to wait for laboratory results and gather animals again for treatment.</p>

Table 2.13. Factors affecting implementation of a performance-based TST programme in the sheep and cattle industries in the UK. FSG: First season grazer(s).

CHAPTER THREE

CHAPTER THREE: VALIDATION OF THE WEIGH-BAND IN ESTIMATING LIVWEIGHT GAIN IN A PERFORMANCE-BASED TARGETED SELECTIVE ANTHELMINTIC TREATMENT PROGRAMME IN FIRST SEASON GRAZING CATTLE IN THE UK

3.1 INTRODUCTION

An accurate assessment of liveweight is necessary if calf liveweight gain is to be calculated accurately and used as a threshold for anthelmintic treatment. Cattle weigh-scales are expensive and often not available on farm, particularly where youngstock may be grazing at pasture and gathered in the field for handling. With this in mind, cattle weigh-bands, which measure heart girth and relate this to liveweight, have been devised and used in practice in order to estimate cattle liveweight. The few studies published investigating targeted selective anthelmintic treatment (TST) using liveweight gain in cattle, have used weigh-scales that may not always be available in the field (Greer *et al.*, 2010; McAnulty *et al.*, 2011; Höglund *et al.*, 2011). Realistically, if a liveweight gain threshold were to be recommended for use on farms in the UK, the weigh-band must estimate liveweight and hence liveweight gain accurately.

3.1.1 Review of the Literature

Numerous indirect measurements of cattle have been investigated in order to ascertain which best equates to actual liveweight (Johansson and Hildeman, 1954; Enevoldsen and Kristensen, 1997; Heinrichs *et al.*, 1992; Dingwell *et al.*, 2006). Heinrichs *et al.* (1992) concluded that in management situations where liveweight cannot be measured, other traits, such as heart-girth, can be used to estimate liveweight accurately. Prediction equations for liveweight have used heart-girth more often than other body dimensions because this measurement has exhibited the strongest correlation to liveweight (Heinrichs *et al.*, 2007). This positive relationship has persisted from very early work; however, the regression equations have changed

over time (Heinrichs *et al.*, 1992; Heinrichs *et al.*, 2007). An accurate and precise equation to estimate liveweight from heart girth measurements is needed if farmers are to utilise estimated liveweight successfully. The regression of heart girth on liveweight had the highest R^2 value (>0.95) compared with any other body trait in the study by Heinrichs *et al.* (1992). The regression of heart girth and liveweight only prove an association between the two, which would be expected as an animal's girth increases with age and therefore liveweight. The objective of Otte *et al.* (1992) was to investigate the agreement between liveweight and liveweight estimation from heart-girth measurement, rather than just the association between the two, in order to understand in which circumstances the two could be used interchangeably. Not surprisingly, the paper found that liveweight estimation by weigh-band is less consistent than by the weigh-scale method; however, in young animals ($<200\text{kg}$) the repeatability is better. Again, unsurprisingly the inter-observer differences are likely to be larger in liveweight estimation by weigh-band than by weigh-scale. The most pertinent findings by the authors were that the weigh-band could be a viable alternative to a weigh-scale in trials where liveweight is the dependent variable (Otte *et al.*, 1992). It was concluded that a weigh-band could give an accurate assessment of the mean liveweight of a group of cattle; however, the estimate of the variance of the groups would be larger than when estimated by a method with a smaller amount of measurement error.

Heinrichs *et al.* (1992) developed a modified equation to determine liveweight from heart-girth for Holstein heifers in the United States of America. In the study, data were collected from several hundred heifers repeatedly over time from birth to twenty-four months of age, and equations were developed. The equation developed is shown below:

$$\text{LW (kg)} = b_0 + b_1\text{HG} + b_2\text{HG}^2 + b_3\text{HG}^3 + e$$

where LW, Liveweight (kg); HG, Heart-girth (cm); b_0 , intercept; b_1 , b_2 , b_3 , regression coefficients; and e , residual. The equation is:

$$\text{LW (kg)} = 65.36 - (1.966 \times \text{HG}) + (0.01959 \times \text{HG}^2) + (0.00001691 \times \text{HG}^3)$$

Heinrichs *et al.* (2007) recently questioned the equation's relevance and accuracy on modern dairy heifers in the USA. However, the authors concluded that prediction of liveweight from heart-girth measurements obtained from a weigh-band were accurate compared to actual liveweight and highly repeatable for multiple measurements by one person or measurement by many individuals. In addition, the equation published by Heinrichs *et al.* (1992) was found to predict Holstein liveweight from heart-girth satisfactorily for a variety of heifers and herd management decisions, particularly for animals with a liveweight >150 kg. The equation does not account for different body condition scores, which the weigh-band used in this study does take into account.

This study aimed to evaluate the use and accuracy of the weigh-band when estimating liveweight in youngstock in the UK, when compared with the gold-standard weigh-scales.

3.2 MATERIALS AND METHODS

Animals were weighed at The University of Glasgow Veterinary School and on Farm C3 using digital weigh-scales (IAE) and mechanical weigh-scales (Ritchie[®], spring balance model 327G), respectively. Each animal was body condition scored (BCS) (1-5 point scale with increments of 0.5) and a heart-girth measurement was recorded in centimetres. This method consists of placing a measuring tape around the circumference of the animal just behind the withers as shown in Figure 3.3. A weigh-band was used that is readily accessible to farmers and easy to use, in order to replicate the true ability of on-farm predictive liveweight estimation. The weigh-band used in the study was a Coburn[®] tape for estimating liveweights of beef cattle (The Coburn Company, Inc.). A predicted liveweight, adjusted for BCS, is given on the tape for each girth measurement. Body condition is categorised into four categories on the tape and the liveweight estimate adjusted for each: 1) Thin, no finish, 2) Moderate, poorly finished, 3) Fleishy, properly finished, 4) Very fleishy, over finished. The Standard five point BCS scale was applied to these categories as follows; BCS 1 = weigh-tape category 1; BCS 1.5 = average of weight at weigh-tape category 1 and 2; BCS 2 = weigh-tape category 2; BCS 2.5 = average of weight at weigh-tape category 2 and 3; BCS 3 = weigh-tape category 3; BCS 3.5 = average of weight at weigh-tape

category 3 and 4; BCS 4 and BCS 4.5 = weigh-tape category 4 and, as a BCS of 5 in growing dairy cattle is unlikely, this category was not included. Whenever possible, the same individual measured heart-girth and BCS.

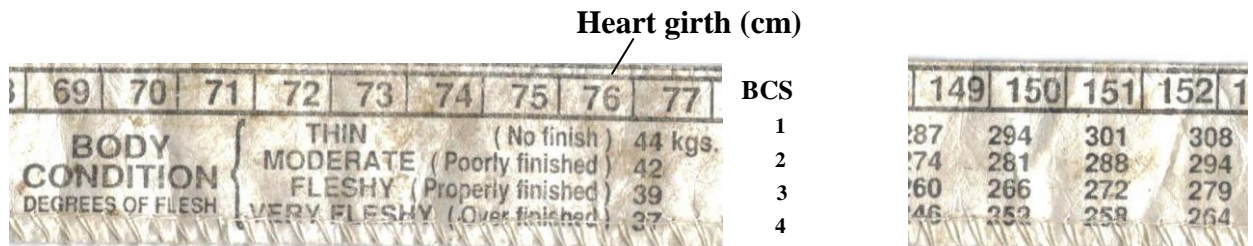


Figure 3.1 Coburn® tape for estimating liveweights of beef cattle (The Coburn Company, Inc.) illustrating weights from heart girth (cm) used for different body condition scores (BCS).

3.2.1 Experimental Animals

The study animals were cattle present on Farm C3 over two years (n=236) and those present at The University of Glasgow Veterinary School over one year (n=18) that fell into the expected age category for a first season grazing animal (6 weeks to 20 months of age). Dairy, dairy x beef and beef animals were included in the study and some animals repeatedly sampled at different ages. Animals on Farm C3 were in good health. Animals at The University of Glasgow Veterinary School were present at the university for numerous reasons including ill-health.



Figure 3.2 Ritchie weigh crush on Farm C3.



Figure 3.3 Heart girth weigh band on Farm C3.

3.2.2 Statistical Analysis

Correlation and agreement between: 1) liveweight and weigh-band estimated liveweight, 2) weigh-band estimated liveweight and calculated liveweight from Heinrichs *et al.* (1992) and 3) liveweight and calculated liveweight from Heinrichs *et al.* (1992) was undertaken using a Pearson Correlation Test (Minitab) and a Bland and Altman plot (Microsoft Excel), respectively. As agreement is not measured by regression or correlation, a Bland and Altman plot was used to plot the difference between weigh-band and weigh-scale liveweights against the mean of the measurements (Bland and Altman, 1999). The mean of the two measurements is used in order to avoid the artefact that occurs when the difference between two measurements is plotted against the value of one of the individual measurements (Rothwell, 2000).

3.3 RESULTS

Table 3.1 shows the estimated liveweight from heart-girth measurements and liveweights from weigh-scales for animals on both farms. The differences (weigh-scale

minus weigh-band) ranged from -56 to -14 kg in animals at The University of Glasgow Veterinary School (n=18) and -119 to 47 kg in animals on Farm C3 (n = 236). On average, the weigh-band estimate was higher by 22.8 kg. Heinrichs *et al.* (2007) showed an 8% difference in weigh-band to weigh-scale liveweight thus, the estimated liveweight of a heavier animal would have a larger variation in kilograms than a lighter animal. Because of this, the data analysis was carried out on a \log_{10} scale, which more easily allows for the assessment of relative differences. On the \log_{10} transformed data the weigh-band estimate exceeded the weigh-scale reading by an average of 0.033 (antilog 0.93) i.e. the liveweight estimate by tape was 7% higher on average than the respective estimate obtained by the scales.

	Mean ±SD	Min	Max	Log Mean ±SD	Log min	Log max
Weigh-band (kg)	316 ± 83.8	140	543	2.48 ± 0.12	2.15	2.73
Weigh-scale (kg)	294 ± 79.5	120	535	2.45 ± 0.12	2.07	2.73
Difference between weigh-scale and weigh-band (kg)	-22.8 ± 30.6	-119	47	-0.033 ± 0.043	-0.15	0.058

Table 3.1. Mean liveweight for weigh-scale and weigh-band measurements.

3.3.1 Weigh-scale and Weigh-band

3.3.1.1 Correlation

A regression analysis between liveweight using the weigh-band or weigh-scales on both farms (n=254) is shown in Figure 3.4.

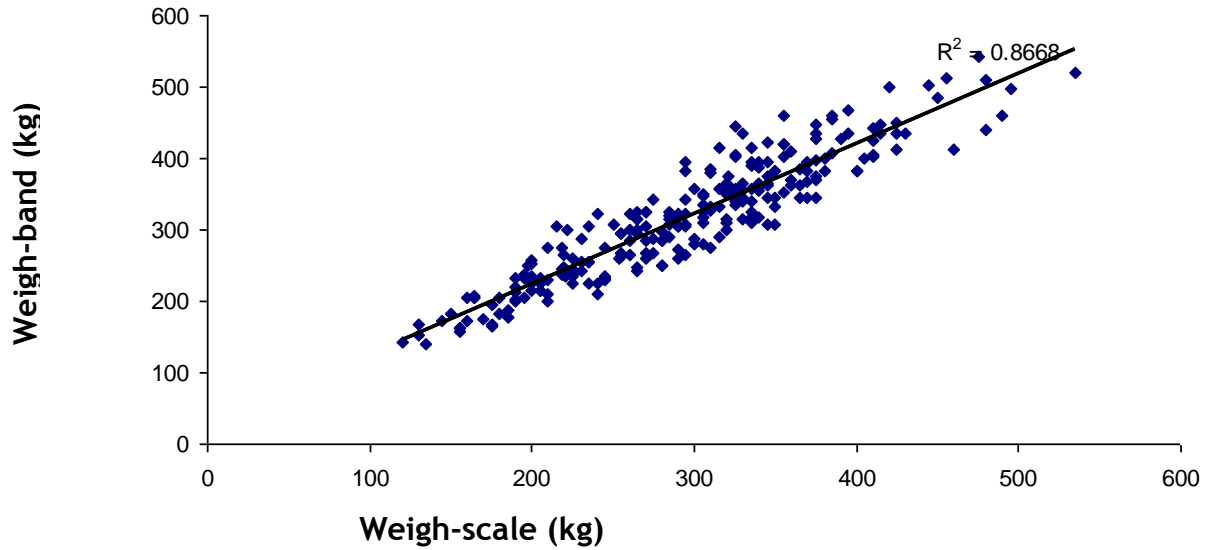


Figure 3.4. Regression of weigh-scale and weigh-band liveweights on Farm C3 and at The University of Glasgow Veterinary School (n=254).

A strong correlation between the weigh-band and weigh-scale measurements is demonstrated. The Pearson's correlation test was $r = 0.92$.

3.3.1.2 Agreement

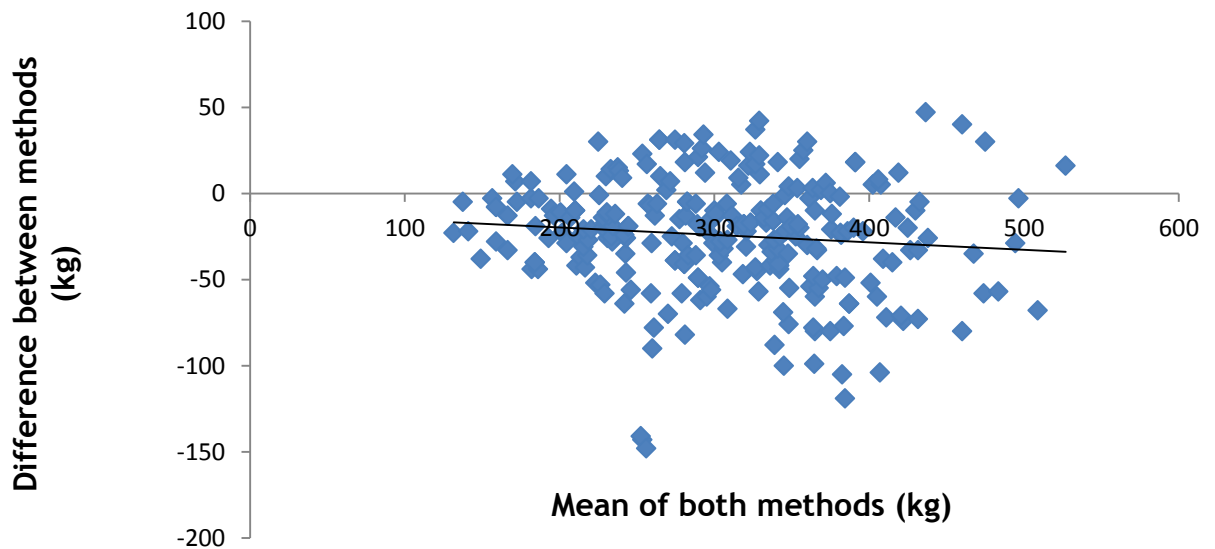


Figure 3.5. Bland and Altman plot of the liveweight of animals (n = 254) obtained by weigh-scale or weigh-band. Slope = -0.0432 ($P=0.10$ ANOVA). Intercept = -11.0 ($P=0.17$ ANOVA).

The plot in Figure 3.5 shows that the weigh-scale liveweight was constantly lower than the weigh-band liveweight at all liveweights. The plot also demonstrates that at extremes of liveweight the correlation is consistent, although there is a weak trend at higher liveweights for more variation in the association between the two methods (smooth black line).

3.3.2 Weigh-band and Calculated Liveweight using Heinrichs' Method

Out of interest, the weigh-band liveweight and liveweight calculated from the formula in Heinrichs *et al.* (1992) were compared using data from all animals on all farms on both years of the study (2009 and 2010) and animals at The University of Glasgow.

3.3.2.1 Correlation

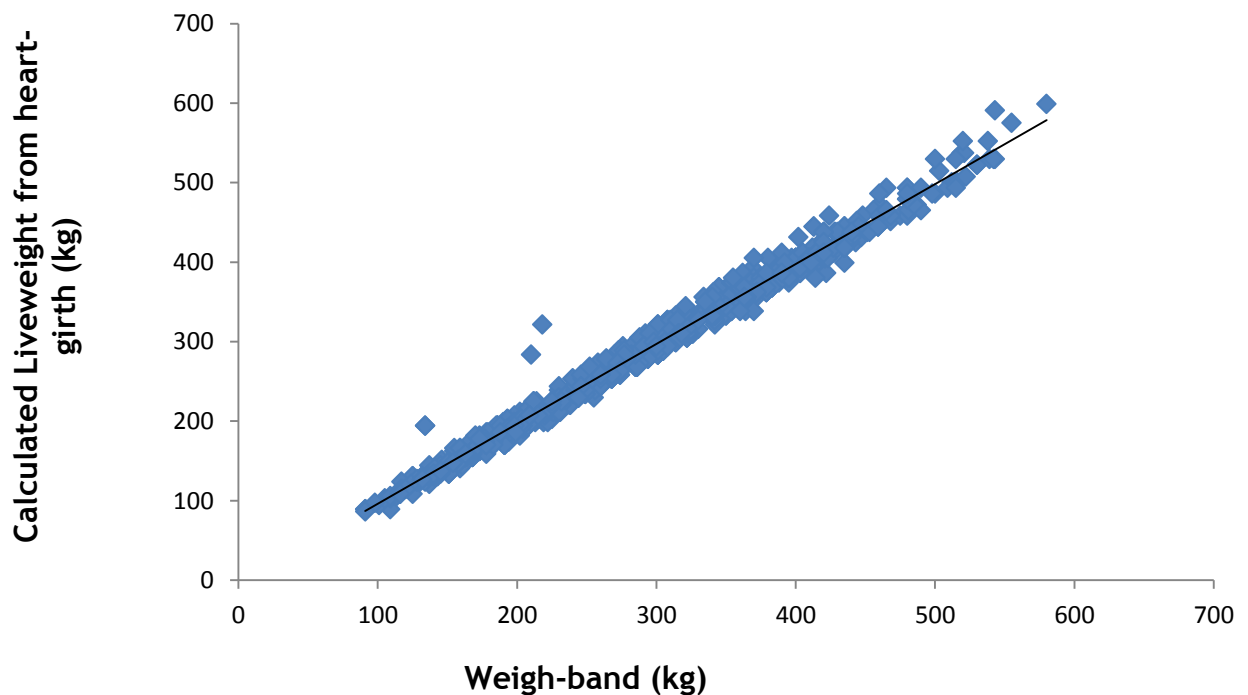


Figure 3.6. Regression of weigh-band and calculation of liveweight from heart-girth using Heinrichs *et al.* (1992) formula on all animals on all farms in the study in Chapters Two and Five and at The University of Glasgow.

There is good correlation between the two values. The Pearson's correlation was $r = 0.99$.

3.3.2.2 Agreement

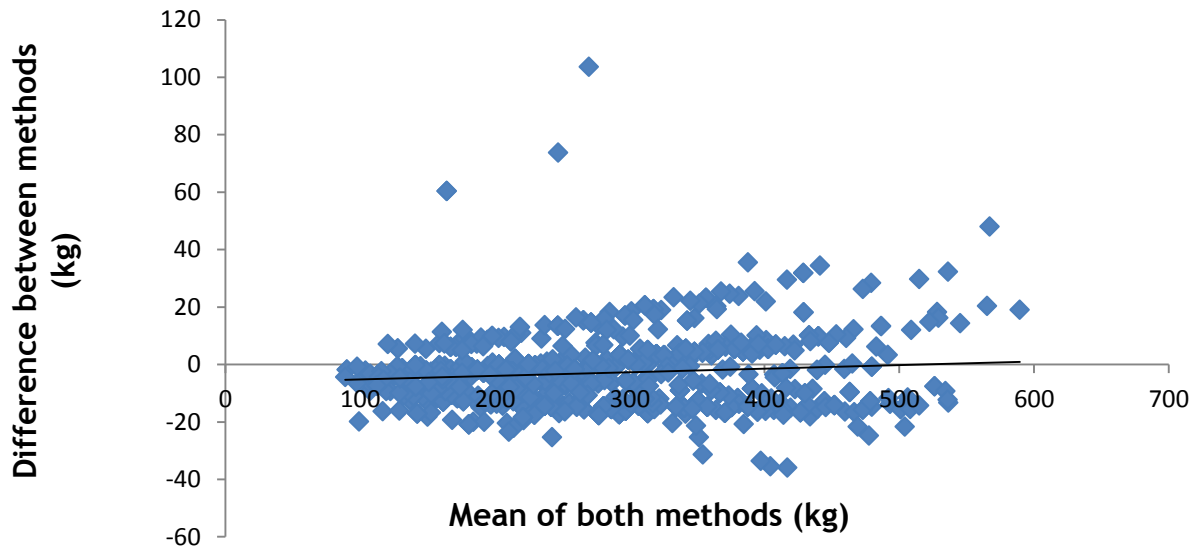


Figure 3.7. Bland and Altman plot of the liveweight of animals obtained by calculation from Heinrichs *et al.* (1992) or weigh-band. Slope = 0.012 ($P < 0.01$ ANOVA). Intercept = -6.508 ($P < 0.01$ ANOVA).

The two methods of estimating liveweight using heart-girth produced very similar estimations of liveweight. Given the close agreement and correlation between the weigh-band and the formula, it is assumed that the weigh-band uses the formula (Heinrichs *et al.*, 1992) in its calculation of liveweight but adjusts for body condition score also.

3.3.3 Weigh-scale and Calculated Liveweight using Heinrich's Method

Again out of interest, the weigh-scale liveweight and liveweight calculated from the formula in Heinrichs *et al.* (1992) were compared using the same data set as in 3.3.1. The calculation from the formula does not take into account body condition score, unlike the weigh-band.

3.3.3.1 Correlation

A regression analysis was performed using a liveweight calculated directly from the formula in Heinrichs *et al.* (1992) (not directly used in the study) and true liveweight with the weigh-scale (Figure 3.8). The Pearson's correlation was $r = 0.95$.

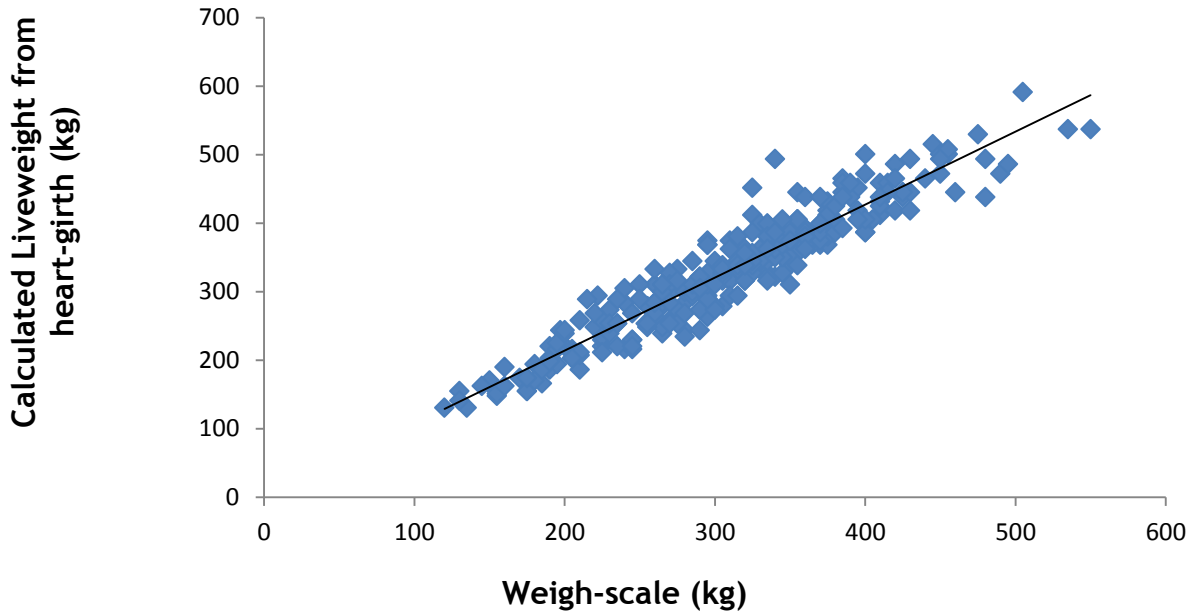


Figure 3.8. Regression of weigh-scale and calculation of liveweight from heart-girth using Heinrichs *et al.* (1992) formula on all animals on Farm C3 in the study in Chapters Two and Five and animals at the University of Glasgow (n = 254).

