Studies on the Population Dynamics of *Teladorsagia circumcincta*

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Abstract

An initial study was conducted to investigate the recovery rates of *Teladorsagia circumcincta* infective larvae from small herbage samples, as most methods have been developed for isolating infective larvae from large, field samples. A direct count technique (DCT) was developed, enabling high levels (on average 80-90%) of infective larvae seeded to be recovered. This technique was used to investigate the influence of temperature, relative humidity and light intensity on larval migration, using growth chambers. The effect of two different sward types was also investigated, using a young, growing sward and a mature, tillered sward. The results suggest that initial migration onto the sward may be very rapid and independent of external stimuli. The majority of the larvae were recovered from the bottom third of the sward. A greater number of larvae were recovered from the mature sward than from the young sward, which may be due to the tillering of the sward, so creating pockets where moisture could be trapped and so altering the micro-environment. The optimum conditions for migration of *T. circumcincta* vary according to the time after seeding that the pots were harvested.

Lambs fistulated at the oesophagus were used to investigate larval intake using trays of rye grass seeded with known numbers of infective larvae and to investigate whether grazing behaviour influenced larval intake. The influence of sward height was also investigated, using a 3cm and a 6cm sward. The bite depth was shown to differ on different height swards but this did not have a significant effect on the number of larvae ingested.

The periparturient ewe is thought to be the major source of contamination for the young growing lamb. To investigate the period of greatest susceptibility to *T. circumcincta*, three groups of ewes were given a single dose of 10,000 infective larvae either 7, 28 or 56 days *post partum* (DPP). Their faecal egg count and blood parameters were measured over a period of 56 days post infection (PI). A group of worm naïve and barren ewes were also included to act as positive and negative controls. The groups found to be most susceptible were the ewes given the larvae 7 and 28 DPP, and the ewes given the larvae 56 DPP were the most refractory to infection, showing a similar level of infection to that of the barren ewes.

One theory as to why young, growing animals to do not mount an effective immune response is that there is competition for available nutrients, especially

protein, between growth, repair of the damaged intestinal tract due to parasite infection and the immune response. If sufficient protein was available in the diet of these animals, it is hypothesised that this competition would be eliminated. To test this theory, two experiments were conducted, one using lambs of approximately 3 ½ months of age at the start of the study and one using lambs of approximately 5 ½ months of age. The lambs were divided into three groups and fed either a basal ration (MP), high protein ration (HP) or the basal ration supplemented with methionine, an essential sulphur amino acid, protected from degradation in the rumen. There was no evidence of either increased resilience, the ability of an animal to perform despite parasite challenge, or increased resistance, the ability to suppress establishment of parasites, resulting from either the increased protein ration or the supplementation of methionine.

The use of computer models offers a very cost effective method to examine management strategies and investigate the development of anthelmintic resistance. In order to simulate what level of reduction in faecal egg count would need to be achieved in the ewe by the addition of dietary protein, to have a significant impact on the infection of the lambs, an anthelmintic bolus of varying efficacy was administered, then a ten year simulation run. A model developed by Drs. Barnes and Dobson was used, with weather data supplied by the Rothamstead Institute used to parameterise the model for UK conditions. The management strategy modelled was that used by one of the Institute's farms, using 120 ewes and their twin lambs. The results obtained show a very similar pattern to experimental data generated over a three year period by Barrett (1987), but of a much smaller magnitude. Nevertheless, the predictor models are an extremely useful and versatile tool.

Declaration

The work described in this thesis was conducted at the Parasitology Division of the Moredun Research Institute, Edinburgh. The growth chamber studies described in Chapter 3 and the oesophageal fistulate studies described in Chapter 4 were conducted at the Macaulay Land Use Research Institute, Aberdeen. Some of the eosinophil and post-mortem tissue data were obtained in collaboration with colleagues at the Institute. Meteorological data were kindly supplied by the Rothamsted Institute, Herfordshire. Nevertheless, most of the work presented in this thesis was conducted by myself, and where conjoint experiments were undertaken, a full role was played in the design of the experiments and interpretation of the results.