3.3.3.2 Agreement

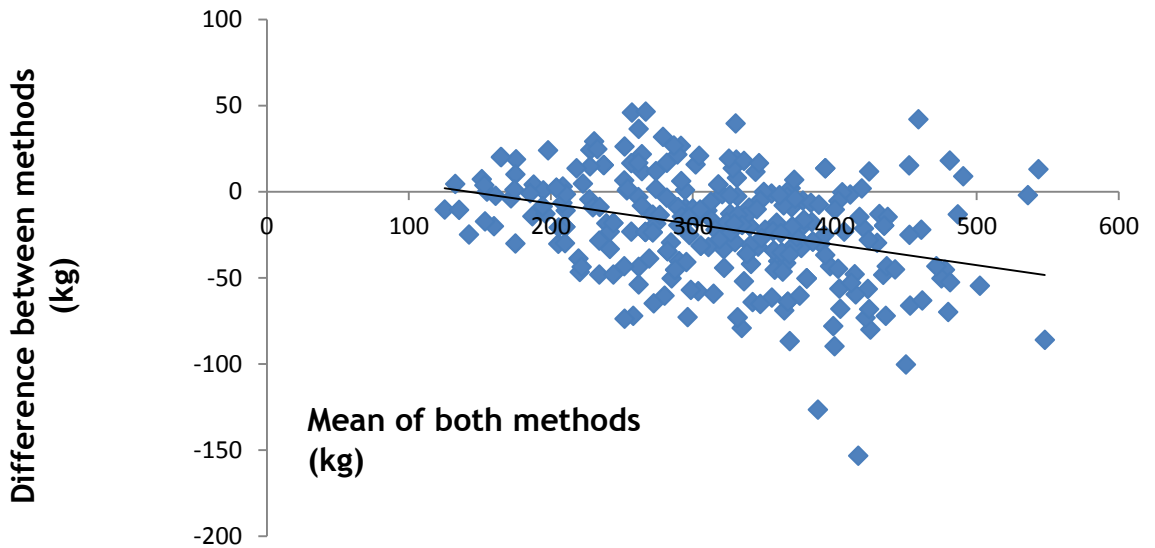


Figure 3.9. Bland and Altman plot of the liveweight of animals obtained by calculation from Heinrichs *et al.* (1992) or weigh-scale. Slope = -0.2370 (P<0.01 ANOVA). Intercept = 17.357 (P<0.01 ANOVA).

The plot in Figure 3.9 shows that the calculated liveweight using Heinrich’s equation overestimated liveweights when compared to weigh-scale measurements at higher liveweights.

3.3.4 Effect of BCS on Weigh-band and Weigh-scale Correlation

Regression analyses (Figure 3.10) were performed for different body condition scores (BCS on a 5 point scale using 0.5 increments) in order to assess the association between weigh-scale and weigh-band liveweight on thin or fat animals. The weigh-band would adjust the estimated liveweight for different BCS but the calculation in Heinrichs *et al.* (1992) would not account for this. No animals in the study were deemed to have a BCS of 4.5 or over. Body condition score of the animals did not appear to significantly affect the correlation between weigh-band and weigh-scale.

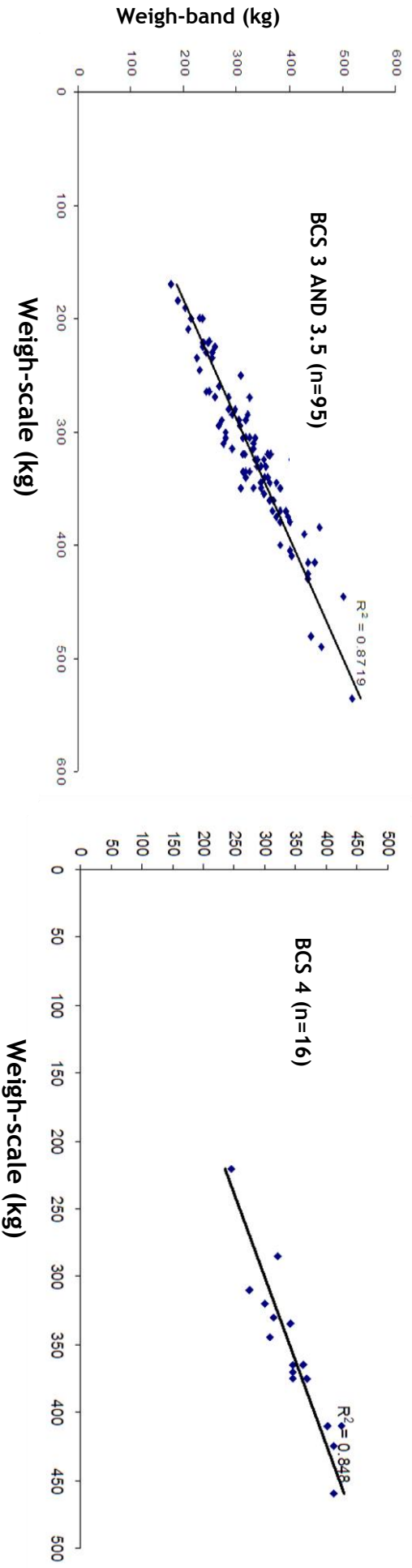
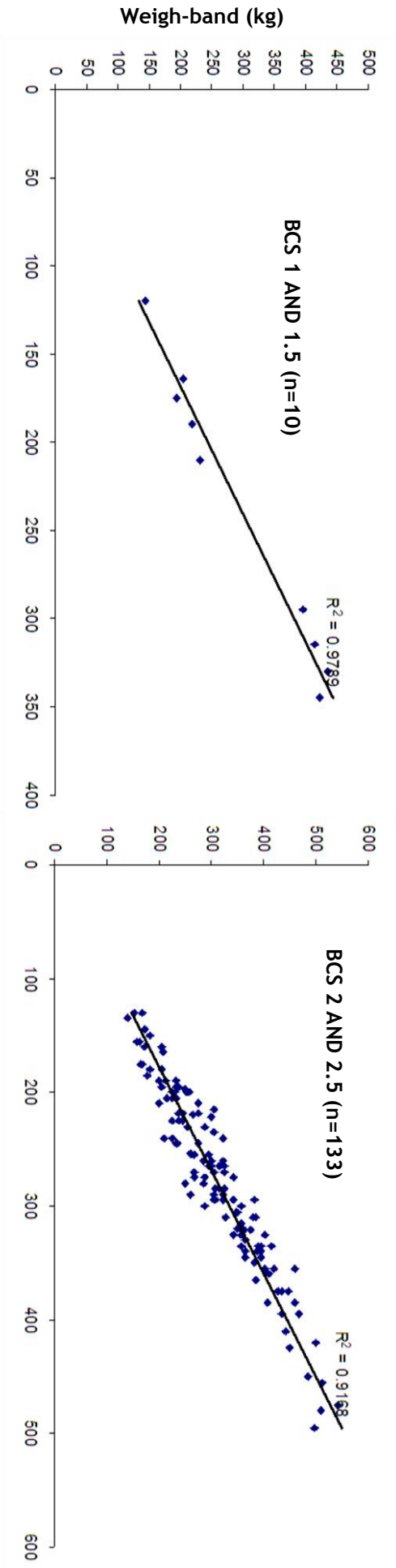


Figure 3.10. Regression of weigh-scale and weigh-band on Farm C3 and at The University of Glasgow Veterinary School for different Body Condition Scores (BCS)

3.4 DISCUSSION

If liveweight gain is to be used in a performance-based TST programme it is necessary to investigate whether methods used on farm are accurate in estimating liveweight gain when no weigh-scales are available. It is clear from the literature (Dingwell *et al.*, 2006; Heinrichs *et al.*, 2007) and this small study that the correlation between liveweight estimated from a weigh-band (using heart-girth) and the measured liveweight of the animal is good. The correlation in this study was lower ($R^2=0.87$) than cited in the literature (Otte *et al.*, 1992; Heinrichs *et al.*, 1992; Dingwell *et al.*, 2006; Heinrichs *et al.*, 2007); however, there was a larger variety of breeds, including beef breeds, in this study and only dairy breeds, particularly Holstein, were measured in the large-scale studies of cattle measurements (Heinrichs *et al.*, 1992; Dingwell *et al.*, 2006; Heinrichs *et al.*, 2007).

Heinrichs *et al.* (1992) expected a higher variance in estimated liveweight if different operators used the weigh-band, but in a further study (Heinrichs *et al.*, 2007) this variance was found to be minimal. Repeatability between two heart-girth measurements by an individual observer on the same animal using a blind heart-girth tape was > 0.99 . Repeatability was also > 0.99 between two measurements by different observers using blind weigh-bands on the same animal (Heinrichs *et al.*, 2007). On farm, it would be usual for the farmer to be the only operator of the weigh-band and so this factor should not be a problem in implementation of this method in the field.

This current study found that the weigh-band was likely to estimate a liveweight of 7% over true liveweight. If farmers use this estimated weight for dosing with anthelmintic then the under-dosing of animals would be significantly reduced, consistent with the responsible use of anthelmintics (discussed in detail in Chapter 4). Heinrichs *et al.* (2007) showed an 8% difference in weigh-band to weigh-scale liveweight (i.e. 4% under or 4% over). The paper showed that in an animal with a true liveweight of 250 kg, the weigh-band would be expected to predict liveweight in the range of 240-261 kg, about two-thirds of the time. The weigh-band predicted liveweight was expected to be within 11 kg (or 4.25%) of the animals' true liveweight. In the current study, the mean difference between weigh-band and liveweight was

22.8 kg. This difference is likely to be small enough not to have any practical significance in any management or feeding decisions on farm where liveweight is used, but it could be large enough to significantly affect a liveweight gain calculation. For example, a 150 kg animal, growing at 0.75 kg/day, would expect to have gained 26.25 kg in five weeks or 52.5 kg in ten weeks. If, at the first visit, the weigh-band liveweight reflected true liveweight but at the second visit the weigh-band predicted a liveweight of 22.8 kg over true liveweight, then liveweight gain would be predicted as 1.23 kg/day after five weeks or 1.07 kg/day after ten weeks, instead of the true value of 0.75 kg/day. The longer the period of time between liveweight estimation, the less the weigh-band error affects liveweight gain. Having said this, as the weigh-band is used at each visit, the example above is likely to be extreme as it's the liveweight weigh-band difference that is important, not whether the weigh-band reflects the true weight of each animal. It is likely that if the skeletal conformation of an animal, and hence heart-girth measurement, does not accurately predict liveweight on one occasion that this will be repeated on another occasion, minimising a large variation in liveweight gain predictions. Otte *et al.* (1992) showed no indication of a consistent difference between liveweight gain (kg) by weigh-scale or weigh-band ($P > 0.2$), although they did note some large discrepancies between the two methods in some animals. As the difference in liveweight between two different time points is what is calculated, the correlation rather than the association of liveweight and weigh band is actually what is important.

The weigh-band and calculation of liveweight from the formula in Heinrichs *et al.* (1992) correlated strongly ($R^2 = 0.99$). Although there is no information regarding which formula the Coburn[®] tape for estimating liveweights of beef cattle (The Coburn Company, Inc.) use, it is highly likely that the weights in the weigh-band have been derived from the formula shown in this study. Calculation of liveweight from the formula (Heinrichs *et al.*, 1992) correlated better with true liveweights ($R^2 = 0.90$) than the weigh-band ($R^2 = 0.87$). It was expected that the weigh-band, as it adjusts liveweight according to body condition score, would correlate higher. However, the condition scores on the weigh-tape were for beef cattle in the USA and therefore may have been interpreted wrongly when used in dairy and dairy x beef calves in the UK. The highest correlation between weigh-band and true liveweight value was found when cattle had a BCS of 2.5 or lower. Most calves in their first year at grass in the UK

would fall into this category. Eliminating body condition score estimation appears not to reduce correlation between heart-girth and liveweight and would be simpler for farmers to implement on farm. In order to calculate liveweight gain efficiently at the crush-side, a computer (either hand-held or laptop) is likely to be required. If this is the case, adding the formula from Heinrichs *et al.* (1992) into an equation on the computer is a relatively straightforward task. As more and more farmers are using technology, either on 'smart phones' in the form of 'applications' (where useful tools/computer programmes are downloaded to the phone), or on hand-held devices, it could be possible to integrate a liveweight gain predictor into this technology in the future.

3.5 CONCLUSION

Given that many farmers do not possess weigh-scales on farm, use of heart-girth measurements to estimate liveweight gain is the best option available to farmers currently. The effect of the error of the weigh-band on liveweight gain will be larger over a shorter time period. In the context of TST, a slight over-estimate of the live weight would be consistent with the principles of responsible use of anthelmintics in that under-dosing would be less likely to occur. A crush-side computer would be useful to farmers if liveweight gains were to be used in a targeted selective anthelmintic treatment programme.

CHAPTER FOUR

CHAPTER FOUR: DEVELOPING A PERFORMANCE-BASED TARGETED SELECTIVE ANTHELMINTIC TREATMENT REGIME FOR DAIRY CALVES IN THEIR FIRST GRAZING SEASON IN THE UK

4.1 INTRODUCTION

Chapter Two described control measures for gastrointestinal parasites in first season grazing calves on three conventional farms through the application of a range of highly effective anthelmintics. However, there are a number of concerns about whether the anthelmintic strategies employed on many farms are sustainable. Firstly, many consumers are concerned about the dependence on biocides in livestock farming. For example, dependence on routine anthelmintic treatment is not generally accepted in organic livestock production in many European countries, and in countries such as Denmark, prophylactic treatment of cattle is prohibited (Anonymous, Ecological Production Directive, Danish Government, No. 210, 1998). Secondly, there is concern that gastrointestinal nematode infections may become more difficult to control as a result of emerging anthelmintic resistance. To date, gastrointestinal nematodes in cattle that are refractory to anthelmintic treatment have been reported in the southern hemisphere, such as in New Zealand (Waghorn *et al.*, 2006), Australia (Rendell, 2010) and in intensive cattle-rearing areas of Latin America (Anziani *et al.*, 2001; Anziani *et al.*, 2004; Suarez and Cristel, 2007), but there is now evidence that anthelmintic resistance is emerging in Europe (Demeler *et al.*, 2007). Accordingly, there is an urgent need to fine-tune the ways in which anthelmintics are used, to avoid escalating problems with anthelmintic resistance.

4.2 ANTHELMINTIC RESISTANCE

Anthelmintic resistance is heritable and present when there is a greater frequency of individual parasites within a population able to tolerate doses of an anthelmintic than in a normal population (Pritchard, 1980). A diagnosis of anthelmintic resistance is generally defined as treatment that reduces either parasite

egg counts or adult worm burdens by less than ninety-five per cent (Coles *et al.*, 1992). Once anthelmintic resistance is present in a parasite it would be assumed that the anthelmintic group would become ineffective. However, there is evidence that if resistance genes are not highly prevalent in parasite populations then the withdrawal of the drug can lead to a reversion to susceptibility, which can be rapid even in field situations (Waller *et al.*, 1988; Anderson *et al.*, 1991). However, re-introduction of the offending drug results in an equally rapid return to resistance. Theoretically, it would seem that the forces that drive these processes would depend on how far along the sigmoid curve to homozygous resistance the parasite population had travelled. This is because there is clear evidence that if this has reached the end-point and resistance genes have become fixed in the parasite genome, then susceptibility takes a long time (if ever) to return (Le Jambre *et al.*, 1981; Waller *et al.*, 1990). Nevertheless, with the benefit of hindsight and the use of computer modelling, the use of combination products may be the way to ensure the preservation of anthelmintic efficacy. Computer simulations have shown that the use of combinations of anthelmintics, in which all components possess individual high levels of efficacy, are the most powerful means of maintaining long term drug efficacy (Barnes *et al.*, 1995). Combination products against nematodes are not commercially available for use in cattle in the UK but are used commonly in other countries, such as New Zealand.

4.2.1 Anthelmintic Resistance in Small Ruminants

Anthelmintic resistance is recognised as a major problem affecting small ruminant production world-wide (Jackson and Coop, 2000; Wolstenholme *et al.*, 2004; Kaplan, 2004). The use, and occasionally indiscriminate use, of anthelmintics has led to the development and dissemination of anthelmintic resistance in small ruminants in the UK (Bartley *et al.*, 2003; Mitchell *et al.*, 2010) and the emergence of multi-drug resistant populations in the UK now gives some farmers limited options for parasite control (Sargison *et al.*, 2005). The current understanding of the selection, transmission and prevalence of anthelmintic resistance is based heavily on the small ruminant sector, and this has led to the development of working groups aiming to tackle the development of resistance; these are as follows:

1). The Sustainable Control of Parasites in Sheep (SCOPS) was launched in 2003 by representatives of the UK sheep industry and provided guidelines for sheep producers (Abbot *et al.*, 2009). In general producers are advised to:

- Ensure treatments are targeted towards animals that will benefit from treatment and applied at an appropriate time;
- Effectively treat and quarantine new stock;
- Ensure correct dose rates;
- Check drenching equipment;
- Check the efficacy of treatments;
- Avoid under-dosing stock by accurate weight measurement;
- Reduce dependence on anthelmintics.

The guidelines were designed to help worm control advisors devise strategies that utilise anthelmintics when necessary and in a way that targets parasite species more accurately. The main aim of SCOPS is to avoid widespread anthelmintic resistance to the ML class of anthelmintics and to prolong the effectiveness of any new anthelmintic compounds (Taylor, 2012).

2). PARASOL (PARAsite SOLutions) is a European Union sponsored research project that has seventeen participants, from Europe and Africa, with a research focus on various aspects of anthelmintic resistance in ruminants. The project, which is co-ordinated by Professor Jozef Vercruysse from the University of Ghent, has six research work-packages that cover different areas including improved sustainable control strategies in ruminants, the diagnosis of resistance, the mechanisms associated with anthelmintic resistance and improving bioavailability as a means of enhancing efficacy against resistant isolates. The key element within the more applied research work packages in PARASOL is the use of targeted selective treatments (TST) to target anthelmintic treatments towards those animals that will most benefit from them, or those that are most responsible for pasture contamination. This approach to the optimisation of anthelmintic usage aims to maintain a susceptible parasite population *in refugia* and thus help to conserve anthelmintic susceptible genes within the parasite population as a whole (Vercruysse *et al.*, 2009).

3). COWS (Control of Worms Sustainably in Cattle) was launched in the UK in May 2010 and produced a technical manual for veterinarians and advisors involved with investigating an apparent increase in treatment failure and suspected anthelmintic resistance in cattle (Taylor, 2010i; Taylor, 2010ii); this is discussed below.

4.2.2 Anthelmintic Resistance in Cattle

There was a long held belief that anthelmintic resistance was not likely to be a problem in cattle due to the fact that they are generally extensively grazed, mount a more effective immune response (lower egg counts than sheep and goats) and adult animals tend not to be treated. However, the expansion in the numbers of intensively farmed enterprises has led to an increased reliance on anthelmintics to maintain productivity and control parasitism (Waghorn *et al.*, 2006, Gasbarre and Smith, 2009). This, combined with the use of delivery systems that extend anthelmintic bioavailability, may have increased selection for resistance over the past decade. Table 4.1 gives examples of anthelmintic resistance reports in the literature from around the world. Anthelmintic resistance, predominantly to macrocyclic lactones in *Cooperia* spp., but also to benzimidazoles in *Ostertagia ostertagi*, has been reported in New Zealand (Pomroy, 2006). A survey of sixty-two New Zealand beef cattle farms found that 94% of the enterprises had treatment efficacies of $\leq 95\%$ against one class of anthelmintic but, more worryingly, 74% had resistance against the ML and BZ classes (Waghorn *et al.*, 2006). Resistance to macrocyclic lactone anthelmintics in *Cooperia* species has been reported in northern mainland Europe (Demeler *et al.*, 2007). Most instances of macrocyclic lactone resistance in *Cooperia* spp. in cattle were reported following the identification of positive faecal worm egg counts after use of pour-on treatments (West *et al.*, 1994; Loveridge *et al.*, 2003; Coles *et al.*, 2008).

Country	Anthelmintic Class	Route of Administration	No. of farms	Gastrointestinal parasite	Reference
United Kingdom	ML	Injection	1	<i>Cooperia</i> spp.	Stafford and Coles, 1999
	ML	Pour-on	2	<i>Cooperia</i> spp.	Sargison <i>et al.</i> , 2009
	ML	Injection	4	<i>Cooperia</i> spp.	McArthur <i>et al.</i> , 2011
	ML	Pour-on	1	<i>Trichostrongyle</i>	Orpin, 2010
Belgium	ML	Injection	1	<i>Cooperia</i> spp.	El-Abdellati <i>et al.</i> , 2010
	LEV	Injection	1	<i>Ostertagi</i> spp.	Geerts, <i>et al.</i> , 1987
Belgium, Germany, Sweden	ML	Injection	10	<i>Cooperia</i> spp., <i>Ostertagi</i> spp.	Demeler <i>et al.</i> , 2009
Australia	ML, BZ, LEV	Pour-on, oral	13	<i>Cooperia</i> spp., <i>Ostertagi</i> spp., <i>Trichostrongylus</i> spp.	Rendell, 2010
Argentina	ML, BZ	Injection, oral	16	<i>Cooperia</i> spp., <i>Ostertagi</i> spp.	Suarez and Cristel, 2007
Brazil	ML, BZ, LEV	Injection	>22	<i>Cooperia</i> spp.	Soutello <i>et al.</i> , 2007
United States	ML	Injection	1	<i>Cooperia</i> spp., <i>Ostertagi</i> spp.	Edmonds <i>et al.</i> , 2010
New Zealand	ML, BZ	Oral	59	<i>Cooperia</i> spp.	Waghorn <i>et al.</i> , 2006

Table 4.1. Examples of published cases of anthelmintic resistance/product failure in gastrointestinal parasites from the UK and world-wide. ML, Macrocytic lactones; LEV levamisoles; BZ, Benzimidazoles.

There are two reasons to question whether these reports are due to true resistance or treatment failure. Firstly, the efficacy of pour-on anthelmintics against the dose-limiting *Cooperia* spp. has been challenged (Bisset *et al.*, 1990), suggesting that poor absorption (Eagleson and Allerton, 1992; Hennessy and Alvinerie, 2002; Gokbulut *et al.*, 2012), or the licking off (Sallovitz *et al.*, 2005; Imperiale *et al.*, 2009) of pour-on macrocyclic lactone (ML) anthelmintics provides a more likely explanation for positive post treatment faecal egg counts than acquired resistance (McKenna, 1995). Administering ML by injection results in significantly higher maximum concentrations of drug (C_{max}) in plasma and decreased variability around the C_{max} (Hennessy and Alvinerie, 2002) than when the same active compound is administered as a pour-on. Not surprisingly then, injectable administration has been shown to result in higher efficacy than when the same active is administered as a pour-on (Eagleson and Allerton, 1992). A recent study (Gokbulut *et al.*, 2012) investigating pour-on ivermectin in cattle showed that heavy rainfall could result in subtherapeutic plasma concentrations of ivermectin following pour-on administration, which may increase the risk of drug resistance in parasites. Furthermore, exposure to direct sunlight reduced the plasma persistence of ivermectin. This may suggest the need for shorter treatment intervals after pour-on administration in grazing cattle because anthelmintic activity depends on the duration of parasite exposure to effective concentrations of the active compounds (Gokbulut *et al.*, 2012). Shedding of *Cooperia* spp. eggs during the prepatent period following topical macrocyclic lactone anthelmintic treatment provides evidence of underexposure of female nematodes within the host, and this situation has been shown experimentally to select for anthelmintic resistance (Van Zeveren *et al.*, 2007).

Secondly, the accurate determination of resistance status in gastrointestinal nematodes infecting cattle is more difficult than in small ruminants. The faecal egg count reduction test (FECRT) provides an estimate of treatment efficacy based on egg counts before and after treatment (McKenna, 1990) or between treated and untreated groups (Coles *et al.*, 2006). The FECRT was originally standardised for small ruminants and Coles *et al.* (2006) recommend that if FEC is <150 epg then a different method than the modified McMaster is necessary. The World Association for the Advancement of Veterinary Parasitology (WAAVP) set guidelines for percentage efficacy thresholds for diagnosing anthelmintic resistance in ruminants. For ML

anthelmintics, these were quoted as a mean FEC reduction of less than 95%, with a lower 95% confidence interval of less than 90% (Coles *et al.*, 1992). The egg output in cattle faeces tends to be lower, which makes accurate calculation of drug efficacy problematic (Demeler *et al.*, 2010; Taylor *et al.*, 2002). Also, some nematode species show a strong density-dependence in egg production (Sangster *et al.*, 1979; Smith *et al.*, 1987), which is likely to further impede detection of resistance based on a faecal egg count reduction test (FECRT) (Taylor *et al.*, 2002). Another significant issue when testing ML anthelmintic efficacy in cattle is the wide variety of molecules and delivery formulations available within the ML family. In small ruminants, it is normal practice in a FECRT to use an oral dose of ivermectin (Sutherland *et al.*, 2002). The situation in cattle is further complicated by the use of different routes of administration of test compounds. The administration of ML compounds by subcutaneous injection in the neck or topical application onto the shoulders/back of animals can result in a temporary suppression of egg production (Mason and McKay, 2006), and for this reason interpretation of post-treatment counts can be difficult; thus, post-treatment sampling needs to be tailored to the mode of administration of the active drug being tested (Sutherland *et al.*, 1999; Sutherland and Leathwick, 2011).

The first case of ivermectin resistance in UK cattle was reported by Stafford and Coles in 1999 in *Cooperia* spp., and further reports of anthelmintic resistance or apparent product failure have also been reported by Sargison *et al.* (2009), Sargison *et al.* (2010), Orpin (2010) and McArthur *et al.* (2011). Sargison *et al.* (2009) reported the apparent treatment failure of doramectin as a pour-on preparation compared with an injectable preparation on two farms in Scotland, using the FECRT to report treatment efficacy. Sargison *et al.* (2010) also investigated the apparent treatment failure of pour-on doramectin on another farm in Scotland using a modified McMaster method, whereby each egg counted represented 25 epg. Orpin (2010) described a <95% reduction in FEC post-treatment in 6/6 animals on one farm in England following administration of pour-on avermectin. However, two of the six animals had FEC < 150 epg pre-treatment.

The Moredun Research Institute, Edinburgh, recently undertook a questionnaire study of helminth management practices on Scottish cattle farms. As part of this study, farmers were asked to participate in a FECRT to assess the efficacy of

ivermectin on their farm. The data from the first four farms that participated in the FECRT showed that three of the farms would be categorised as harbouring ivermectin resistant nematodes, as a FEC reduction of less than 95% in epg was achieved at each site (McArthur *et al.*, 2011). The study used an injectable preparation of ivermectin and a modification of the salt flotation method described by Jackson (1974), which has a sensitivity of 1 egg per gram (epg), which was necessary as some of the pre-treatment FEC were less than 150 epg.

The development of anthelmintics in cattle has been driven by the requirement for ease of administration and infrequency of application to animals that may not be easy to handle. Advances in formulation development enabled the introduction of a range of products that were delivered by either subcutaneous injection or by topical application. The benzimidazole and levamisole groups of anthelmintics have low bioavailability by both of these application methods (Hennessy, 1997) but the introduction of the macrocyclic lactone group overcame this, and injectable (Goudie, 1993) and pour-on preparations (Shoop, *et al.*, 1996; Yazwinski, *et al.*, 1999) began to dominate the market. In a survey of parasite control methods on seventy-two beef farms in south-west England, topical treatment using MLs was shown to be the most common method of anthelmintic administration (Barton *et al.*, 2006) and three quarters of respondents from the recent Moredun Research Institute questionnaire on farm management practices in Scotland stated that they had used MLs in the previous twelve months (McArthur *et al.*, 2011).

The increase in reported anthelmintic resistance in cattle parasites may be due to the selection for resistant parasites occurring more rapidly in recent years, greater spread and dissemination of resistant parasites, more testing for anthelmintic resistance in cattle, or a combination of all of the above. With this apparent increase in anthelmintic resistance there is a need to investigate potential risk factors associated with the presence or absence of anthelmintic resistance and ensure that producers follow the best advice and recommendations where applicable. Based on their findings, Sargison *et al.* (2009) recommended that cattle farmers in the UK should be encouraged to consider the risk of selection for anthelmintic resistance in their herds, and to follow their sheep farmer counterparts by adopting strategies aimed at preserving susceptible nematodes *in refugia* that will preserve anthelmintic

efficacy. It is generally accepted that there is a need to incorporate integrated parasite management practices with appropriate and practicable use of efficient treatments. COWS (Control of Worms Sustainably in Cattle) aimed to encourage veterinarians and advisors in the cattle industry to help farmers to follow guidelines in order to prevent treatment failure and anthelmintic resistance in cattle. In general the guidelines advise to treat animals when necessary with a dose for the right weight of animal and utilise epidemiological knowledge of the parasites in pasture management. Monitoring gastrointestinal parasite risk is encouraged with the use of faecal egg counts (Taylor, 2010i; Taylor, 2010ii).

4.3 TARGETED SELECTIVE ANTHELMINTIC TREATMENT

Drench frequency is a contributing factor in the development of anthelmintic resistance (Van Wyk, 2001) and therefore any strategy that can reduce the numbers of treatments administered to a herd is beneficial. Targeted treatments (TT) can be defined as whole group treatment given at the most appropriate times, bearing in mind the need to maintain refugia. These treatments can be further enhanced by targeted selective treatment (TST) of only those animals that will benefit the most from treatment, leaving the rest of the group untreated (Kenyon *et al.*, 2009). Maintaining a population of parasites *in refugia*, in order to maintain both phenotypic and genotypic susceptibility, is an important strategy in order to avoid the development of anthelmintic resistance (Van Wyk, 2001; Soulsby, 2007). Refugia is the proportion of the parasite population at any one time that is unexposed to anthelmintic treatment and that can be ingested by a host: it commonly resides within untreated animals or as free-living stages on the pasture. Parasites that avoid anthelmintic treatment are under no chemical selection pressure and, therefore, parasites with susceptible genes can persist and are able to dilute the genes for resistance that are generated and propagated in the progeny of survivors in treated stock (Van Wyk *et al.*, 2002). Martin *et al.* (1981) applied the concept of refugia to a population of gastrointestinal nematodes over thirty years ago but the concept has only relatively recently been embraced in parasitological research (Van Wyk, 2001). In fact, Van Wyk (2001) goes so far as to say that refugia could be the most important factor determining the rate of development of resistance and should be considered above all else throughout the development and implementation of any control

strategies. There are a number of factors that affect the management of refugic parasite communities. These can be classified into parasitic, host, environment or management associated factors (Jackson and Waller, 2008) and are summarised in Table 4.2.

Factors Determining Refugia	
Parasitic	Frequency of resistant alleles Biotic potential Longevity
Host	Resilience to infection Immunity
Environment	Drought Temperature
Farm Management	Dose and move strategies

Table 4.2. Factors determining refugia in parasite populations.

The higher the frequency of resistant alleles in the parasite population the more likely that resistant strains will be produced and the opportunity to maintain a susceptible population *in refugia* decreases (Gaba *et al.*, 2006). The shorter the time the parasite survives on pasture, for example in periods of drought or high temperatures, the higher the contribution of resistant parasites by the host to the parasite population. Intuitively, the more eggs a single resistant worm can produce the higher the potential spread of resistant strains. Animals resilient to parasite infection, in theory, require less anthelmintic treatments, thus increasing refugia (Bisset *et al.*, 2001). As youngstock become immune, their contribution to parasite eggs on the pasture reduces. Thus, during the grazing season an increasing number of animals may need to be left untreated in order to increase refugia. The practice of dose and move, where animals are treated and moved immediately to new pasture, has been shown to significantly increase resistance within a parasite population (Michel, 1985; Waghorn *et al.*, 2009) and this strategy has been highly discouraged within the SCOPS and COWS initiatives (Taylor, 2010ii). Molento *et al.* (2004) suggested that animals should be moved prior to drenching and treatment delayed until the desired levels of refugia have built up on the new pasture to ensure that unselected parasites were transferred to the pasture.

Preferentially animals that benefit most from treatment only would be treated, as the common grazing of animals that have different treatment regimes and different susceptibilities to infection are likely to provide a continual turnover of parasites *in refugia*. In Greece and Ethiopia it is common to graze animals together that have been subjected to different treatment regimes and this may well provide an explanation for the lack of anthelmintic resistance observed in the Greek mainland (Papadopoulos *et al.*, 2001) and Ethiopia (Sissay *et al.*, 2006). The concept of refugia can be applied to slow development of resistance through the uses of approaches such as dilution of resistant with susceptible parasites when the proportion of resistance alleles is high, and targeted treatments and selectively targeted treatments when resistant alleles are less common (Kenyon *et al.*, 2009), such as is assumed to be the case in cattle in the UK.

4.3.1 Potential Markers as Indicators for Targeted Selective Anthelmintic Treatment

An ideal biomarker for use in a targeted selective treatment (TST) programme would be cost-effective, simple to use, require minimal operator training and allow decisions to be made promptly. Ideally, the marker should indicate animals within the group that would benefit most from treatment and identify animals resilient to the parasite challenge. Strategies that have been suggested for use in targeted treatment (TT), where whole group treatment is given at the most appropriate times, are the use of serum pepsinogen levels in first season grazers (Charlier *et al.*, 2011), *O. ostertagi*-specific antibodies levels in milk (Charlier *et al.*, 2009iii) and faecal egg counts (Taylor, 2010i). Only a handful of studies have investigated the use of biomarkers in a TST approach. Johannes Charlier, working at Ghent University, has noted that the individual variation between *O. ostertagi* antibody concentration in milk is significant enough to advocate its use in a TST approach (Charlier *et al.*, 2010i; Charlier *et al.*, 2010ii; Charlier *et al.*, 2011). Mejia *et al.* (2011) recently advocated a TST approach using faecal egg counts in adult cattle within the first month of lactation. Notably, both studies relate to adult dairy cattle.

One of the aims of the study in Chapter Two was to investigate biomarkers as potential TST indicators. As resilient animals generally grow well, despite parasite challenge (Barger, 1985), the correlation between liveweight gain and biomarker was assessed. Fructosamine concentration had not previously been investigated in cattle in relation to gastrointestinal parasitism. Work in sheep has shown a potential for fructosamine as a useful indication of the intensity of parasitic infection and as a possible indication of lambs that subsequently acquire an above-average number of parasites (Stear *et al.*, 2001). No evidence was found in this study to indicate that there is a correlation between fructosamine concentration and liveweight gain. Thus, it was concluded, that this biomarker could not be implemented into a TST regime where calves that would benefit the most from treatment must be identified. Faecal egg count, pepsinogen concentration and *O. ostertagi* antibody were also found not to correlate with liveweight gain. The obvious limitation of these tests is that generally there is a period between sampling, results and then treatment. Animals are required to be gathered and handled at least twice which poses practical difficulties. As liveweight gain can indicate the effect of gastrointestinal parasitism on the host, then using this as a biomarker could be a sensitive, cheap, crush-side test for the identification of animals requiring treatment. It is relevant to farm economics and the effect of parasites present. Many studies have shown the adverse effect of gastrointestinal parasitism on liveweight gain in animals in their first grazing season (Coop *et al.*, 1977; Ploeger and Kloosterman, 1993). The majority of information regarding the use of liveweight gain in relation to a TST approach has been gained from experimental (Kenyon and Jackson, 2012) and on-farm (Stafford *et al.*, 2009; Busin *et al.*, 2013) studies in small ruminants.

4.3.2 Performance-based Targeted Selective Anthelmintic Treatment in Small Ruminants

Out of necessity, the small ruminant industry has been exploring alternate anthelmintic treatment strategies for much longer than the cattle industry (Kenyon and Jackson, 2012). A successful TST indicator in small ruminants implemented in climates where *Haemonchus contortus* poses significant challenge is the FAMACHA[®] system. The haematophagous parasite, which causes anaemia, lead to the development of the FAMACHA[®] system, which utilises conjunctival colour as an

indicator for anthelmintic treatment (Bath *et al.*, 1996; Van Wyk and Bath, 2002). As gastrointestinal parasites in temperate regions generally cause production loss, liveweight gains have been the focus of research in relation to TST programmes in these climates. In New Zealand, one approach has been to leave a proportion of the heaviest animals untreated at each drenching occasion. Leathwick *et al.* (2006i) left the heaviest ten per cent of a flock untreated and showed no difference in liveweight gain compared with a flock where all animals were treated. In another study, when the heaviest fifteen per cent of a flock were left untreated there was a slower development of resistance in *Teladorsagia* spp. (Leathwick *et al.*, 2006ii). This approach requires batches of animals of similar ages, which only occurs on dairy farms with a strict calving season, such as in New Zealand. In France, TST was applied on the basis of low daily weight gain and/or the highest FEC within the whole flock over one grazing season (Gaba *et al.*, 2010). When compared with a whole-flock treatment group, the TST group received 90% less anthelmintic but maintained similar growth rates, but the use of two targets is more complicated in the field. In the UK, the fastest growing lambs were left untreated on a commercial farm (Stafford *et al.*, 2009). To identify those animals to leave untreated, a random subset of 20–25 lambs were weighed and an average weight gain obtained. Lambs were left untreated if they were in the top 25% of their peer group in weight gain and were also deemed to be in good body condition, with no evidence of breech soiling. This TST approach reduced anthelmintic treatment by 4.7% compared with the standard anthelmintic treatment, that was two or three anthelmintic treatments per lamb in a year. The percentage of lambs left untreated averaged 8.5%. The liveweight gain and FEC of the untreated group did not differ from the treated group.

In Scotland, the use of a threshold of liveweight gain as an indicator for treatment has been further refined by taking into account some of the external influences that may affect lamb liveweight gain, such as herbage availability and quality, to give the efficiency of nutrient utilisation in an animal (Greer *et al.*, 2009). The results of the efficiency calculation were used to predict the liveweight gains of each animal, and treatment was given depending on whether the animal had reached the stated weight. The model, termed the Happy Factor™, was able to successfully distinguish between animals that would benefit most from anthelmintic treatment. Busin *et al.* (2013) used the Happy Factor™ to implement a TST programme on a

commercial Scottish sheep flock. Findings suggest a 50% reduction in anthelmintic usage in the TST group compared to the routinely treated group with no difference in the liveweight gains achieved in both groups.

4.3.3 Performance-based Targeted Selective Anthelmintic Treatment in First season Grazing Calves

In New Zealand, a performance-based TST approach was investigated on two different herds of Friesian and Friesian x Jersey first season grazing calves (FSG) (Greer *et al.*, 2010; McAnulty *et al.*, 2011). On each farm, the FSG calves were separated into two different groups: 1) Regular anthelmintic treatment (monthly drenching) and 2) TST administered to individuals failing to reach predetermined liveweight gains, shown in Table 4.3. Both groups on each farm were grazed together. On one farm, anthelmintic usage was reduced by 72% and on the other farm it was reduced by 47% in the TST groups compared to the calves treated regularly (McAnulty *et al.*, 2011). Liveweight gain in both groups on both farms did not differ significantly.

Season	Target growth rate (kg/day)	
	Friesian	Friesian x Jersey
Spring	0.62	0.59
Summer	0.68	0.64
Autumn	0.62	0.60
Winter	0.30	0.30

Table 4.3. Target liveweight gain for FSG in TST group (Greer *et al.*, 2011).

European and New Zealand dairy industries differ in a few aspects. Cattle in New Zealand graze year-round whereas in Europe, and particularly Scotland, it is necessary to house cattle during the winter months and feed conserved forage. When considering a TST approach in New Zealand it was necessary to present targeted liveweight gains throughout the year, which is unnecessary in Europe. In general, cattle in New Zealand are smaller than the cattle in Europe and so target liveweight gains in New Zealand will reflect the lighter liveweight at which cattle are served. A recent study in Sweden investigating TST in FSG can be more easily extrapolated to the UK, involving a grazing trial conducted over three years (Höglund *et al.*, 2011

unpublished data). FSG were separated into three different treatment groups: 1) no parasite control, 2) suppressive treatment (ST) by monthly doramectin (Dectomax[®], Elanco) injections, or 3) TST dependant on liveweight gain. The threshold for treatment was given as liveweight gain inferior to the average of the poorer half of the group treated suppressively, an approach impossible to replicate under real farming conditions. Using a target liveweight gain extrapolated from calves on the same farm would give a liveweight gain appropriate to the nutrition, management and cattle breeds on farm but may be unrealistically high or low depending on the conditions of the group used in the calculation. In all three years, the ST group had significantly higher liveweight gains (0.42-0.61 kg/day) than the TST group (0.33-0.51 kg/day), which in turn, had significantly higher weight gains than the group with no treatment (0.25-0.43 kg/day), although the authors did note that the TST group did not suffer major production loss. It should be noted that the liveweight gains were low in all groups compared to what would be expected in the UK. The use of anthelmintics was more than halved in the TST group compared with the ST group; however, there were no signs of selection for anthelmintic resistance in the ST group (Höglund, personal communication). Faecal egg counts in the TST group and the group not treated with anthelmintics were similar. Höglund *et al.* (2011) suggests that “*the TST approach failed to prevent dangerous levels of pasture contamination*”; however, from a refugia point-of-view, the TST approach succeeded. There have been no published studies in the UK investigating the use of TST in first season grazers.

4.4 DEVELOPMENT OF A THRESHOLD FOR A PERFORMANCE-BASED TARGETED SELECTIVE TREATMENT STRATEGY IN FIRST SEASON GRAZING CALVES IN SCOTLAND

First season grazing animals on dairy farms in the UK are composed of dairy replacements and calves to be fattened for beef. It need not be pointed out that a beef animal requires a good growth rate in order to reach the target market liveweight with minimal expenditure, and this is always the aim of a producer. However, dairy replacements also need to be healthy, well-grown individuals with good fertility that are well-equipped to join the herd at first calving, and this should be the aim of every dairy producer. To achieve the optimal target of calving at two years of age (24 months), dairy heifers need to be served by fifteen months of age (450 days) at 55-60% of their expected adult liveweight (Wathes *et al.*, 2008). Calving

at an earlier age not only reduces rearing costs but allows cows to reach first lactation sooner and, over the course of their life, provide more economic benefit and less environmental impact. There is increasing pressure on the dairy industry to improve its environmental impact. In England, there has been an industry-led initiative since 2008 called “The Road Map” which targets greenhouse gas emissions, energy and water usage and biodiversity impacts. Preliminary results from the 2011 Dairy Roadmap Report (Defra, 2011) details increasing evidence that the farmers who are the most efficient producers, are also those with the smallest carbon footprint. Modelling work by Garnsworthy (2004), suggests that if first calving is delayed until twenty-seven months of age, the number of heifers on farm will increase by twelve per cent but methane emissions by replacements increases by thirty per cent. This is because the heifers are larger, and so the total herd emission increases by six per cent. Dairy heifers calving between twenty-two and twenty-five months of age were more successful in conceiving at first service and survived for longer in a herd (Wathes *et al.*, 2008). A short herd lifespan is a significant economic cost to the dairy industry. Fertility has fallen in recent years: UK figures for first service conception rates were around 60% in the 1970s but only 40% by 2000 (Royal *et al.*, 2000). Poor fertility is thus the major limiting factor determining longevity. Furthermore, the low number of offspring produced per cow limits the availability of potential replacement heifers born within a herd, whilst at the same time the decrease in fertility increases the requirement for more. Animals that failed to conceive at fifteen months were lighter at nine months of age than those that did conceive (Bourne *et al.*, 2007). This data suggests that the age at which animals calve is indeed affected by early growth rates and that animals that have difficulty in conceiving are often poorly developed at six to nine months. Poorly grown animals have low conception rates as both heifers and cows resulting in very poor lifetime productivity and are thus uneconomic to keep (Wathes *et al.*, 2008). The age at first calving also affects the net costs of raising replacement heifers; reducing the age at first calving by one month lowered the cost of replacement programs by 4.3% (Tozer and Heinrichs, 2001). Therefore, the effects of having a heifer calve at a particular age need to be considered in relation to rearing costs, as well as her subsequent fertility, productivity, and longevity in the herd (Brickell and Wathes, 2011).

In the UK, the average dairy cow would be expected to accomplish a liveweight of approximately 650 kg and so the expected liveweight at 15 months would be ~ 380 kg. Good parasite control over the grazing season is important in order to achieve the required liveweight gains of between 0.7 - 0.8 kg/day at pasture (Van Amburgh *et al.*, 1998) required to achieve this target.

Table 4.4 shows data from the 2009 study described in Chapter Two. The estimated age at calving (assuming holding to first-service) was estimated on all farms. The FSG in Farms C1, C2 and O3 reached the ideal weight for serving within the study period and so estimated calving age was easily estimated. On Farms C3, O1 and O2 it was estimated using the average liveweight gain achieved over the grazing season from housing. These estimated calving ages were compared with the answer given from farmer interview and questionnaire.

Farm	Mean liveweight at housing (kg)	Age at housing (days)	Liveweight gain over grazing season (kg/day)	Estimated age at serving assuming 0.75 kg/day liveweight gain at housing (months)	Estimated age at calving (months)	Reported age at calving from farmer interview (months)
C1	390	407	0.77	14.5	23.5	27
C2	384	421	0.72	15	24	30
C3	303	319	0.93	13.3	22.3	26
O1	307	454	0.46	20.4	29.4	30
O2	281	365	0.57	17.9	26.9	30
O3	512	626	0.80	17.3	26.3	28

Table 4.4. Data from the 2009 study showing mean liveweight and age of the FSG on each farm at housing (visit 4). The liveweight gain required to reach 55-60% of expected liveweight (~600kg) at 15 months of age was calculated. The estimated age at calving was predicted using liveweight gain over grazing season and compared with the answer given from farmer interview.