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Abbreviations

CP	crude protein
DCT	direct count technique
DPP	days *post partum*
EDTA	Ethylenediaminetetracetate
epg	eggs per gramme of faeces
EL_4	early fourth-stage larva
FEC	faecal egg count
FECRT	faecal egg count reduction test
g	gravity
GL	globule leukocyte
HP	high protein
L_1	first-stage larva
L_2	second-stage larva
L_3	third-stage larva
L_4	fourth-stage larva
LL_4	late fourth-stage larva
LP	low protein
ML_4	mid fourth-stage larva
MMC	mucosal mast cell
MHC	major histocompatibility complex
NRDP	non rumen degradable protein
OD	optical density
PBS	phosphate buffered saline
PI	post-infection
PPR	peri-parturient rise
rpm	revolutions per minute
SEM	standard error of the mean

Chapter 1

General Introduction

1.1 Introduction

The nematode parasites of small and large ruminants are of great importance for economic, welfare and environmental reasons. The economic impact is large and worldwide, the total sales of antiparasitic agents in livestock alone being estimated at US $1465 million for 1996 (Witty, 1999). Gastrointestinal parasitism, if left untreated, can cause weight loss, reduction in wool and milk yields and in severe cases, death of the affected animal. The treatment of these parasites has led to increasing concern over pesticide residues in the environment affecting non target species (McKellar, 1997).

In temperate areas such as northern Britain the most prevalent gastrointestinal nematodes of small ruminants are *Teladorsagia (Ostertagia) circumcincta*, *Trichostrongylus vitrinus* and *Nematodirus battus* (Parnell, Rayski, Dunn & MacKintosh, 1954; Boag & Thomas, 1971, 1977; Taylor & Cawthorne, 1972; Thomas & Boag, 1972, 1973; Reid & Armour, 1975; Waller & Thomas, 1978). Other species such as *Trichostrongylus colubriformis*, *Trichostrongylus axei*, *Haemonchus contortus*, *Oesophagostomum venulosum*, *Cooperia curticei*, *Nematodius fillicollis*, *Nematodirus spathiger*, *Chabertia ovina*, *Trichuris ovis*, *Bunostomum trigonocephalum* and *Strongyloides papillosus* tend to occur less frequently and are generally not associated with outbreaks of disease. *Teladorsagia circumcincta* (Ransom, 1907), formerly known as *Ostertagia circumcincta* (Stadelmann, 1894) is the most economically significant to the UK sheep industry (Urquhart *et al.*, 1991) though *T. vitrinus* may be one of the main causes of helminthiasis in grazing lambs in their first winter (Parnell *et al.*, 1954).

1.2 Life Cycle

1.2.1 Teladorsagia circumcincta life cycle

T. circumcincta follows the direct, simple typical trichostrongylid life cycle as shown in Figure 1.1. The adult worms dwell in the abomasum, closely connected with the mucosal tissue and after mating the gravid female lays eggs which pass out in the faeces. Under optimum conditions, occurring between 18-26°C, the time taken from eggs passing onto pasture to hatching is around 2 weeks. The first larval stage

(L_1) develops within the egg and hatches. This larval stage feeds on bacteria present within the faeces and moults to the second larval stage (L_2). This stage also feeds on bacteria until it undergoes ecdysis to reach the infective, third stage larvae (L_3). The cuticle of the L_2 is retained around the infective larvae hence this stage cannot feed. This sheath protects against adverse environmental conditions, especially desiccation, enabling the larvae to overwinter on pasture and in the soil (Urquhart, Armour, Duncan, Dunn & Jennings, 1991). This larval stage migrates onto the grass sward where it is ingested by a grazing sheep or lamb. The infective larvae exsheaths in the rumen and then invades the abomasal gastric glands, where it undergoes further moults to the fourth (L_4) and fifth (L_5) larval stages, before emerging. Once the L_5 matures, becomes sexually active and mates, the cycle begins again, taking on average three weeks (Armour, Jarrett & Jennings, 1966).

1.2.2 Trichostrongylus spp. life cycle

These species too follows the typical trichostrongylid life cycle as shown in Figure 1.1, but infective larvae exsheath in the abomasum before reaching their predilection site, the anterior small intestine. The larvae then invade the base of the villa and undergo their first moult.