The conventional farms, in theory, can achieve a first calving age of twenty-four months and under with liveweight gains of between 0.72 and 0.93 kg/day over the grazing season. The FSG on the organic farms O1 and O2, in theory, cannot calve down at two years of age or younger with the liveweight gains achieved over the

grazing season. Farm O3 was an anomaly as the FSG were 14.5 months at turnout. In fact, four of the FSG in the group were moved to run with a bull at visit 3. All farmers on all farms overestimated the age at first calving on their farm compared to the predicted age. This is likely due to the assumption in this study that the animals held to their first service (approximately 40% will have) and the fact that farmers are probably voluntarily serving at higher weights.

It is clear that a target liveweight gain for use in a performance-based targeted selective anthelmintic programme should aim to achieve a twenty-four months age at first calving. The rearing period, however, should not be too short as a lowered weight at first calving may ultimately decrease milk yield (Van Adrichem and Shaw, 1977). Three different threshold liveweight gains, 0.70 kg/day, 0.75 kg/day and 0.80 kg/day were used to estimate the percentage of FSG on each farm in 2009 that would have been treated at visit 2 (5-10 weeks post-turnout) with these three different thresholds. The percentage of FSG on each farm that would have been treated if a performance-based TST programme had been implemented on the farms for the three different thresholds are illustrated in Figures 4.1, 4.2 and 4.3.

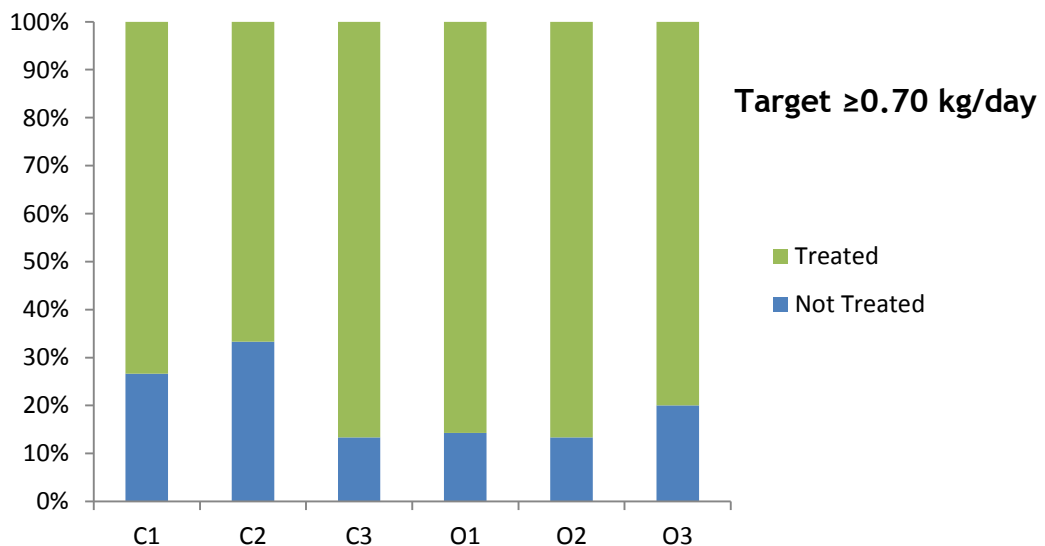


Figure 4.1. Number of calves on each farm in 2009 that would have been treated with anthelmintic at visit 2 (5-10 weeks post-turnout) if a performance-based TST programme had been implemented with a target liveweight gain of ≥ 0.70 kg/day.

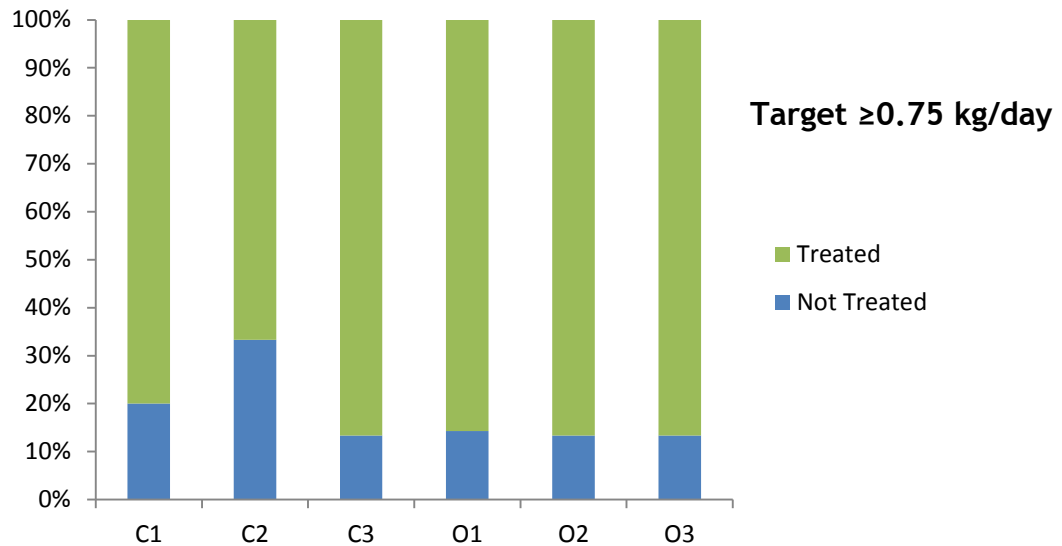


Figure 4.2. Number of calves on each farm in 2009 that would have been treated with anthelmintic at visit 2 (5-10 weeks post-turnout) if a performance-based TST programme had been implemented with a target liveweight gain of ≥ 0.75 kg/day.

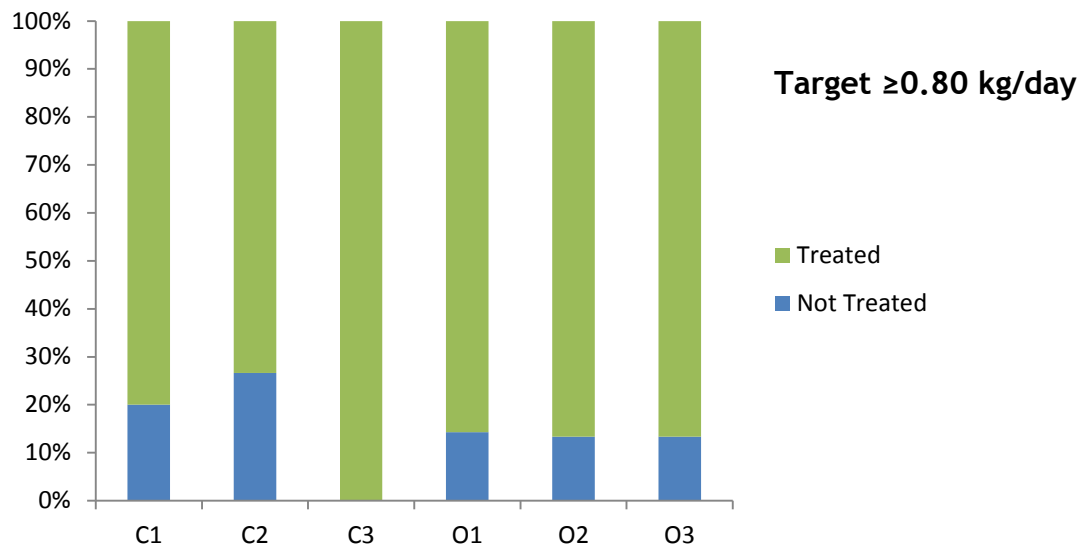


Figure 4.3. Number of calves on each farm in 2009 that would have been treated with anthelmintic at visit 2 (5-10 weeks post-turnout) if a performance-based TST programme had been implemented with a target liveweight gain of ≥ 0.80 kg/day.

The three different thresholds had minimal effects as to the percentage of animals in the group that would have been treated with anthelmintic. A retrospective analysis of three trials, conducted in Sweden between 1997 and 2004, was performed to determine if daily liveweight gain could be used as a TST indicator for calves in their first grazing season (Höglund *et al.*, 2009). Groups of ten first season grazing calves (FSG) were turned out onto pasture in mid-May of each season and grazed for approximately twenty weeks. The authors separated the grazing season into three time zones: 1) early - three to four weeks post-turnout, 2) middle - six to eight weeks

post-turnout and 3) late - at housing. Daily liveweight gains from the middle time zone, i.e. six to eight weeks post-turnout, were found to be good predictors of liveweight at housing. Analysis of the data using a reporter operator curve (ROC) suggested an appropriate threshold for anthelmintic treatment to be liveweight gain of 0.75 kg/day. Similarly, recent studies in New Zealand (McAnulty and Greer, 2011) suggested that, for animals above 130 kg liveweight, an appropriate target liveweight gain at grass is 700-800 g/day for use in a TST regime. Taking all of this information into account, the target liveweight gain to be implemented into a TST regime in this study was set at 0.75 kg/day.

It should be feasible for most farmers to record individual liveweight gains in their cattle, making this a practicable marker for decision-making purposes. The long-term aim of TST is to minimise the numbers of wholeherd anthelmintic treatments, by directing treatments towards only those animals that are likely to suffer from disease and/or production loss. This will reduce the opportunities for any associated environmental and health risks, while maintaining agricultural productivity. However, in order to create low-input and sustainable programme for nematode control, TST strategies also need development and validation under practical farming conditions.

CHAPTER FIVE

CHAPTER FIVE: TARGETED ANTHELMINTIC TREATMENT USING LIVEWEIGHT GAIN IN FIRST SEASON GRAZING DAIRY CALVES IN SCOTLAND

5.1 INTRODUCTION

Chapter Two described the difference in anthelmintic treatment on three organic and three conventional dairy farms on the west coast of Scotland in 2009. Two of the conventional farms treated all animals during their first year at grass with long-acting anthelmintics from the macrocyclic lactone (ML) class of anthelmintics and one used an intra-ruminal bolus from the benzimidazole class (BZ). The organic farms performed faecal egg counts (FEC) when calves appeared to have reduced liveweight gain, poor coat appearance and/or diarrhoea during mid to late summer. Based on FEC, the calves were treated with anthelmintics from the BZ class, and one farm with the ML class, of anthelmintics. Arguably, this approach to parasite control was suboptimal in the organic farms in particular. The current study looked to; 1) target anthelmintic treatment towards animals that would benefit the most, thus reducing anthelmintic use whilst maintaining liveweight gain on conventional farms and 2) use liveweight gain as a marker for anthelmintic treatment rather than FEC on organic dairy farms, reducing subclinical parasitic gastroenteritis.

Targeted selective anthelmintic treatment (TST) using liveweight gain has been successfully applied in small ruminants, resulting in no overall reduction in liveweight gain and reducing anthelmintic resistance development in the parasite population compared with suppressively treated animals (Leathwick *et al.*, 2006ii; Kenyon *et al.*, 2009; Busin *et al.*, 2013). There is limited published literature regarding the use of performance-based TST approaches being used in cattle in the field (Kenyon and Jackson, 2012). Analysis of published trial data using receiver operating curve (ROC) analysis suggested that an appropriate threshold for liveweight gain in a TST regime would be 0.75 kg/day (Höglund *et al.*, 2009). Commenting on Höglund *et al.* (2009) Kenyon and Jackson (2012) suggested that if the theoretical results from this paper

were reproducible in the field it would offer farmers a simple and effective way to implement a TST approach in FSG calves.

5.1.1 Objectives

The main objectives of the study were to implement and evaluate a liveweight gain TST approach in first season dairy calves in the field. The specific objectives were to; 1) use and evaluate a farmer-friendly and cheap method of estimating liveweight of cattle; 2) reduce unnecessary and ineffective anthelmintic use; 3) target anthelmintic treatments to animals that would gain the most benefit; 4) assess the feasibility of a performance based targeted anthelmintic approach in the field.

5.2 MATERIALS AND METHODS

5.2.1 Farm Selection

Three dairy farms in Scotland were recruited to the study; two organic and one conventional (Farm O1, Farm O2 and Farm C3).

Farm	Location	Enterprises	No. milking cows	Total no. of FSG (no. of dairy replacements)
Organic 1	West Central Scotland	Dairy, Beef	66	20 (9)
Organic 2	South West Scotland	Dairy, Beef, Sheep	105	41 (20)
Conventional 3	West Central Scotland	Dairy, Beef, Sheep	70	43 (10)

Table 5.1. Overview of the three farms involved in the 2010 study.

The general features of the participating farms are shown in Table 5.1 and have been described in detail in Chapter 2.2. The three farms were recruited from a total of six farms involved in a monitoring study of gastrointestinal parasitism the previous year (described in Chapter Two). As published data of a TST trial in the field

is lacking, it was felt that the two commercial conventional dairy farms (Farm C1 and Farm C2) could not be included in the 2010 trial study. Farm C3 is The University of Glasgow Veterinary School farm and was therefore willing to be included in the study. Organic Farm 3 was invited to participate in the 2010 study but declined, due to the necessity of handling FSG throughout the grazing season.

5.2.2 Experimental Animals

All first season grazers (FSG) on-farm were included in the study (Farm O1 n = 20, Farm O2 n = 41, Farm C3 n = 43). In the previous year, all animals included in the study had been screened so as to identify any animal persistently infected with bovine diarrhoea virus (BVD PI). Given the small number of animals (n = 15) on each farm in 2009 this was deemed necessary as one BVD PI could have significantly skewed results. No animals were screened before the current study to identify BVD PIs as screening would not ordinarily occur in the field unless indicated. All animals on Farms C3 and O1 received two doses of Bovilis Huskvac™ (MSD) before turnout to prevent subsequent parasitic bronchitis.

The FSG on each farm grazed in sub-groups. Farms C3 and O2 separated their FSG into two groups. Farm O1 separated the FSG into four management groups; two of those groups were grazing with older animals. This is shown in Table 5.2.

Farm	Number of FSG	Breeds Included in FSG Group*	Management Group	Number of Animals in Management group	Number of FSG in Management group
C3	43	HF, HOLX, HFDX, AAX, BBX	1	20	18
			2	25	25
O1	20	BF, AYR, BS, AAX, BrBX	1	18	2
			2	5	5
			3	9	9
			4	8	4
O2	41	AYR, AAX	1	20	20
			2	21	21

Table 5.2. Management groups of the FSG on each farm. * Breeds in FSG group as whole, not separated between different management groups. AAX, Aberdeen Angus cross; AYR, Ayrshire; BrBX, British Blue cross; BF, British Friesian; BS, Brown Swiss; HF, Holstein-Friesian; HOLX, Holstein cross; HRDX Hereford cross.

Figure 5.1 illustrates the liveweights of the FSG at turnout. The FSG on Farm C3 were older and larger in size than the FSG on Farms O1 and O2.

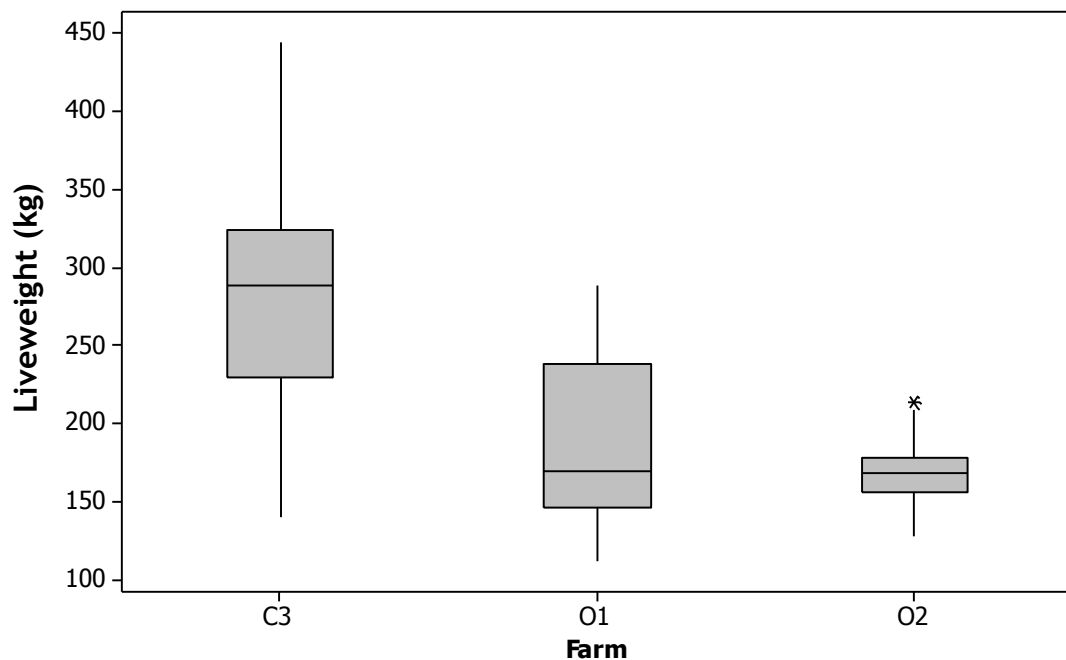


Figure 5.1. Box and whisker plot of liveweight at visit 1 using a weigh-band on each farm.

5.2.3 Experimental Design

Farms were visited at turnout and then monthly until housing (except for September on Farms O1 and O2 as the farmers requested no visit this month due to extra work on the land). Dates of sampling visits on each farm are shown in Table 5.3. At Visit 1 on all farms, each FSG had an estimated liveweight calculated by weigh-band (Coburn[®] weigh tape). On Farm C3, all FSG were also weighed on Ritchie[®] mechanical weigh-scales. Figure 5.2 illustrates the experimental design which is summarised below. At visit 3, eight to ten weeks post-turnout, all FSG had their liveweight gains from turnout calculated. If the liveweight gain of an individual animal was < 0.75 kg/day they were treated with eprinomectin (Eprinex[™] pour-on, Merial). A computer was used at the crush side to readily calculate liveweight gains making it necessary to bring the animals through the crush only once. At visit 4, four weeks later, the liveweight gains of the FSG over the previous four weeks were calculated. Animals that had not been treated previously and were growing <0.75 kg/day were treated with eprinomectin. Eprinomectin has a persistency of activity of twenty-eight days (Cramer *et al.*, 2000) thus, animals previously treated at visit 3 that had a liveweight gain < 0.75 kg/day between visits 3 and 4 were not treated

again. All animals growing ≥ 0.75 kg/day were not treated with an anthelmintic. As farmers from farms O1 and O2 had requested a months respite from sampling in September, no treatment was planned for this visit on Farm C3 either. No treatment was planned for visit 6 at housing unless it was felt that there was a high risk of Type II ostertagiosis.

Farm	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6
O1	04/05/2010	07/06/2010	06/07/2010	03/08/2010		08/10/2010
O2	30/04/2010	04/06/2010	08/07/2010	12/08/2010		20/10/2010
C3	12/05/2010	17/06/2010	09/07/2010	11/08/2010	01/09/2010	01/10/2010

Table 5.3. Dates of sampling visits for each farm.

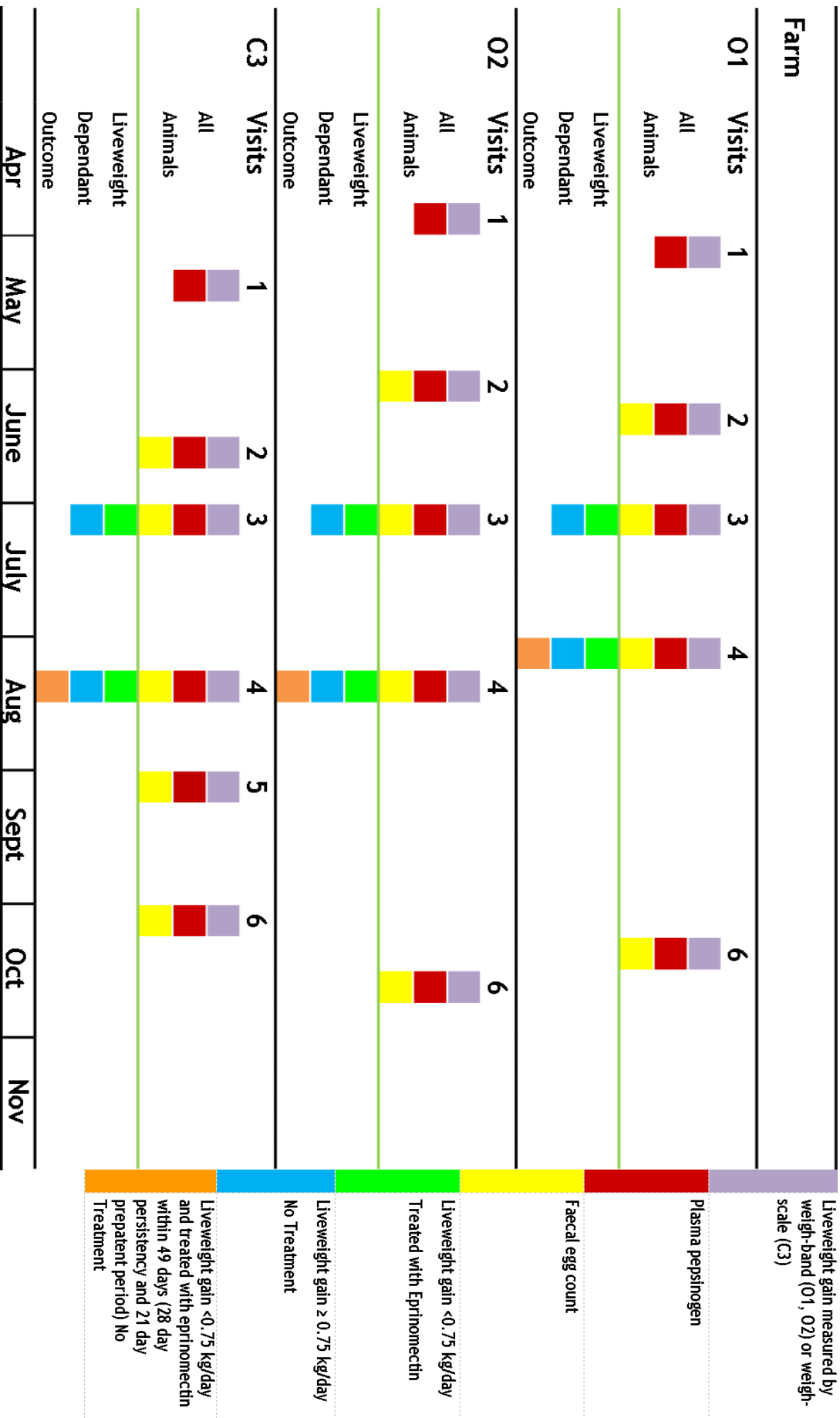


Figure 5.2. Diagrammatic representation of timings of visits on each farm and application of anthelmintic in all FSG. At visit 3 the liveweight gain of each animal was calculated from visit 1. If the animal was growing at <0.75 kg/day it was treated with eprinomectin pour-on, shown in green. No treatment was given to an animal growing ≥0.75 kg/day. At visit 4 the liveweight gain between visits 3 and 4 was calculated. Only animals not previously treated with eprinomectin and growing <0.75 kg/day were treated.

5.2.4 Laboratory Analysis

Each calf had a blood sample obtained via jugular or coccygeal venepuncture for plasma pepsinogen analysis (all visits) and a faecal sample taken *per rectum* obtained at visits 2, 3, 4, 5 and 6 for faecal egg count, lungworm and liver fluke monitoring. Larval culture was performed on faeces at visit 3. Laboratory techniques have been described in detail in Chapter 2.2.11. Plasma pepsinogen and faecal egg counts were analysed as both have been advocated in targeted treatment protocols in youngstock. It was felt that monitoring of plasma fructosamine and *O. ostertagi* antibody concentrations would not add significant value to the study.

5.2.5 Statistical Analysis

The Spearman's rank correlation test was used on non-normally distributed data to investigate association with liveweight gain. Statistical analysis of the data was performed using Microsoft Excel and Minitab 16 for Windows.

5.2.6 Meteorology

The study was conducted during the grazing season of 2010. The temperature and precipitation during this grazing season are shown in Figure 5.3. In 2010, the driest spring was experienced since 1984 and ranks equal eleventh driest in the UK from 1910. May and June were particularly dry months in the west of Scotland, with less than fifty per cent of the average monthly rainfall ordinarily recorded. July was much wetter than normal in the west and north of Scotland, with over two hundred per cent of average rainfall widely. Temperatures were slightly cooler than average early spring (Met Office, UK). High rainfall during July will have broken up faecal pats thus disseminating infective larvae over the pasture making mid-summer 2010 high risk for gastrointestinal parasitism.

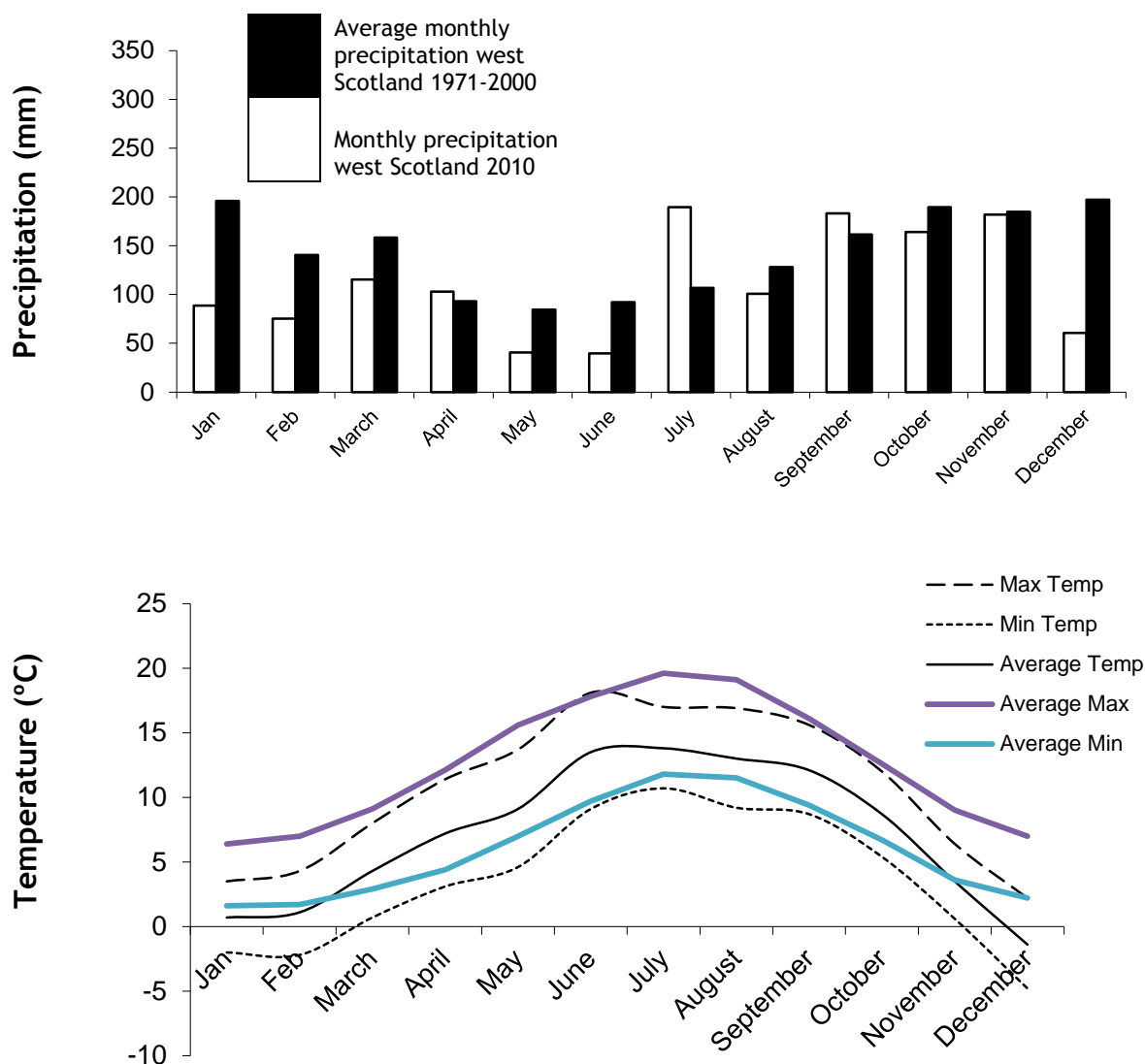


Figure 5.3. Meteorological data from the Paisley weather station, west Scotland in 2010. Top figure shows the comparison between total monthly precipitation for the west of Scotland (open bars) and the average of the total monthly precipitation for the west of Scotland between 1971 and 2000 (closed bars). Bottom figure shows maximum (large dashed), minimum (small dashed) and average (smooth line) monthly temperature, in the west of Scotland. The purple line shows maximum and blue line minimum average monthly temperature for the west of Scotland between 1971 and 2000.

5.3 RESULTS

An overall summary of the results are tabulated in Table 5.5 and summarised further graphically below.

5.3.1 Liveweight Gain

Mean liveweight gain (\pm Standard Deviation) over the grazing season was 0.75 ± 0.23 kg/day on Farm C3, 0.69 ± 0.28 kg/day on Farm O1 and 0.82 ± 0.13 kg/day on Farm O2.

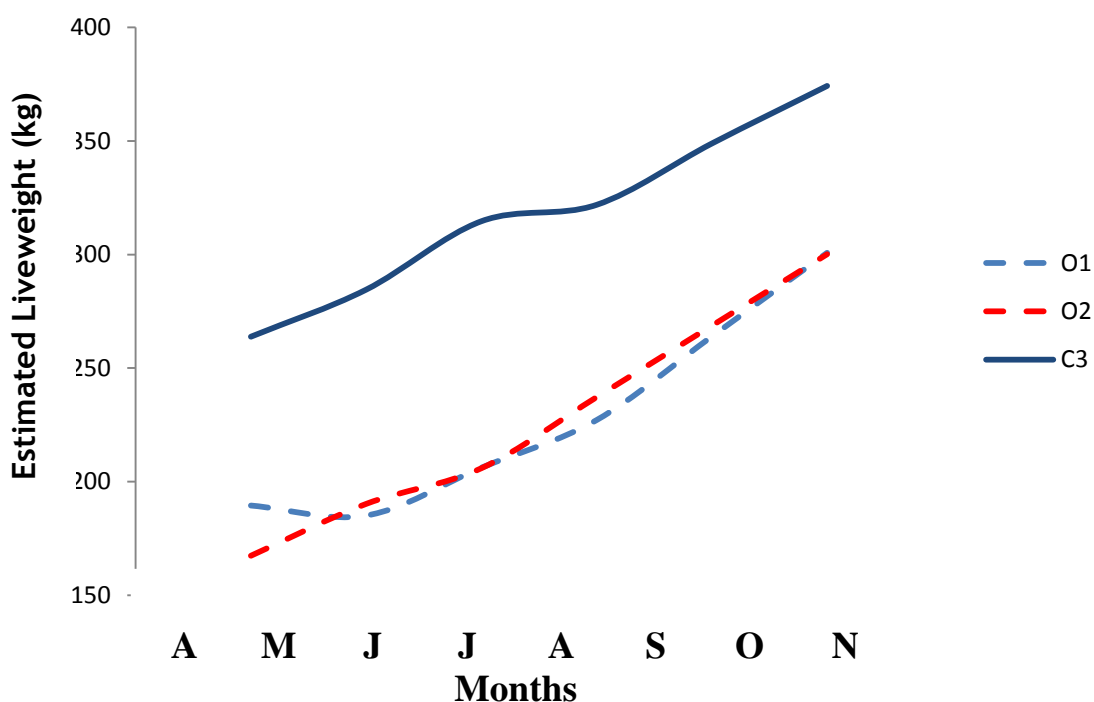


Figure 5.4. Growth curves of FSG on the three study farms over the grazing season.

The growth curves of all three farms are shown in Figure 5.4. In the middle of the grazing season the growth curve for farm C3 tapers and becomes almost horizontal. Early in the grazing season the FSG on Farm O1 reduced their liveweight before resuming growth at the beginning of June.

5.3.2 Targeted Selective Anthelmintic Treatment

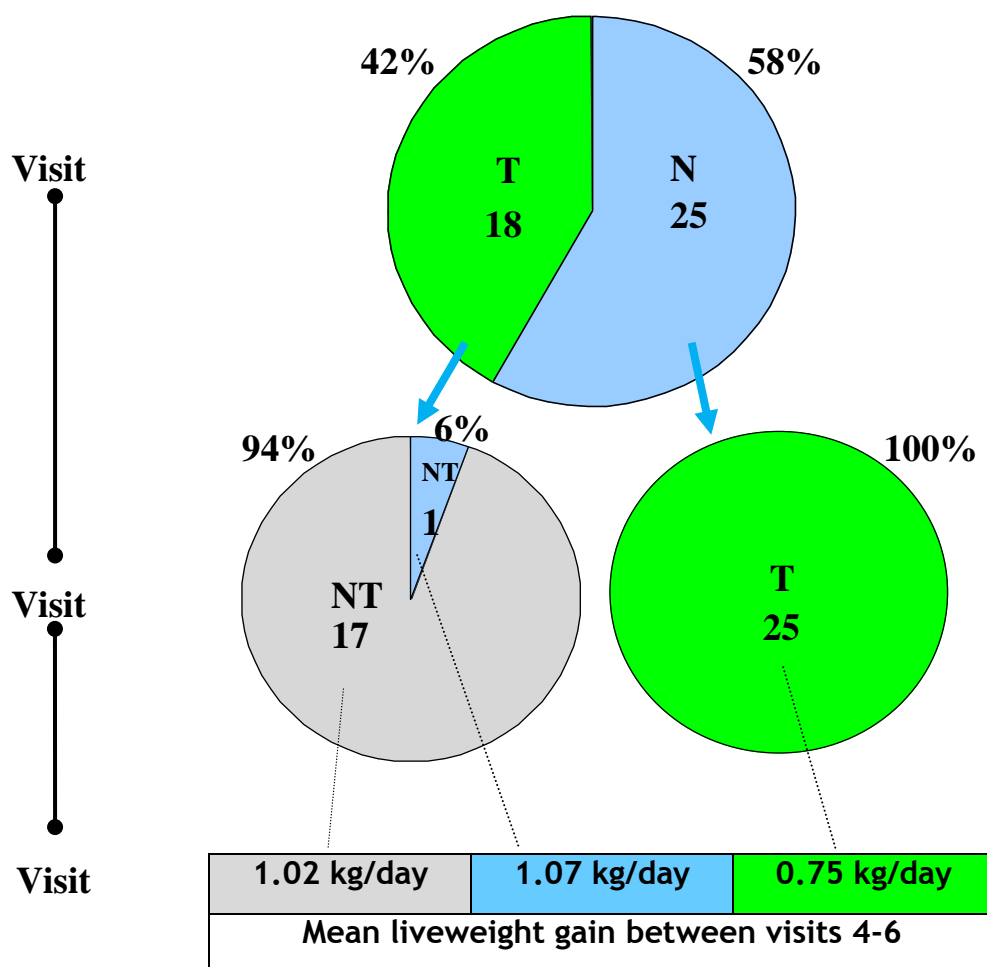
By the end of the grazing season all FSG on Farm C3 had been treated once with eprinomectin, 18/19 had been treated once on Farm O1 and 37/41 had been treated once on Farm O2. Table 5.4 gives a breakdown of the number of FSG treated at visit 3 or 4 on each farm.

Visits 1-3						
Farm	Liveweight Measurement	<0.75 kg/day		≥0.75 kg/day		Total
C3	Weigh-scale	18		25		43
O1	Weigh-band	17		2		19
O2	Weigh band	29		12		41
Visits 3 - 4						
		<0.75 kg/day		≥0.75 kg/day		
C3	Weigh-scale	17	1	25	0	
O1	Weigh-band	11	6	1	1	
O2	Weigh-band	22	7	8	4	

Table 5.4. Number of FSG on each farm growing <0.75 kg/day or ≥0.75 kg/day between visits 1-3 and 3-4.

5.3.2.1 Conventional Farm 3

Figure 5.5 illustrates that at visit 3, 18/43 FSG were not growing at the target rate of 0.75 kg/day and were treated with eprinomectin. At visit 4, four weeks later, only one of these calves had reached the target liveweight gain. Twenty-five FSG were not treated with eprinomectin at visit 3 and by visit 4 none of these calves over the past month had been able to maintain the target growth rate so all were treated with eprinomectin. The average liveweight gain of this group from visit 4 to housing was 0.75 kg/day.



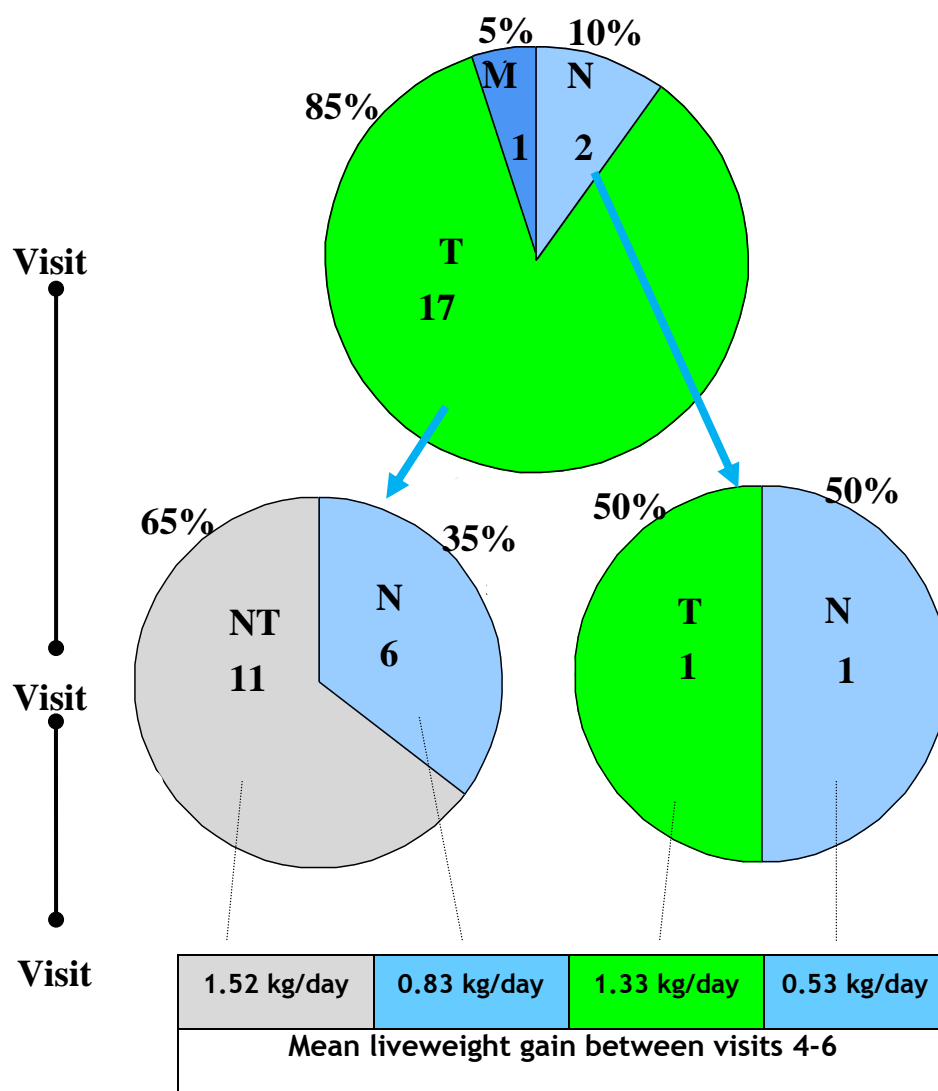
T	Treated with Eprinomectin Liveweight gain <0.75kg/day
NT	Not Treated Liveweight gain \geq 0.75 kg/day
NT	Not Treated Liveweight gain <0.75 kg/day

Figure 5.5. Illustration of the number and percentage of FSG on Farm C3 treated with eprinomectin at visits 3 and 4 and subsequent liveweight gain. T, Treated; NT; Not treated; Numbers of calves in each group are displayed in pie chart segment.

5.3.2.2 Organic Farm 1

Figure 5.6 illustrates that at visit 3, 17/19 FSG (one calf could not be identified) were not growing at the target growth rate and were treated with eprinomectin. Of the calves that were treated, by visit 4, six had

reached the target growth rate. Two calves were not treated at visit 3 and by visit 4 one calf required treatment and one did not.

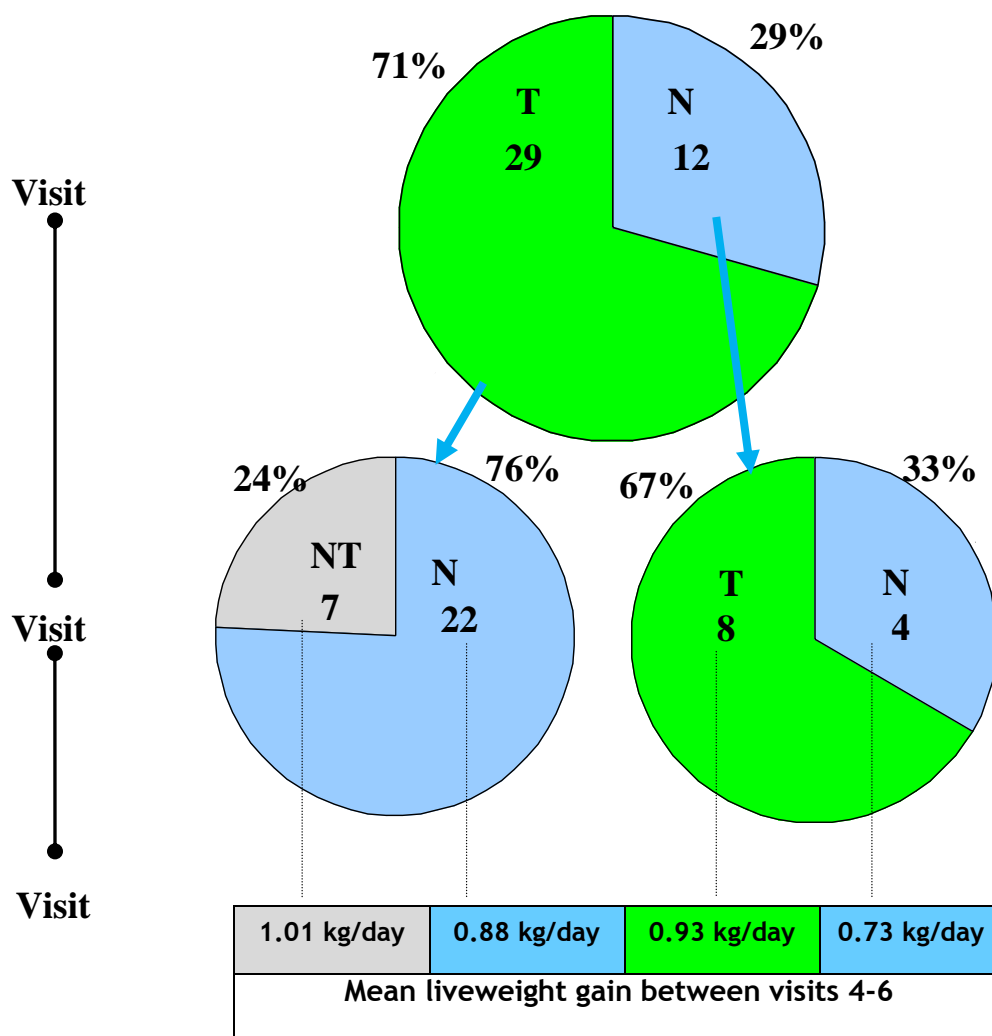


T	Treated with Eprinomectin Liveweight gain <0.75kg/day
NT	Not Treated Liveweight gain ≥0.75 kg/day
NT	Not Treated Liveweight gain <0.75 kg/day
M	FSG not identified

Figure 5.6. Illustration of the number and percentage of FSG on farm O1 treated with eprinomectin at visits 3 and 4 and subsequent liveweight gain. T, Treated; NT; Not treated; Numbers of calves in each group are displayed in pie chart segment.

5.3.2.3 Organic Farm 2

Figure 5.7 illustrates that at visit 3, 29/43 FSG did not reach their target growth rate and were treated with eprinomectin.



T	Treated with Eprinomectin Liveweight gain <0.75kg/day
NT	Not Treated Liveweight gain ≥0.75 kg/day
NT	Not Treated Liveweight gain <0.75 kg/day

Figure 5.7. Illustration of the number and percentage of FSG on Farm O2 treated with eprinomectin at visits 3 and 4 and subsequent liveweight gain. T, Treated; NT; Not treated; Numbers of calves in each group are displayed in pie chart segment.

Twenty-two of these FSG had reached the target growth rate at visit 4, leaving seven in the group that had not. Of the twelve calves that were not treated at visit 3, eight of these required treatment at visit 4 leaving four calves not requiring any anthelmintic treatment over the grazing season (~10% of the FSG group).

Farm	Visit	EWt (kg) ± SD		Wt (kg) ± SD		BCS (range)		Pep (iu/l) ± SD		FEC(epg) (range)		Visits	EGR (kg/day) ± SD		GR (kg/day) ± SD	
C3	1	277	± 79	264	± 81	3.0	(1.5-4)	0.6	± 0.2			1-6	0.99	± 0.26	0.75	± 0.23
	2	292	± 71	285	± 75	3.0	(2-4)	1.1	± 1.3	0	(0)	1-2	0.42	± 0.55	0.61	± 0.70
	3	334	± 75	315	± 71	3.0	(1.5-4)	1.0	± 0.5	0	(0-50)	2-3	1.60	± 0.94	1.00	± 0.51
	4	363	± 77	322	± 70	3.0	(1-4)	1.1	± 0.5	0	(0-150)	3-4	0.90	± 0.85	0.31	± 0.33
	5	378	± 69	349	± 69	3.0	(2-4)	1.6	± 0.6	0	(0-50)	4-5	0.56	± 1.07	0.93	± 0.23
	6	420	± 68	374	± 65	3.0	(2-4)	1.3	± 0.5	0	(0-350)	5-6	1.54	± 1.15	0.95	± 0.72
O1	1	190	± 12			2.8	(1-4.5)	0.7	± 0.1			1-6	0.69	± 0.28		
	2	185	± 44			2.8	(1-5)	1.7	± 0.9	0	(0)	1-2	-0.13	± 0.57		
	3	206	± 46			1.9	(1-4)	2.3	± 1.1	358	(0-1150)	2-3	0.74	± 0.50		
	4	227	± 57			2.0	(1-4)	2.0	± 0.8	0	(0-150)	3-4	0.60	± 0.68		
	6	301	± 77			2.7	(1-4)	3.2	± 1.7			4-6	1.20	± 0.97		
O2	1	167	± 20			3.0	(2.5-4)	0.5	± 0.1			1-6	0.82	± 0.13		
	2	190	± 21			4.0	(3-4)	0.9	± 0.4	0	(0-550)	1-2	0.63	± 0.48		
	3	206	± 24			3.0	(2-4)	1.5	± 0.5	0	(0-50)	2-3	0.71	± 0.53		
	4	237	± 27			3.0	(2-4)	1.5	± 0.5	0	(0-100)	3-4	0.95	± 0.60		
	6	300	± 33			3.0	(1.5-4)	2.0	± 0.6			4-6	0.90	± 0.32		

Table 5.5. Summary of data collected from all farms at each visit. EWt, mean liveweight from weigh-band; Wt, mean Liveweight by weigh-scale; BCS, median body condition score; Pep, mean plasma pepsinogen; FEC, median Faecal egg count; EGR, mean liveweight gain by weigh-band; GR, mean liveweight gain by weigh-scale.