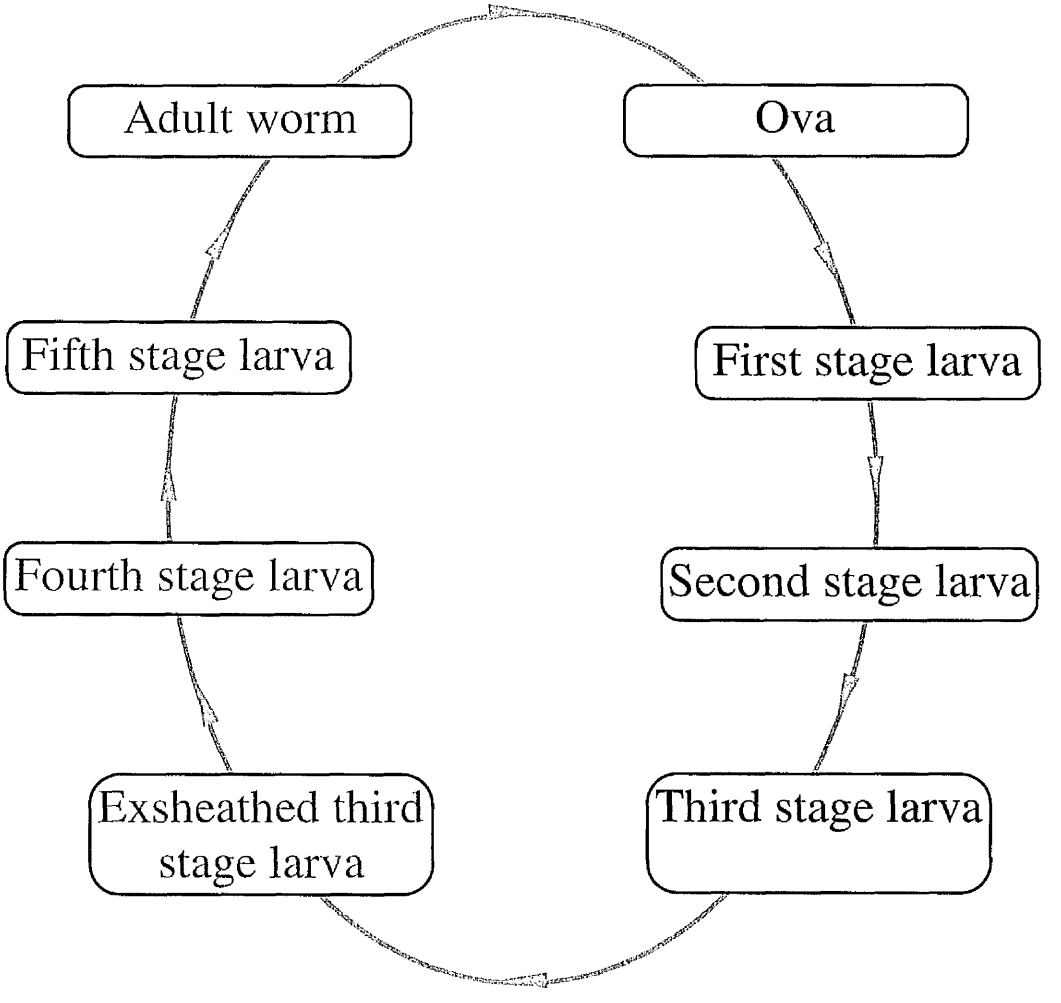


Figure 1.1 *Typical trichostrongylid life-cycle*

1.2.3 Nematodirus spp. life cycle

The life cycle of *Nematodirus*, a nematode that primarily affects the young lamb is slightly different, in that development up to and including the third stage larva occurs whilst still remaining within the egg. A period of chilling followed by a rise in temperature is required before the infective larvae hatch, ensuring that the larvae are present on the pasture when the susceptible lambs first start grazing (Boag & Thomas, 1975).

1.3 Larval development

Once the egg has been passed out in the faeces, environmental conditions, primarily temperature and humidity, but also rain, sunlight and wind, determine how fast the larvae will develop into the third stage, infective larvae (Stromberg, 1997) . The ideal conditions for rapid development of *T. circumcincta* are high levels of humidity (70-90%) and a temperature range of 16-26 °C with an optimum hatching temperature of around 16°C (Pandey, Chaer & Dakkak, 1989, 1993). Development is faster at higher temperatures but the larvae become hyperactive and mortality increases as a result of depleted lipid reserves. At lower temperatures (<10°C) development from egg to L_3 does not usually take place and the movement and metabolism of developed L_3 is minimal (Urquhart *et al.*, 1991).