5.3.3 Parasitic Biomarkers

5.3.3.1 Pepsinogen

Plasma pepsinogen concentrations increased on all farms during the grazing season reaching a peak late in the summer, as illustrated in Figure 5.8. Farm O1 had high plasma pepsinogen concentrations at housing (3.2 ± 1.7 iu/l).

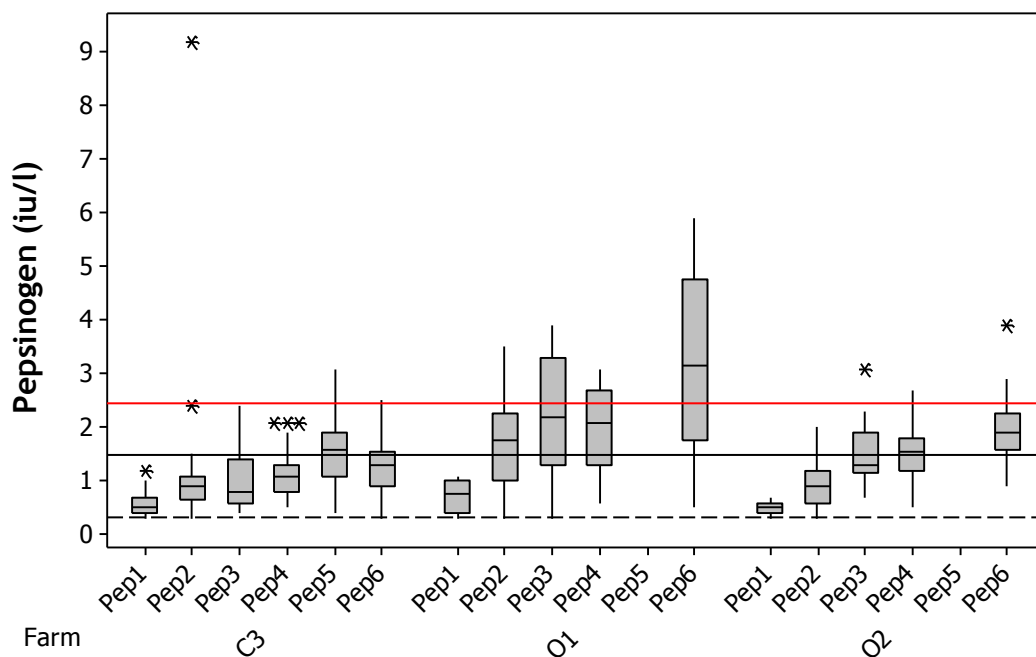


Figure 5.8. Box and whisker plot showing the plasma pepsinogen values on all farms on all visits. Values above; top red line indicates abomasal parasitism likely to be associated with clinical disease; middle line indicates abomasal parasitism likely to cause impaired liveweight gain; bottom dashed line indicates exposure to abomasal parasitism (SAC Laboratory).

As shown previously in Chapter Two, plasma pepsinogen concentrations showed no correlation with growth rate (Figure 5.9). The Spearman's rank correlation was -0.27.

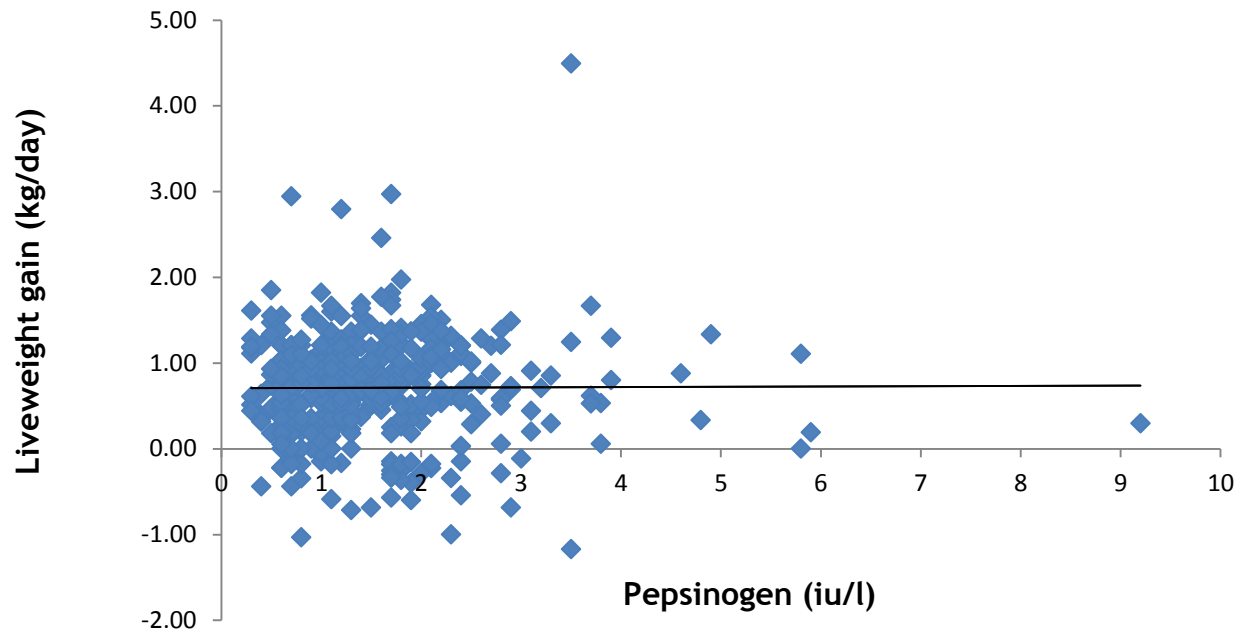


Figure 5.9. Correlation between plasma pepsinogen concentration and liveweight gain.

5.3.3.2 Faecal Egg Count

Faecal egg counts on all farms at each visit are illustrated in Figure 5.10. Farm O1 recorded a mean faecal egg count at visit 3 (>200 epg). Faecal egg counts on Farms O1 and O2 at visit 6 were spoiled in the laboratory.

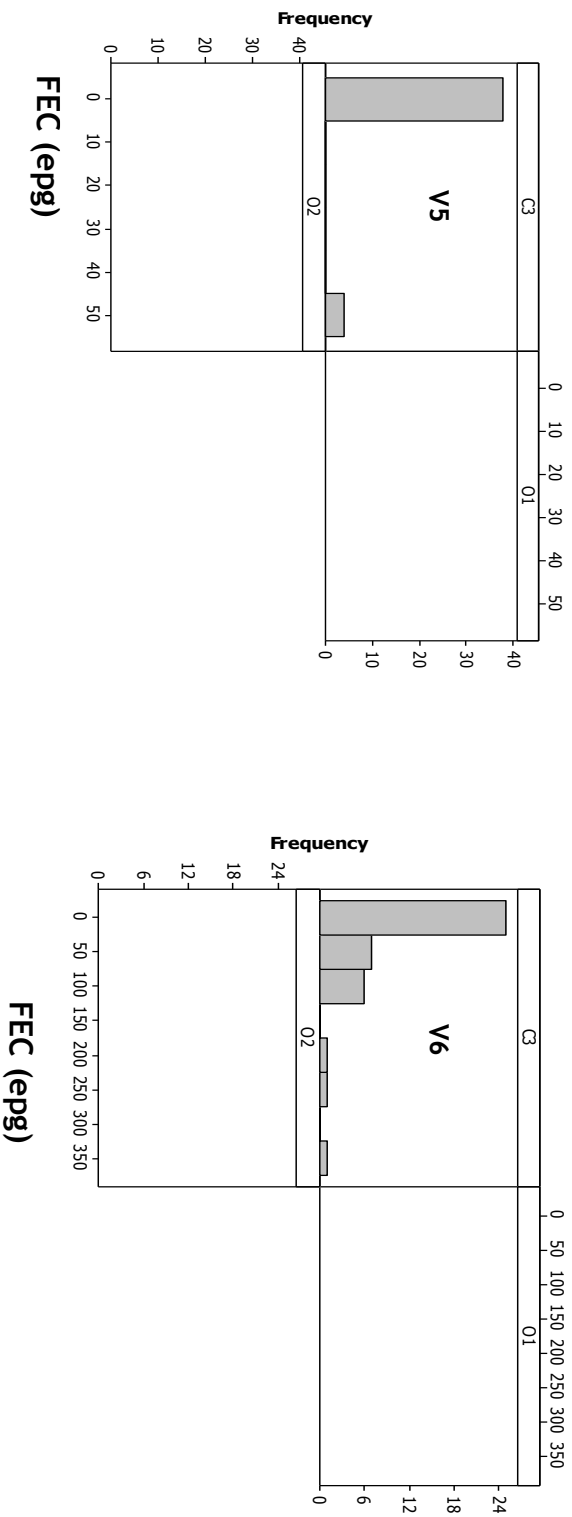
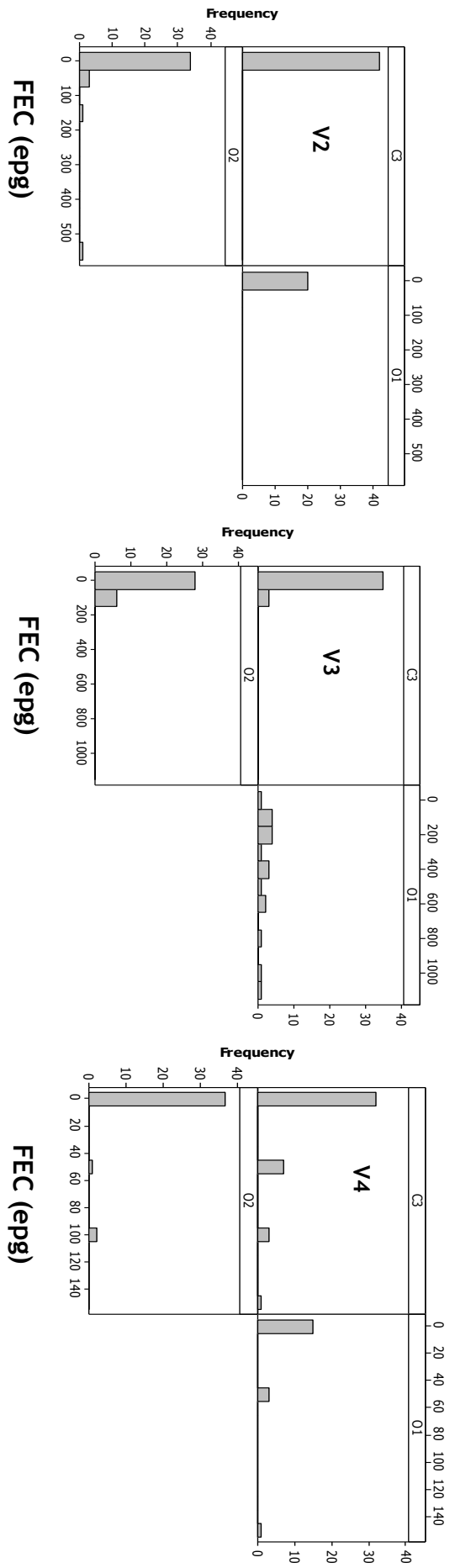


Figure 5.10. Histograms of faecal egg count (FEC) for each farm at visits 2-6

As shown previously in Chapter Two, faecal egg counts showed no correlation with growth rate (Figure 5.11).

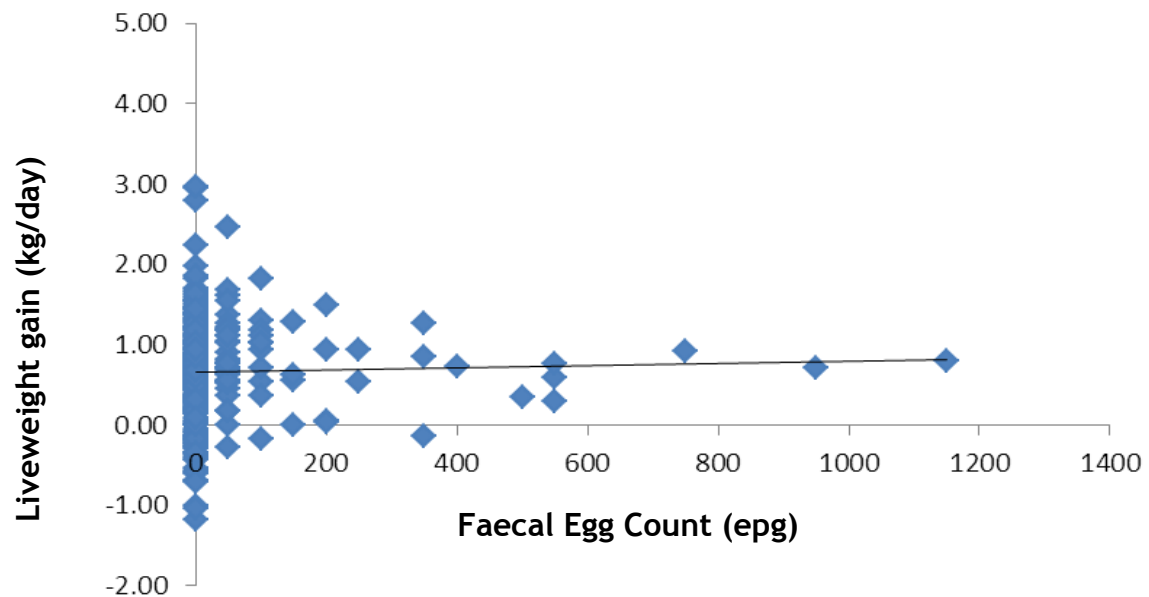


Figure 5.11. Correlation of faecal egg count and growth rate.

5.3.3.3 Larval Culture

Larval speciation was undertaken at visit 3, mid-grazing season before anthelmintic treatment. The results are tabulated in Table 5.6. The majority of larvae cultured were *C. oncophora*.

Farm	<i>O. ostertagi</i>	<i>C. oncophora</i>
C3	5	23
	18%	82%
O1	6	22
	21%	79%
O2	2	51
	4%	96%

Table 5.6. Numbers and percentage of *O. ostertagi* and *C. oncophora* larvae at visit 3 on each farm.

5.4 DISCUSSION

The target liveweight gain for the first season grazers (FSG) was 0.75 kg/day over the grazing season. Farms C3 and O2 achieved this target on average over the entire grazing season. Farm O1 did not reach this target but did increase liveweight gain by 50% from the previous year (see Chapter Two). As mentioned in Chapter Two, Farm O1 consisted of some breeds of cattle which are smaller than the typical UK Holstein-Friesian or Ayrshire breeds.

5.4.1 Performance-based Targeted Selective Anthelmintic Treatment

Table 5.7 summarises liveweight gain and anthelmintic exposure on each farm in 2009 and 2010. On Farm C3, the liveweight gain reduced by 19% in 2010 compared to the previous year.

	2009	2010	% difference
Farm	Liveweight Gain (kg/day)		
C3	0.93	0.75	-19
O1	0.46	0.69	+50
O2	0.57	0.82	+44
	Anthelmintic Exposure (days/calf)		% difference
C3	120	28	-77
O1	28.2	26.5	-6
O2	2	25.2	+1160

Table 5.7 Liveweight gain and anthelmintic exposure (calculated as persistency in days of anthelmintic per calf) on each farm in 2009 and 2010.

Possible reasons for this are: 1) in 2010 the FSG went out to grass earlier than the group the previous year and were therefore not given supplementary feed indoors; 2) In 2009 the group consisted of only beef-cross animals; 3) increased gastrointestinal parasite challenge; 4) respiratory disease. The mean liveweight gain of the FSG between visits 3 and 4 was unusually low at 0.31 kg/day. The animals presented with purulent nasal discharge and pyrexia to the veterinary surgeon shortly

after visit 4 and were treated with antibiotics. It is likely that respiratory infection affected the liveweight gains of the FSG during this time. As part of a regular monitoring programme, a bulk milk sample was taken on 25/09/2010 and was found to be positive for IBR virus antibodies (Percentage Positivity was 17%). It is likely that a combination of all of the reasons given above account for the reduction in liveweight gain in 2010. The consideration of alternative reasons for poor liveweight gains in youngstock should always be considered when liveweight is going to be used as an indication for anthelmintic treatment.

Farms O1 and O2 increased the liveweight gains of the FSG in 2010 by 50% and 44% respectively. Farm O2 exposed the FSG to 1160% more days of anthelmintic than in 2009; however, ~ 10% of the group were left untreated in 2010. On both farms, the FSG that had no anthelmintic treatment showed the lowest liveweight gains (O1, 0.53 kg/day; O2 0.73 kg/day) between visits 4 and 6. After discussion with the farmers on each farm regarding these FSG, no anthelmintic treatment was applied as both farmers felt that these calves showed no other signs of clinical disease (dull coats and diarrhoea).

5.4.2 Biomarkers

Faecal egg counts and plasma pepsinogen concentrations were analysed throughout the grazing season and an evaluation of their correlation with liveweight gain was undertaken as both have been advocated for use in targeted selective anthelmintic regimes (Charlier *et al.*, 2011; Taylor, 2010i).

5.4.2.1 Faecal Egg Counts

An analysis of the number of calves that would have been treated with anthelmintic at visit 3 if faecal egg counts were implemented in a TST (where individual animals are treated) regime was performed. The results are presented in Figure 5.12 and show only ten FSG had FEC of ≥ 250 epg, all on Farm O1. Fifty-four calves, growing < 0.75 kg/day would not have been treated with anthelmintic. If faecal egg counts had been applied in a targeted treatment (TT) approach (where all

animals in a group are treated) at visit 3, only the FSG on Farm O1 would have been treated as the mean FEC was ≥ 250 epg.

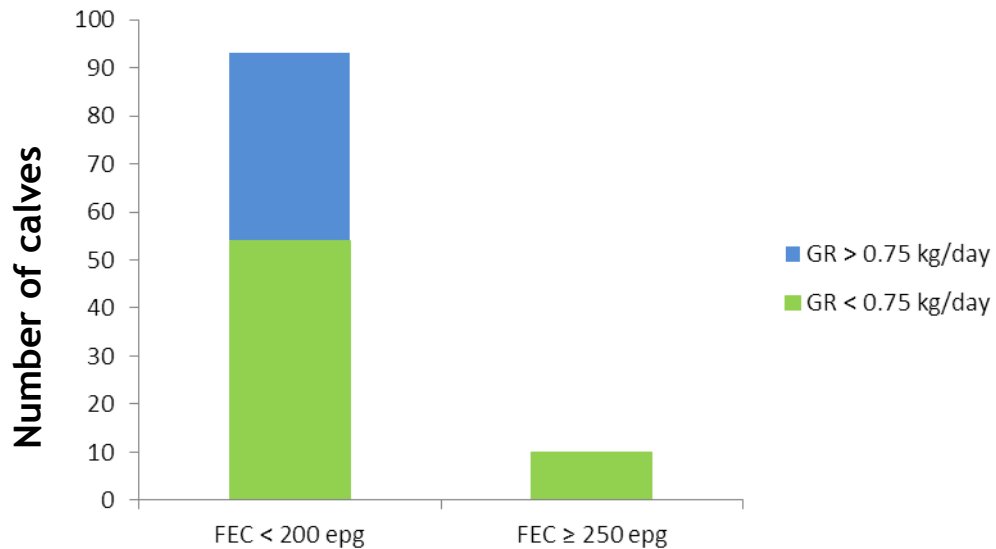


Figure 5.12. Bar chart of number of FSG with FEC of <250 epg or ≥ 250 epg at visit 3 that were growing at <0.75 kg/day and treated with eprinomectin (green) or growing at ≥ 0.75 kg/day and not treated (blue).

Given the results of the correlation between faecal egg counts and liveweight gain, it comes as no surprise that different animals are chosen for treatment when the two methods are used separately in a TST regime. This discrepancy is likely due to the natural resilience some calves possess, where growth rate is uncompromised in the face of gastrointestinal parasite challenge. Some calves will grow well despite a worm burden, where adult worms are producing eggs which are subsequently recorded in faecal egg counts.

5.4.2.2 Pepsinogen

Charlier *et al.* (2011) discussed the use of plasma pepsinogen concentrations at housing as a useful indicator of gastrointestinal challenge for the next year of first season grazers. Plasma pepsinogen levels were separated into three different groups (1, <1.2 iu/l; 2, 1.2-3.5 iu/l; 3, >3.5 iu/l) and recommendations provided, taking into account length of grazing season and anthelmintic programme used on farm, for the next group of FSG the subsequent year. Looking at this study, mean plasma pepsinogen concentrations at housing in 2009 for Farm C3, O1 and O2 were 0.54 iu/l,

0.87 iu/l and 1.15 iu/l respectively (All fall into Group 1 above). So following the recommendations by Charlier *et al.* (2011), it would be recommended that Farm C3 in 2010 (which had a short grazing season and used prophylactic anthelmintic) should increase the grazing season and/or reduce anthelmintic use for their FSG. In retrospect, both of these recommendations were in fact implemented in the 2010 study. Similar recommendation would also have been made for FSG in 2010 on Farms O1 and O2. However, neither could be implemented on these farms as reducing anthelmintic use may have seriously compromise production and welfare, and the grazing season could not be extended. It should be noted however, that the reproducibility of plasma pepsinogen concentration between laboratories was found to be poor and so implementing advice from this study (Charlier *et al.*, 2011) using plasma pepsinogen concentration from a different laboratory than the one used in the paper is likely to be flawed.

5.5 CONCLUSION

Applying a performance-based targeted anthelmintic regime treatment in the field is possible and using it on farms where anthelmintic treatment was already minimal, such as organic farms, increased liveweight gains in first season grazers without significantly increasing anthelmintic treatment. Applying a TST regime to a conventional farm where previously a suppressive anthelmintic treatment had been applied may have reduced liveweight gains in the first season grazers (FSG) but maintained it at an acceptable level. However, there was only one conventional farm in the study and respiratory disease in the FSG was apparent. Using a performance-based TST approach in the field requires regular handling of first season grazers which is labour intensive and time-consuming. Faecal egg count and plasma pepsinogen concentration were found not to correlate with liveweight gain.

CHAPTER SIX

CHAPTER SIX: GENERAL DISCUSSION

The work described in this thesis was designed to investigate the current impact of parasitic gastroenteritis on organic and conventional dairy farms in first season grazing youngstock in Scotland, and to examine potential markers of significant parasite challenge within individual calves, in order to target these calves with an anthelmintic treatment. It was felt particularly that any recommendations should be practical and easily implemented on-farm, and optimise anthelmintic usage, with regard to animal health, welfare and performance on both organic and conventional farms.

The salient findings were:

- Calves experiencing their first grazing season under organic farming systems had lower liveweight gains over the grazing season compared with calves reared in conventional systems;
- Markers of gastrointestinal parasite challenge provided evidence that the lower liveweight gains in the organically reared calves were most likely due to gastrointestinal parasitism;
- Faecal egg counts, plasma pepsinogen concentration, plasma fructosamine concentration and plasma *Ostertagia ostertagi* antibodies do not correlate with liveweight gain;
- By using sub-optimal liveweight gain as a treatment trigger, in a targeted treatment approach towards individual animals within a group, liveweight gains in organic youngstock were higher than those observed under the previous anthelmintic management regimes.