Under ideal conditions the infective larvae can develop from the egg in around 2 weeks (Urquhart *et al.*, 1991). Depending on environmental conditions the larvae will either remain dormant in the mat at the base of the herbage or the upper layers of soil or migrate up the sward until ingested by a grazing lamb. The ability of the larvae to become quiescent and survive on the pasture until more favourable conditions occur enables them to over-winter on pasture or in the soil until the next crop of susceptible lambs start to graze the following spring .

There is evidence to suggest that infective larvae develop differently when subjected to different environmental conditions. Rossanigo & Gruner (1996) showed that under optimal developmental conditions *T. circumcincta* and *T. colubriformis* infective larvae grew longer, mainly due to an increased length of intestinal cells, and that these longer larvae migrated faster out of an agar block and exsheathed faster. In the case of *T. circumcincta* there was also a significantly lower establishment rate in lambs with the shorter infective larvae, although the adult worms showed no significant difference in size or fecundity.

1.4 Factors affecting larval migration

There are two different components influencing the migration rates, the initial migration from the soil onto the base of the sward and the migration up and down the sward. The larvae are capable of migrating downwards into the soil as well as onto the sward, as shown by studies on the cattle parasites *O. ostertagi* (Fincher &

Stewart, 1979; Al Saqur, Bairden, Armour & Gettinby, 1982; Krecek & Murrell, 1988) and *Cooperia oncophora* (Fincher & Stewart, 1979) and in the sheep parasites *Teladorsagia* spp., *Trichostrongylus* spp. and *Nematodirus spathiger* (Furman, 1944; Crofton, 1948; Sturrock, 1961; Gruner, Mauleon & Sauve, 1982; Callinan & Westcott, 1986). The soil is thought to be an important reservoir where the infective larvae can over winter then emerge back onto the pasture when the weather becomes more favourable (Al Saqur *et al.*, 1982).

Due to the technical difficulties involved in directly measuring parameters such as the moisture content on the sward, most of the measurements involved are indirect such as rainfall or relative humidity and these technical limitations can make separating out the individual effects of the environment problematic.

1.4.1 Influence of moisture

This is thought to be the most important parameter in determining the migration of larvae in the pasture as the larvae move on the sward in a moisture film, using the surface tension of the film to move vertically (Crofton, 1954; Stromberg, 1997; Niezen, Robertson, Waghorn & Charleston, 1998b). If this dries out then the larvae either retreat with the edge of the moisture film or are left stranded on the sward, leaving them very vulnerable to desiccation. The moisture film position depends on the density of the sward, the height of the sward and the relative humidity of the microclimate surrounding the sward (Niezen *et al.*, 1998b).

1.4.2 Influence of temperature

The largest influence of temperature may be its effect on the moisture content of the sward. The greatest level of migration occurs at dawn and dusk (Rees, 1950), although this again may be a function of the moisture film movement. Different larval species have different optimum temperature ranges, as would be expected due to their different geographical ranges. Species such as *H. contortus* show optimum migration at higher temperatures than those species adapted to a more temperate climate such as *T. circumcincta* or *T. colubriformis*.

1.4.3 Influence of light intensity and light wavelength

As with the temperature, the greatest effect of light intensity may be due to its influence on the moisture film. Rees (1950), working in the field, reported maximum migration rates at dawn and dusk, however, in many field experiments the light intensity is not a variable, and not measured, making comparisons difficult.

1.4.4 Influence of relative humidity

Peak migration rates have been reported as occurring at between 74% (Rees, 1950) to around 90% relative humidity (RH) (Silangwa & Todd, 1964; Callinan & Westcott, 1986). Pandey *et al.* (1989, 1993) when looking at the development and survival of eggs and larvae found that higher levels of RH, of around 75-95% were detrimental and that lower RH of around 35-65% were more like the optimum conditions required.

1.4.5 Influence of sward height and density

There are few data on sward density effects, both Kauzal (1941) and Silangwa & Todd (1964) recovered greater numbers of larvae from a 'dense' sward than from a 'sparse' sward, although no figures were given as to density of seeds sown. Both authors speculated that the denser the sward the greater the probability of the larvae 'finding' a blade.

1.4.6 Influence of larval species

There is conflicting evidence as to whether there is a species difference in migration rates under the same environmental conditions. Some authors report no significant difference between *Trichostrongylus* spp. and *Teladorsagia* spp. (Callinan & Westcott, 1986; Niezen *et al.*, 1998) while others report differences between *H. contortus*, *Trichostrongylus* spp. and *Teladorsagia* spp. (Rogers, 1940) and differences between *H. contortus* and *H. placei* (Krecek, Groeneveld & van Wyk, 1991; Krecek, Groeneveld & Maritz, 1992; Krecek, Hartman, Groeneveld & Thorne, 1995). It would be expected that parasites with different geographical distributions would react differently to temperature, for example. This seems to be supported by Rogers (1940) who reported reduced migration rates for *H. contortus* on wet swards.

1.4.7 Influence of plant species

There is evidence suggesting that different migration rates are achieved on different sward species (Crofton, 1948; Tarshis, 1958; Silangwa & Todd, 1964; Moss & Vlassof, 1993; Niezen, Charleston, Hodgson, Miller, Waghorn & Robertson, 1998c) and that levels of parasitism in lambs grazing different herbage species also differ (Scales, Knight & Saville, 1995) but it is not clear what these differences are attributable to. It has also been shown that the external morphology of the leaf can influence the existence and thickness of water films (Wallace, 1959), although the effect this may have on infective larvae has not been investigated. It is likely that different plant species create different micro-climates and this, along with distinct leaf morphologies, may explain the different migration rates.

Work done in New Zealand on the migration rates of both *T. circumcincta* and *T. colubriformis* found the greatest migration on Yorkshire fog, ryegrass and cocksfoot with the lowest migration on white clover and lucerne (Niezen, Charleston, Hodgson, Miller, Waghorn & Robertson, 1998c). In most of the species a greater larval density, as measured by the numbers of larvae recovered per kilogram dry weight of herbage, was observed on the top of the swards.

1.4.8 Influence of bite depth and grazing behaviour

It is known that when there is sufficient herbage present on a pasture, sheep will not graze around an area contaminated with faeces (Crofton, 1948; Gruner & Sauve, 1982; Hutchings, Kyriazakis, Anderson, Gordon & Coop, 1998). However, this situation changes when the herbage becomes more sparse and the sheep are forced to graze in these areas, which has obvious consequences for the ingestion of infective larvae. Also it appears that the age of the faeces has an effect on grazing behaviour, with the freshest faeces being avoided the most. Conversely these faeces are the least likely to be contaminated with infective larvae. There was a difference in the grazing behaviour of the parasitised and non-parasitised animals, with the parasitised animals being more selective in their sward choice and taking smaller bites, although this may of course be a function of the parasite induced anorexia .

1.4.9 Influence of diurnal rhythms

Recent work has shown that there is no influence on the larvae of any diurnal rhythm (Ferre and Beecham, personal communication). The *T. circumcincta* larvae were left for differing periods of time in light and darkness and then placed on the soil of a pot and left to migrate. There was no difference in migration rates between any of the treatments.

1.5 Pathophysiological effects of T. circumcincta on the host.

The effect of infection by *T. circumcincta* is to produce a catarrhal gastro-enteritis with associated diarrhoea, dehydration, anaemia, inappetence and subsequent weight loss.

The inhibition of gastric acid secretion and increase in serum pepsinogen and gastrin is a well documented phenomenon associated with *T. circumcincta* infection (Holmes & MacLean, 1971; McLeay, Anderson, Bingley & Titchen, 1973; Anderson, Blake & Titchen, 1976; Coop, Sykes & Angus, 1977; Anderson, Hansky & Titchen, 1981; Anderson, Hansky & Titchen, 1985; Lawton, Reynolds, Hodgkinson, Pomroy & Simpson, 1996) as is also the case with the abomasal parasites *H. contortus* in sheep (Christie, Brambell & Mapes, 1967; Dakkak, Bueno & Fioramonti, 1981; Fox, Pitt, Gerrelli, Jacobs, Adhikari & Goddard, 1988; Nicholls, Lee, Adrian, Bloom & Care, 1988; Simpson, Lawton, Simcock, Reynolds & Pomroy, 1997) and *O. ostertagi* in cattle (Jennings, Armour, Lawson & Roberts, 1966; Fox, Gerrelli, Pitt, Jacobs, Hart & Simmonds, 1987; Hilderson, Dorny, Berghen, Vercruysse, Fransen & Braem, 1991; Fox, Carroll, Hughes, Uche, Jacobs & Vaillant, 1993) respectively. The mechanism by which this occurs is largely due to the emergence of immature adults from the gastric glands as early as 8 days post infection (Armour *et al.*, 1966). Cells occupied by parasites lose their specialized secretory function such that parietal (HCl secreting) and zymogenic (pepsinogen secreting) cells are replaced by irregular cuboidal epithelium (McKellar, 1993). Emerging parasites spread the damage to surrounding un-parasitized cells which also lose their differentiation; the end result being a thickened hyperplastic gastric mucosa with impaired cell junctional integrity. It was thought that abomasal pH is elevated primarily by the loss of functional parietal cells, although recently Simpson, Simpson, Simcock, Reynolds & Pomroy (1999) have published evidence suggesting