6.1 GASTROINTESTINAL PARASITES IN ORGANICALLY AND CONVENTIONALLY REARED CALVES.

The organically farmed first season grazers in the 2009 study showed high gastrointestinal parasite challenge, indicated by higher faecal egg counts (FEC), higher plasma pepsinogen concentrations and lower liveweight gains when compared with their conventional contemporaries. The major difference between the management of the first season grazers (FSG) on the organic and conventional farms was the administration of anthelmintic. The conventional producers, in this study, exposed FSG to 652% more days of anthelmintic over the grazing season than the organic producers. Essentially, the organic producers fulfilled the ethos of organic production, reducing anthelmintic usage and demonstrating necessity for anthelmintic treatment, with the use of faecal egg counts. One of the organic farms in the study (Farm O1) had implemented a targeted selective treatment programme (TST) where any calves with a FEC \geq 250 epg were treated with anthelmintic, and all other calves were not, despite poor liveweight gains or other clinical signs of gastroenteritis in some of these calves. During the study year of 2009, one calf on that farm (O1) was euthanased due to poor liveweight gain during the grazing period. On post-mortem examination, the calf was found to have abomasal pathology attributed to *Ostertagia ostertagi* and *Dictyocaulus viviparus* adult nematodes were present in the trachea and bronchi. The calf had not been treated with an anthelmintic due to FEC results of $<$ 250 epg on two separate occasions. The other two organic farms (Farms O2 and O3), implemented a targeted treatment regime (TT), where FEC were taken from a subset of the FSG, and once the mean FEC \geq 250 epg, all calves were treated with anthelmintic. Where the organic certifying bodies are concerned, there is acceptance that anthelmintics are often necessary in controlling parasitic gastroenteritis, but producers are advised that in these circumstances, they may treat individual animals with anthelmintics after they have checked that they are infected (for example, through faecal egg counts) and may only use treatments previously agreed in a health plan. With the permission of the organic body, producers may use anthelmintics on a whole herd, or group of animals, but only as part of a disease control programme, which must have been agreed in the health plan. Thus, the use of prophylactic anthelmintics, which aim to reduce pasture contamination early in the grazing season, are accepted if described in the health plan on organic farms. None of the farmers in this study, either because of striving for the 'organic ethos' of reducing

dependency on anthelmintics, or because of misinformation, used anthelmintics prophylactically. Where gastrointestinal parasitism is clearly causing clinical disease, such as in Farm O1, it surely would be prudent to prevent the inevitable rather than to use the 'fire-brigade treatment' approach evident in this study.

The use of faecal egg counts as the sole indication for the necessity of anthelmintic treatment, on all of the organic farms in the study, must be questioned. Where FEC were used in a TST approach on Farm O1, clinical parasitic gastroenteritis leading to the death of a calf, occurred. Farms O2 and O3 ordinarily obtained FEC when they were already suspicious of parasitic gastroenteritis in their FSG, confident that a FEC result that would indicate the need for treatment. As farmers have to pay for the FEC test, it is human nature that they are likely to wait to perform the FEC, so that they possibly only have to pay for one laboratory test. Given the ubiquitous nature of gastrointestinal parasitism, FSG given no anthelmintic treatment early in the grazing season (on contaminated pasture) will inevitably, as a group, produce a mean FEC of ≥ 250 epg; a fact that many organic farmers have surely grasped. It may be true to extrapolate that on some organic farms, the assumed need to use FEC, in order to justify an anthelmintic treatment, exposes FSG calves to longer periods of subclinical parasitic gastroenteritis during the early and mid-grazing season, affecting liveweight gain and likely compromising the welfare of these animals. Furthermore, as FEC have been shown poor correlation with parasite burden or liveweight gain, the use of FEC in a TST regime cannot be recommended for FSG. Although FEC appear to be engrained in 'sustainable' gastrointestinal parasite control, there appears to be no good evidence for their use in targeted anthelmintic approaches in youngstock at grass, either in conventional or organic systems.

The reduced liveweight gains seen in FSG with significant gastrointestinal parasite challenge may be expected to return to a similar liveweight gain as that observed in youngstock not exposed to gastrointestinal parasite challenge when the animals are housed. However, the effects of gastrointestinal nematode infections on growth performance of calves have actually been shown to last during the subsequent housing period (Van Adrichem and Shaw, 1977; Jorgensen *et al.*, 1978; Tornquist and Tolling, 1987). Some compensatory growth has been reported to occur after housing in groups of calves which were severely affected by nematode infections (Jorgensen

et al., 1978) although it would seem unlikely the animals would return to their potential. Poor liveweight gains, both during grazing and then into the housing period, will increase the age at which dairy heifers can be served for the first time, increasing age at first calving. This was demonstrated in this study (Table 4.4). As discussed in this thesis, an age at first calving greater than 25 - 26 months of age, appears to affect subsequent fertility and longevity within a milking herd and increases the environmental impact of the farm from milk production. Both organic and conventional producers should aim for liveweight gains in their dairy replacements that allow the service of heifers at fifteen months of age. Conventional producers should look towards methods of anthelmintic administration in youngstock that will preserve the efficacy of products in the future, whilst maintaining adequate liveweight gains. Organic producers must consider the environmental and welfare considerations of significant gastrointestinal parasite infections in youngstock, and use appropriate methods for the indication of significant parasite challenge and consequent anthelmintic treatments that still support the organic ethos.

6.2 HEART-GIRTH MEASUREMENT AS AN INEXPENSIVE METHOD OF ESTIMATING LIVWEIGHT GAIN IN CALVES DURING THEIR FIRST YEAR AT GRASS

Both Otte *et al.* (1992) and Ploeger *et al.* (1990iv) concluded that measuring liveweight gain, either by weighing calves or by measuring heart-girth, produced quite similar results. It was also clear from the literature (Dingwell *et al.*, 2006; Heinrichs *et al.*, 2007), and this small study, that there is good correlation between liveweight estimated from a weigh-band (using heart-girth) and the measured liveweight of the animal. Therefore, it was felt that the use of a weigh-band on Farms O1 and O2, in 2010 to estimate liveweight gain for the use in a TST programme, was adequate when weigh-scales were not available.

There was concern regarding the significance of the error of the weigh-band, when liveweight is divided by number of days, to produce a liveweight gain figure. The example given in Chapter Three was that a 150 kg animal, growing at 0.75 kg/day, would expect to have gained 26.25kg in five weeks or 52.5 kg in ten weeks. If, at the first visit, the weigh-band liveweight reflected true liveweight but at the second visit the weigh-band predicted a liveweight of 22.8 kg over true liveweight,

then liveweight gain would be predicted as 1.23 kg/day after five weeks or 1.07 kg/day after ten weeks, instead of the true value of 0.75 kg/day. The longer the period of time between liveweight estimation, the less the weigh-band error affects liveweight gain. Having said this, as the weigh-band is used at each visit, the example above is likely to be extreme as it is the liveweight weigh-band difference that is important, not whether the weigh-band reflects the true weight of each animal. Examining the data from 2009 on Farm C3 (both weigh-scale and weigh-band used), did not yield a good correlation between the two methods of calculating liveweight gain ($R^2 = 0.4$). However, the weigh-scale was not available at visit 1, only three visits were possible with the short grazing period on this farm, and so, only the results between visits 2 and 3 could be compared (Figure 6.1), which was a small sample size ($n = 15$). The outlier was likely a misidentified calf.

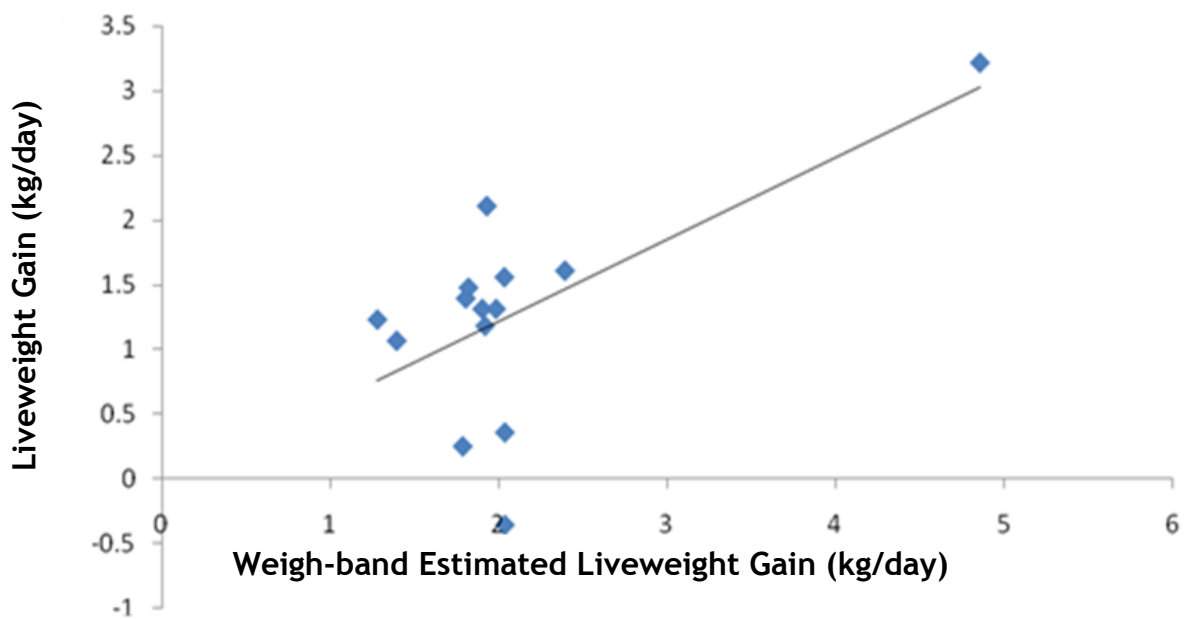


Figure 6.1. Correlation between estimated liveweight gain from weigh-band and liveweight gain using weigh-scales on FSG ($n = 15$) on Farm C3 between visits 2 and 3 in 2009 (Chapter Two).

As Otte *et al.* (1992) and Ploeger *et al.* (1990iv) both showed no indication of a consistent difference between liveweight gain by weigh-scale or weigh-band it was still considered that weigh-band was adequate in estimating liveweight gain.

Weigh-scales, which are considered the gold standard measurement of liveweight, were used on Farm C3 during the 2010 study to calculate liveweight gain used in the TST programme. Out of interest, the estimated liveweight gains were also calculated from the weigh-band and are shown in Table 6.1.

Farm	Visit	EWt (kg) ± SD	Wt (kg) ± SD	Visits	EGR (kg/day) ± SD	GR (kg/day) ± SD
C3	1	277 ± 79	264 ± 81	1-6	0.99 ± 0.26	0.75 ± 0.23
	2	292 ± 71	285 ± 75	1-2	0.42 ± 0.55	0.61 ± 0.70
	3	334 ± 75	315 ± 71	2-3	1.60 ± 0.94	1.00 ± 0.51
	4	363 ± 77	322 ± 70	3-4	0.90 ± 0.85	0.31 ± 0.33
	5	378 ± 69	349 ± 69	4-5	0.56 ± 1.07	0.93 ± 0.23
	6	420 ± 68	374 ± 65	5-6	1.54 ± 1.15	0.95 ± 0.72

Table 6.1. Liveweight and liveweight gain by weigh-scale and weigh-band on Farm C3 during 2010. EWt: liveweight from weigh-band, Wt: Liveweight by weigh-scale, EGR: liveweight gain by weigh-band, GR: liveweight gain by weigh-scale.

The difference in liveweight gain between the two methods was large enough to affect anthelmintic treatment application, using the two different methods of calculating liveweight gain. Figure 6.2 illustrates the number of FSG at visits 3 and 4 on Farm C3 that would have been treated with anthelmintic if estimated, using a weigh-band, rather than liveweight gains from the weigh-scale, to dictate anthelmintic application.

Two more FSG at visit 3 would have been left untreated, but overall the percentage of the FSG treated or not treated is similar to that seen in the study (63% treated by weigh-band compared to 58% by weigh-scale). If the weigh-band had been used to calculate liveweight gain between visits 3 and 4, the results would be very different from those reported in earlier (Figure 5.5). Twelve out of twenty-seven FSG would have been left untreated at both visits, and only four from sixteen FSG treated at visit 3 would have been found to be growing less than the target at visit 4.

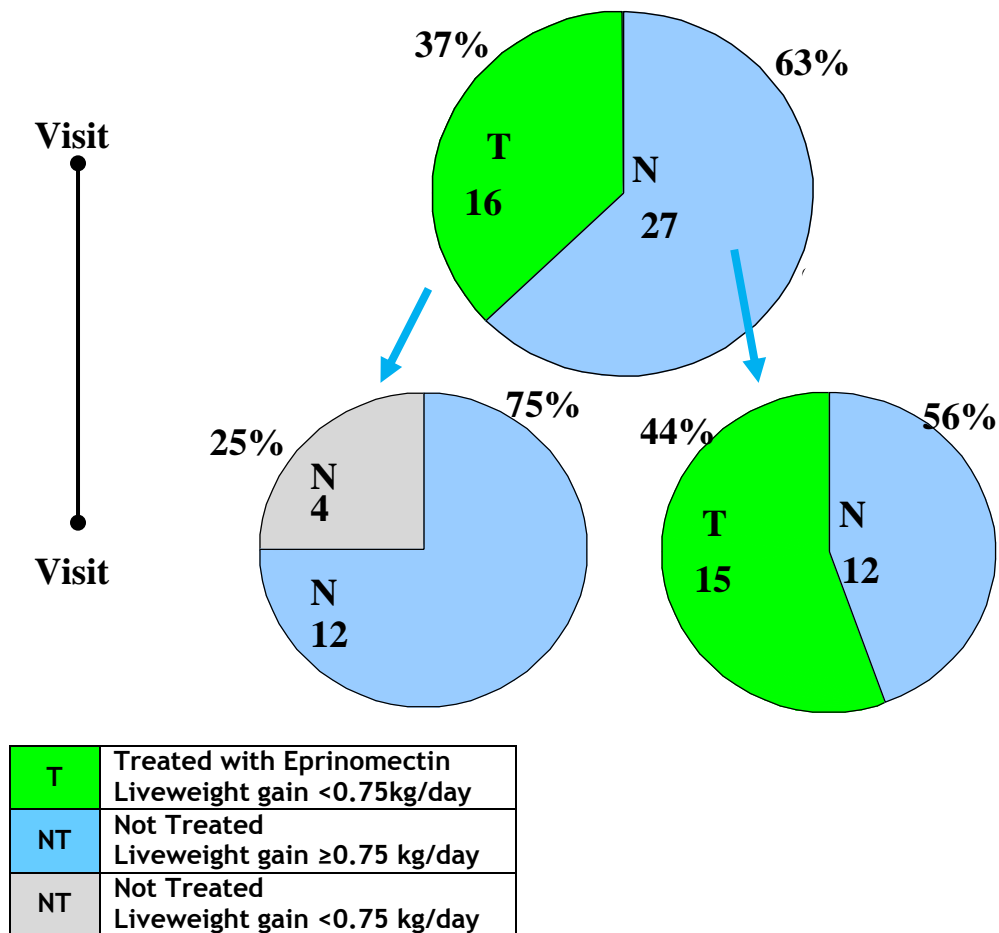


Figure 6.2. Illustration of the number and percentage of FSG that would have been treated with eprinomectin at visits 3 and 4 on Farm C3 if the weigh-band had been used to estimate liveweight. T: Treated, NT: Not treated. Actual numbers of calves in each group are displayed in pie chart segment.

It may be hypothesised that, as the numbers of animals treated at visit 3 were similar for both methods of liveweight calculation, but very different at visit 4, that the time between the visits had a significant effect on the accuracy of liveweight gain calculation when using the weigh-band. As discussed above, the longer the period of time between liveweight estimation, the less the weigh-band error may affect liveweight gain. The number of days between visits 1 and 3 was 63 days and the number of days between 3 and 4 was 28 days. However, when the correlation between weigh-band and weigh-scale liveweights were plotted for the two different time periods, there was no correlation between liveweights using either method between visits 1 and 3 (Figure 6.3) and between visits 3 and 4 (Figure 6.4).

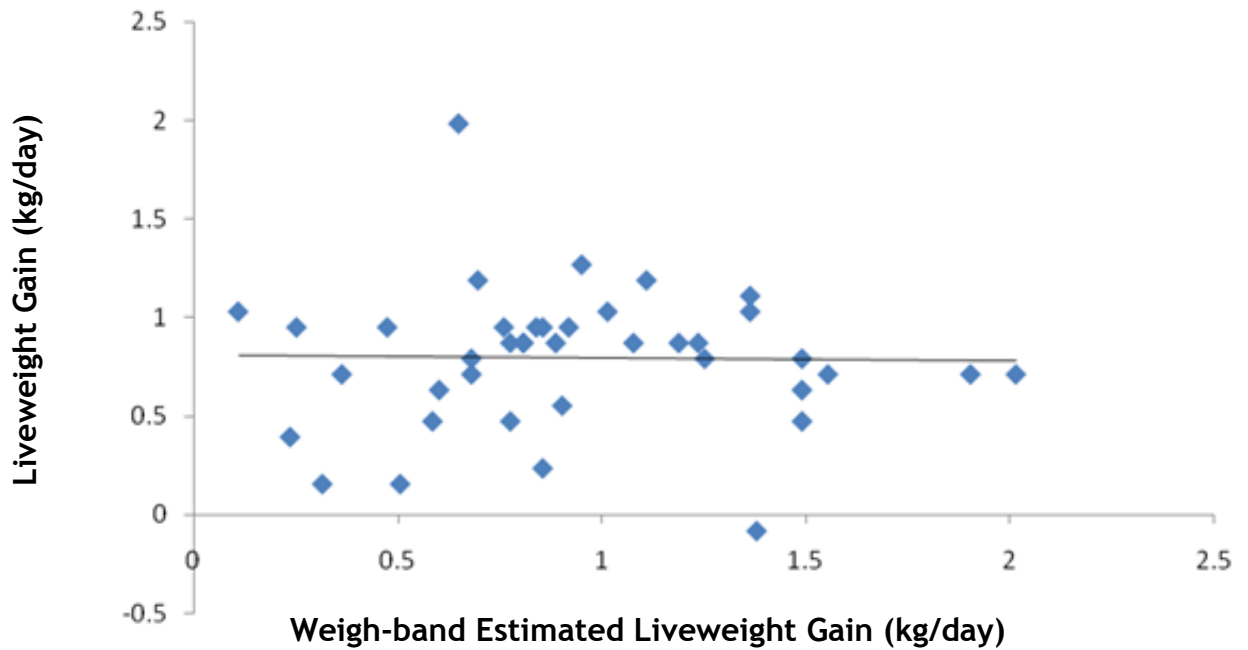


Figure 6.3. Correlation between weigh-band and weigh-scale liveweight gain calculation between visits 1 and 3 (63 days).

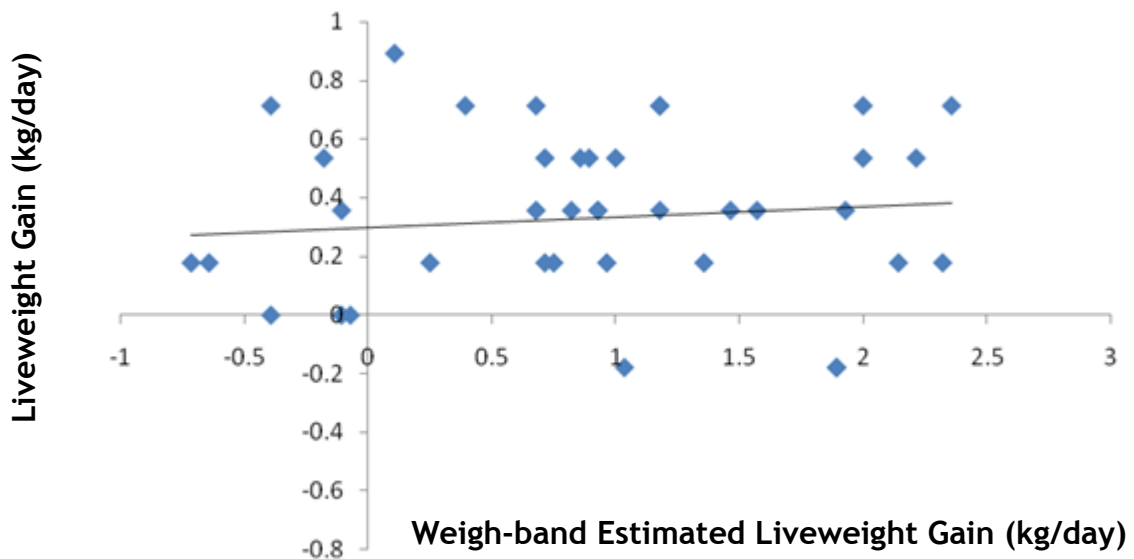


Figure 6.4. Correlation between weigh-band and weigh-scale liveweight gain calculation between visits 3 and 4 (28 days).

The poor correlation between liveweight gain using weigh-band and weigh-scale measurements in this study could be due to the inaccuracy of the weigh-scales used on Farm C3. The weigh scales were calibrated at every visit by taring the scale

to zero and checked for accuracy by weighing on object of known mass. However, it was impossible to adequately check that the scales were accurately weighing livestock at higher liveweights than 80kg (the heaviest mass calibrated). It was necessary to move the weigh-scales by tractor for two miles between the fields of calves at each visit, which had the potential to reduce the accuracy of the weigh-scale measurement. It was noted that the indicator, pointing to the liveweight on the scale, was sticking at some points and required percussion to adjust. It is felt that the weigh-scales, in this circumstance, cannot be assumed to be wholly accurate and likely explain the lack of correlation between the two methods of liveweight gain calculation. Figure 6.5 illustrates the correlation between liveweight on the weigh-scales and liveweight estimated by weigh-band at visit 1. The correlation is good ($R^2 = 0.87$). However, when the correlation between these two methods of liveweight calculation are correlated at later visits, 3 (Figure 6.6) and 6 (Figure 6.7), after numerous tractor movements, the correlation decreases; this is likely to indicate inaccuracy of the weigh-scales or a poorer correlation of heart-girth and liveweight in heavier animals.

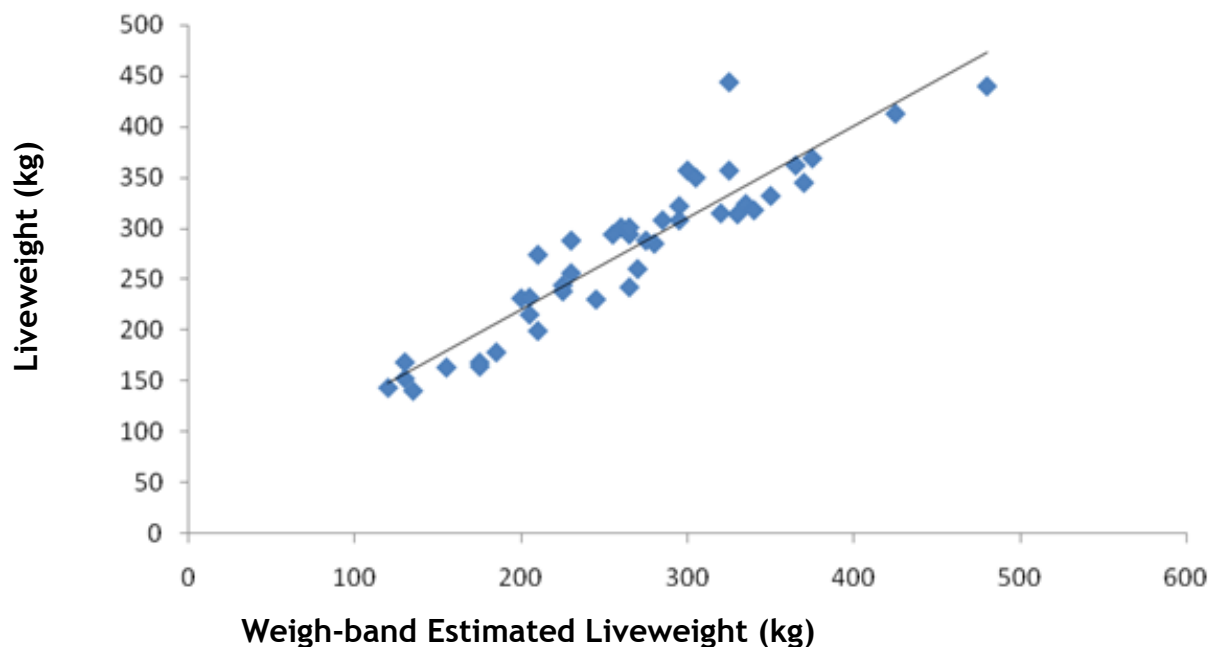


Figure 6.5. Correlation between liveweight and weigh-band estimated liveweight on Farm C3 at visit 1.

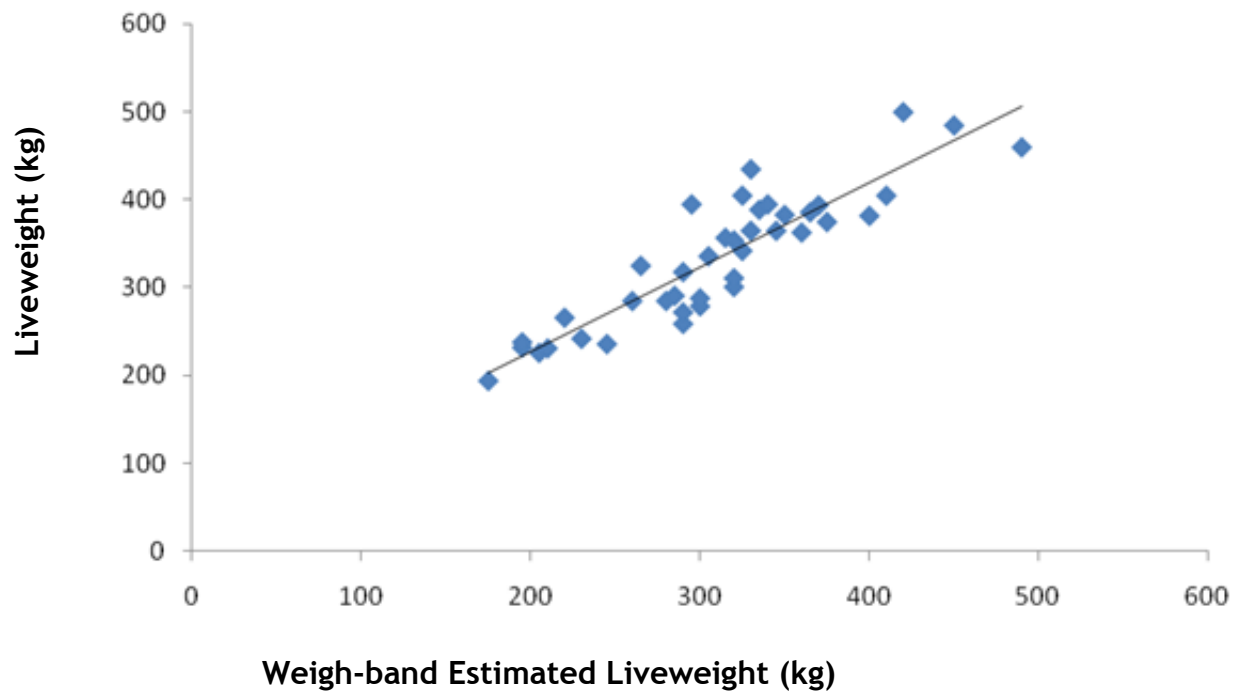


Figure 6.6. Correlation between liveweight and weigh-band estimated liveweight on Farm C3 at visit 3.

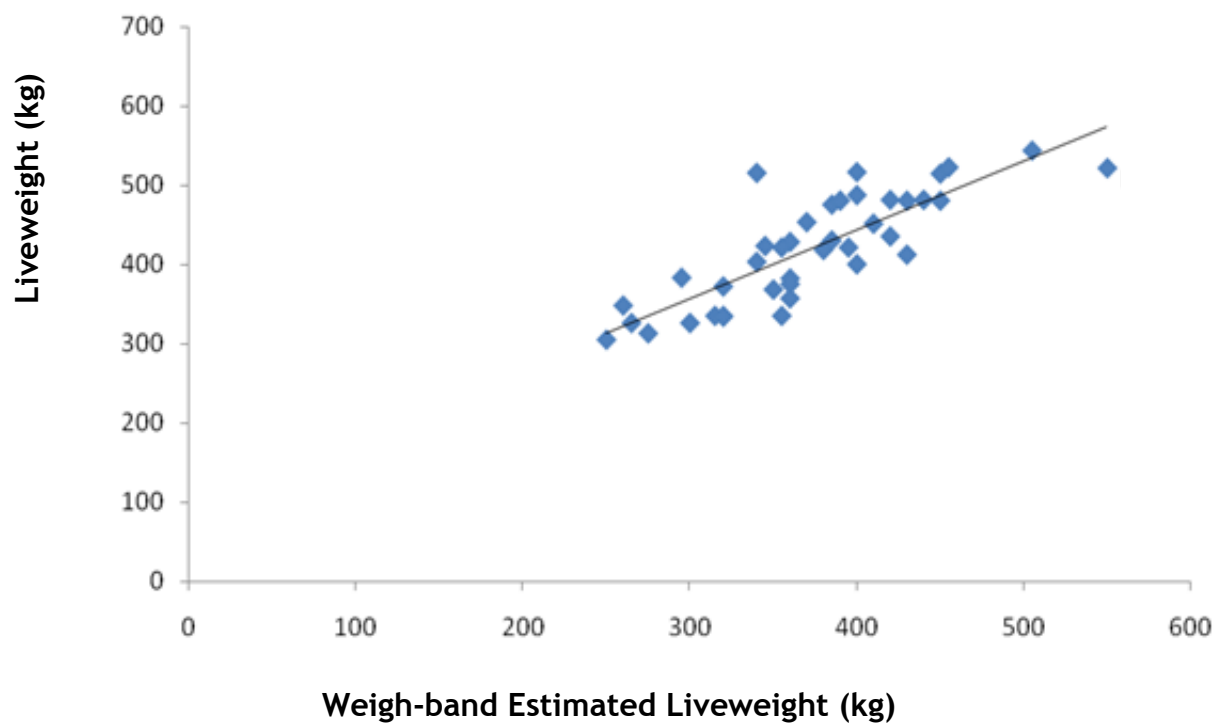


Figure 6.7. Correlation between liveweight and weigh-band estimated liveweight on Farm C3 at visit 6.

Eliminating the body condition score element from the estimation of liveweight gain, using heart-girth measurements, appeared not to reduce the correlation between heart-girth and liveweight. Removing this element from liveweight estimation is simpler for farmers to implement on farm. In order to calculate liveweight gains efficiently at the crush-side, a computer (either hand-held or laptop) is likely to be required, and so it is suggested that adding the formula from Heinrichs *et al.* (1992) into an equation on the computer should be a relatively straightforward task. It should be possible therefore, to use a crush-side 'application' on a smart phone or laptop computer, to further refine the TST programme by taking into account some of the external influences that may affect calf liveweight gain at grass, such as pasture availability and quality. This would give the efficiency of nutrient utilisation in an animal, as is integrated into the 'Happy FactorTM' in sheep (Greer *et al.*, 2009) and this possibility is discussed later in this chapter.

Given that many farmers do not possess weigh-scales on farm, the use of heart-girth measurements to estimate liveweight gain is currently the best option available to farmers. Also, the weigh-scales used on-farm may not always be accurate, and it can be difficult to adequately calibrate and check for accuracy at higher liveweights. The effect of the error of the weigh-band and weigh-scale on liveweight gain will likely be larger over a shorter time period.

6.3 TARGETED SELECTIVE ANTHELMINTIC TREATMENT USING LIVWEIGHT GAIN IN CALVES DURING THEIR FIRST YEAR AT GRASS.

In this study, applying a performance-based targeted anthelmintic treatment regime in the field was shown to be possible, and applying it on farms where anthelmintic treatment was already minimal such as on organic farms, increased liveweight gain in first season grazers. The increase seen in the liveweight gains of these calves suggests reduced levels of subclinical gastroenteritis, which in turn supposes higher levels of animal welfare. Although production performance is not the primary aim of organic production, animal welfare and reducing the environmental impact of farming certainly is. It appears that a performance parameter, at present, best selects individual animals that would most benefit from anthelmintic treatment. This should not be considered by the organic community as the application of an

anthelmintic treatment for the sole purpose of increasing performance, rather, it should be viewed as a marker that is superior and/or easier to use than parasitological measurements such as FEC or plasma pepsinogen concentration.

6.3.1 Refugia and Resistance

Applying a targeted selective treatment (TST) programme means that a proportion of animals remain untreated. The proportion of animals that need to be left untreated, to ensure that an effective parasite population remains in refugia, is the subject of much debate within the scientific community. This proportion will depend on host susceptibility, factors that influence refugia, prevalence of resistance genes and the mechanism of resistance (Kenyon *et al.*, 2009). Given that within a population of grazing animals it is assumed that approximately twenty per cent are grow well despite a gastrointestinal nematode challenge, many TST programmes in small ruminants commonly demonstrate between ten and twenty per cent of the flock remain untreated. In this study, if a percentage of the herd were to be left untreated, it would have made it necessary to have gathered and weighed all animals, calculated the top ten or twenty per cent, and then gathered the calves again for treatment. This rendered the implementation of this method on-farm impractical. A target liveweight gain is easier to implement and, as a bonus, may also focus farmer's attentions onto the expected target liveweight gain of their dairy replacement heifers. There is a dearth of literature regarding performance-based targeted anthelmintic treatment in cattle; however, two independent research groups, one led by Johan Höglund in Sweden and the other by Andrew Greer and Rob McAnulty in New Zealand, suggested a figure of between 0.70 and 0.80 kg/day as a possible target liveweight gain for use in a TST programme. In this thesis, a target liveweight gain of 0.75 kg/day was applied as, in the UK, this is generally accepted as the ideal liveweight gain for dairy replacements, in order to reach the adequate liveweight for serving at fifteen months of age. The advantage of using a target liveweight gain is that it can easily be altered on each farm and even on an annual basis on the same farm. The expected liveweight of an animal, at a certain age, can be used to extrapolate the necessary liveweight gain required, over a period of time, in order to reach a certain weight at a certain age. However, it may be prudent to set a target anthelmintic treatment liveweight gain higher than your expected liveweight

gains, as animals that fall below target will take a while to recover adequate liveweight gains, meaning that they would be below expected liveweight gain for a period of time if both target and expected liveweight gains were the same.

The major disadvantage found while implementing a performance-based TST approach in the field, was the requirement of regular handling of first season grazers, which is labour intensive and time-consuming. It may also be impractical as often crush facilities are not available away from the farm. Farm O3 declined participation in the 2010 study year, due to the requirement for an increased number of visits during the grazing season from 2009. This farm grazed FSG away from the farm where there were no crush facilities, and in 2009, it was necessary to transport a crush before every visit to the field. When considering the implementation of a TST programme, on-farm practicalities are of vital importance. It is highly unlikely that a TST programme with ideal epidemiological, parasitological and environmental considerations could be integrated into current farm management. For example, turning out animals later in the grazing season would significantly reduce parasite challenge, but animals would not be grazing at the time of good grass quality and growth - sound advice from a parasitological view-point, however few farmers would value this recommendation.

6.4 CRITIQUE

6.4.1 Handling of First Season Grazers at Grass

The necessity to gather FSG regularly, in order to either place them on weigh-scales or measure heart-girth, was a major disadvantage in the TST programme for the farmers. On many farms the FSG are turned out to pasture and not handled again until housing. Thus, advising farmers to gather and handle this class of animal on their farm regularly throughout the grazing season may be asking too much of a management change. Many farmers would also find this impossible due to a lack of handling facilities where their youngstock graze. Having said this, some farm animal specific veterinary practices have started offering the services of 'paraprofessionals', such as foot trimmers and ultrasound scanners. Certainly, at least one large veterinary practice in the south of England offers a paraprofessional with a portable

weigh-crush that can be used by farmers as and when required. The practice is particularly keen to promote good replacement heifer rearing management and advocates the use of the weigh-crush to assist in this respect. Parasite management and anthelmintic treatment on-farm, more often than not, does not involve the veterinary practitioner. It may be that a portable weigh-crush, along with heifer rearing advice from the veterinary professional, would be valued by farmers and, anthelmintic advice, possibly encompassing a TST programme, could then be implemented within the package.

6.4.2 Liveweight Target for a Targeted Selective Treatment Programme

The target of 0.75 kg/day liveweight gain, used in this study may well have been set too low. As mentioned previously, it may be prudent to set a target anthelmintic treatment liveweight gain higher than your expected liveweight gains. This is because animals that fall below target will take a while to recover adequate liveweight gains, meaning that they would be below target for a period of time if both target and expected liveweight gains were the same. A target of 0.80 kg/day may have been a better target liveweight gain for farms in the study that previously demonstrated high mean liveweight gains, such as Farm C3. However, this target would probably have been too high a target to expect Farms O1 and O2 to have achieved, given their mean liveweight gains over the previous grazing season. The target liveweight gain can be specific for each farm and even modified annually, depending on the expectations of the farmer and the cattle breeds present on the farm.

It should be noted that it has been demonstrated that the water retention of parasitised ruminants is commonly higher than in parasite-free controls (Halliday *et al.*, 1965). Holmes (1987) suggested that such changes in water retention clearly demonstrated that tissue loss attributable to parasitic infections may not be reliably determined from changes in body weight alone, although the actual significance of this is unknown in the field.

6.4.3 The Use of Eprinomectin

Most of the organic standards bodies recommend the use of anthelmintics from the levamisole or benzimidazole classes, rather than the macrocyclic lactone (ML)

class, due to environmental concerns regarding this class of anthelmintic. Decomposing animal faeces return valuable nutrients to the soil, and dung fauna—fungi, yeasts, bacteria, nematodes, insects and earthworms play an important role in this decomposition process. The natural disintegration of cow dung follows an ecological succession initiated by insects, including dung beetles. These aerate the pat, attracting earthworms, which, with dung beetles, mites and other invertebrates, subsequently disperse the pat into the soil (Stevenson and Dindal, 1987; Holter *et al.*, 1994). Drugs belonging to the benzimidazole and levamisole/morantel groups are thought to be relatively harmless to dung fauna (McKellar, 1997). Macrocyclic lactones do not decompose rapidly once dung has been deposited, particularly on pasture (Schmidt, 1983; Wall and Strong, 1987; Strong and Wall, 1988). It is only when treated faeces are mixed with a large volume of soil, for example, when ploughing into soil as a fertiliser, that ivermectin binds with soil particles and is broken down (Halley *et al.*, 1989). The study presented in this thesis used eprinomectin (Eprinex™, Merial), from the ML class, in the TST programme. The use of this anthelmintic was accepted by the organic standard bodies on both of the organic farms before the study began. There is little information regarding the environmental impact of eprinomectin specifically (Lumaret *et al.*, 2012). Halley *et al.* (2005) showed that there was no effect on earthworms when eprinomectin was deposited in the soil under typical usage conditions. Wardhaugh *et al.* (2001), working on an introduced Australian dung beetle, showed that faeces voided by cattle treated with a pour-on formulation of eprinomectin were associated with high juvenile mortality during the first couple of weeks after treatment. Increased mortality also occurred among newly emerged beetles fed on faeces collected 3 days after eprinomectin treatment and there was evidence of suppressed brood production among those that survived. The authors also ran a model, simulating the effects of drug residues on dung beetle populations, which suggested that, in the absence of immigration, a single treatment of eprinomectin is capable of reducing beetle activity in the next generation by 25–35%. Eprinex™, however has no warning labels, other than for potential aquatic toxicity, along with many other parasiticides in both Europe and the USA, the regions with the strictest regulations (Anonymous, European Medicines Agency). Future acceptance of a TST regime on organic farms would likely require the use of non ML anthelmintics, which do not possess persistency of activity, thus, possibly requiring more regular handling of FSG. However, organic farmers are familiar with this rule

and collection of the FSG throughout the grazing season for anthelmintic treatment is more commonplace on organic farms than on conventional farms.

6.4.4 Further Considerations

The TST programme is advocated as a method to ensure that the gastrointestinal parasites present within some calves, within a group of animals, are not exposed to anthelmintic, hence maintaining a refugium population. However, it should be noted that, in temperate areas, the majority of the parasite population (up to 95% of total worm population) is found on the pasture (Barnes *et al.*, 1988). Parasites on the pasture are also *in refugia* and so this element of the parasite population should always be considered when implementing any anthelmintic treatment regime.

When instigating a TST programme for gastrointestinal parasites, other parasites encountered by grazing stock should be considered. Infectious larvae of *Dictyocaulus viviparus*, can also be found on pasture. Infection with this parasite can cause death or significant disease in all ages of cattle, but particularly in youngstock. Outbreaks of this disease tend to be sporadic and unpredictable. Often, this parasite is controlled by the anthelmintics used to control gastrointestinal parasites, and so leaving some calves untreated, that appear resilient to gastrointestinal nematodes, may leave them exposed to infection. In the case of *Dictyocaulus viviparus*, there is a vaccine that can be recommended for use to reduce the chance of a disease outbreak, and this may be a prudent precaution when implementing a TST programme in an area where this disease is known to occur.

6.5 FUTURE RESEARCH AND IMPLEMENTATION ON-FARM

A computer or smart-phone is likely to be useful in order to calculate liveweight gains at the crush-side and it should be possible to add-in some of the external influences that may affect calf liveweight gain at grass, such as pasture quality and stocking rate. A computer-based algorithm that a farmer could work through in order to advise on appropriate timings of anthelmintic treatment should be possible to produce. In order to provide sound advice, multiple farm-specific factors

would need to be entered into the programme, such as climate, class of animals grazing the pasture the previous year and timings of calf movement to fresh pasture. This could become onerous for farmers, which may result in the programme being underutilised. It may be more prudent to give simple bullet point advice that can then be individually tailored to each farm. It would also be possible to mathematically model various scenarios of TST outcomes using different classes of anthelmintic, different management situations (pasture rotation/stocking density etc.) and target liveweight gain using hypotheses of various numbers of overwintering larvae and different larval survival rates in different weather situations.

A TST programme need not be a complete alternative to the regular treatment of calves seen in most conventional systems. Epidemiological knowledge of gastrointestinal parasites demonstrates the over-wintering of larvae on pasture and ingestion of these by calves at turnout. Allowing contamination of the pasture early in the grazing season until gastrointestinal parasites begin to affect liveweight gain, as shown in this thesis, could be prevented by dosing with an anthelmintic one month post-turnout. This dose of anthelmintic serves to reduce pasture contamination rather than simply treat the infection in the calf, thus, it cannot realistically be targeted (FEC at this stage are likely to be low and *C. oncophora* weighted). Collecting the calves at this stage would allow for body weight to be estimated by weigh-band or weigh-scale. In the middle of the grazing season calves could then be collected and a TST, based on liveweight gain, implemented. This may be an ideal compromise for the organic industry in order to reduce subclinical gastroenteritis. All of the organic farms in the studies presented in this thesis treated all of their calves at least once with an anthelmintic and so, if this is the case, it is more beneficial for this treatment to be early in the grazing season, and may reduce the need for any further anthelmintic treatment in some calves.

6.6 CONCLUSIONS

The acceptance by farmers of TST strategies, and their implementation, may require a high level of input and education to the farming community, veterinary practitioners and organic bodies. For many years, farmers have been encouraged to treat regularly with anthelmintics and strategies, such as dose and move, were

advocated to minimise pasture contamination. The possible need to consider the impact of refugia on the development of anthelmintic resistance, has resulted in a change in the advice offered to farmers. Maintaining an effective refugium for a proportion of the worm population, is now considered one of the most important factors determining the rate of the development of resistance, and should be included in any potential prophylactic control regime suggested for nematode parasites (Kenyon *et al.*, 2009). However, Van Wyk *et al.* (2006) suggested that there are insufficient numbers of advisors with the necessary experience, to support farmers through a management change, and that alternative methods, such as on-farm automated decision support, needs to be developed. In order for refugia-based regimes to gain acceptance in the farming community, it is important that the suggested strategies minimise any loss in animal performance. For this reason, it is imperative that extensive education is made available to both farmers and their advisors to ensure that the complex messages of the new strategies are fully understood. In addition, both farmers and advisors must be made aware of the benefits of setting realistic performance targets and of using regular monitoring of animal performance and anthelmintic efficacy to acquire the necessary understanding required to achieve these targets. It is also crucial that practitioners/farmers and advisors are made aware not only of the cost benefits, and potential disadvantages, accruing from a TST approach, but also those associated with anthelmintic resistance. The primary challenge for researchers is the need to develop strategies that can slow or prevent the development of resistance, whilst still maintaining optimal performance and thus form the basis of sustainable integrated parasite management programmes (Van Wyk *et al.*, 2006).

Kenyon *et al.* (2009) discussed the evidence to date which suggests that targeted treatments or TST strategies make it possible to reduce anthelmintic usage in ruminants in such a way as to slow the development of anthelmintic resistance whilst still maintaining animal performance. However, there is a clear need for the development and validation of sustainable, objective, user-friendly and regionally specific indicators for treatment. In addition, trials should be conducted to develop a better understanding of the proportion of untreated animals necessary to maintain effective refugia under conditions of differing parasite species, environments and animal management regimes.

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APPENDIX



University of Glasgow | Scottish Centre for Production
Animal Health & Food Safety

Farm Details

Name

Farm name

Farm address

County

Postcode

Telephone number

Conventional

Organic

Other (please give details) _____

If the farm is organic;

How many years has the farm held organic status for? _____

Which organic certification body is the farm registered with?

Herd Details

Approximately how many animals are on your farm? (please include breed)

Dairy _____ Beef _____

Other _____ Sheep _____

Pigs _____

What is the herd status of the farm?

Open Closed

Do you buy in bulls / rams?

Yes No

Animals within the herd that have not been out for a grazing season

How many of these first season grazers (FSGs) do you have?

Where any of these animals out at grass at the end of last season?

Within the FSG group how many are?

Dairy replacements _____ Beef _____

Other _____

What vaccinations have these calves received?

Blue Tongue (e.g. Bovillis BTV8)

Blackleg

Respiratory vaccinations (e.g. Rispoval / Bovipast / Bovillis IBR)

Lungworm (e.g. Huskvac)

BVD (e.g. Rispoval 4 / PregSure / Bovillis BVD)

Other _____

Which anthelmintics have you used this year?

Did you treat any other stock?

When did you apply these?

What did you use last year?

When did you apply these?

Grazing Management

Where have the FSGs been grazed so far? (include approx size of field and stocking rates)

What was grazed on these fields last year?

Do you co-graze?

Yes

No

Gut worms on the farm

Do you think that you have ever seen a clinical case of gut worms before?

Yes

No

Have your growth rates been less than you expected some years?

Yes

No

Has your vet ever been involved with worm control on the farm?

Yes

No

Are you happy with your worm control?

Yes

No

Have you got any concerns regarding gastrointestinal parasitism on farm?

What are the main health concerns regarding your whole herd?

Specifically the first season grazers?