that the elevated pH in the abomasum can be caused by excretory/secretory products produced by adult *T. circumcincta* alone, leading to increased circulating pepsinogen levels since pepsinogen conversion to the proteolytic enzyme pepsin is most effective at low pH (Jennings *et al.*, 1966). The exact mechanism for elevated blood pepsinogen is not yet entirely clear and it may be the result of a combination of factors such as direct stimulation of zymogenic cells by the parasite, indirect stimulation via elevated circulating concentrations of hormones and leakage from abomasal fluid between poorly differentiated epithelial cells (McKellar, 1993).

The targeting of the abomasal parietal cells by the parasite is thought to be a mechanism to increase the pH of the abomasum. This resulting rise in abomasal pH is thought to enable the parasites to survive the harsh environment of the abomasum, as the life span of the parasite is reduced below a pH of around 4.5 (Simpson *et al.*, 1999).

The major effect on production in the sheep is the suppression of appetite and a reduction in feed utilisation, also the reduction in mineral deposition in the carcass, most notably calcium and phosphorous. The minimum level of infection sufficient to cause these losses in production is thought to be 1000-1500 infective *T. circumcincta* larvae per day (Coop *et al.*, 1982), although there is evidence to suggest that there is a breed difference (Holmes & MacLean, 1971). This level of infection does not produce any clinical signs of parasitism but is sufficient to cause significant reductions in parameters such as liveweight and wool growth. For these reasons it is desirable to be able to detect sub-clinical parasitism, one such marker for this is the measurement of plasma pepsinogen level. This parameter is associated with the rate of larval intake as opposed to adult worm burden and so can be used in immune animals. In these immune animals there is thought to be a hypersensitivty reaction which causes an inflammation of the mucosal wall.

The faecal egg count usually follows a stereotypical response, regardless of the size of larval challenge and so is not considered a very accurate marker for the level of infection experienced by the animal.

Evidence suggests that an infection with the cattle parasite *Ostertagia ostertagi* stimulates an immune response to, and so offers a degree of protection against *T. circumcincta* in sheep (Coop, Smith, Angus, Graham, Wright & Jackson, 1985b; Sutherland, Brown, Green, Miller & Leathwick, 1999). This could have an

important bearing on grazing management systems, as alternately stocking cattle and sheep could not only help to 'clean' the pasture of infective larvae but also stimulate an immune response in the sheep.

1.6 Factors affecting establishment

The means by which the host regulates its nematode burden during prolonged and continuous exposure to infection include the periodic spontaneous expulsion of the established adult parasite by resistant individuals (Gordon, 1948; Stewart, 1953), a reversible inhibition of larval development which may form part of a process for the continual replacement of adult worms, the inhibition of ovulation of female worms and the development of resistance to reinfection (Michel, 1963).

In natural infections the frequency of gastrointestinal nematode infection follows a negative binomial distribution describing a state of overdispersion (Barger, 1985). The result of this is that a relatively small proportion of the host population harbours a large proportion of the parasite population. Riffkin (1988) has estimated that the most resistant 50% of grazing animals may produce less than 10% of the worm eggs counted, whereas the most susceptible 15% of the flock may be responsible for over 50% of the egg output. The existence of such overdispersed parasite populations illustrates the importance of host immunity to infection. Host immunity to infection can be expressed in one of two ways; innate and acquired. Innate immunity is a non-specific and pre-existing phenomenon which provides a measure of the host's ability to regulate parasite establishment, development, persistence and fecundity. In contrast, acquired immunity is highly specific for a particular pathogen and is an active, adaptive and aggressive response which improves with repeated exposure to the same pathogen (Emery & Wagland, 1991). Many factors can effect the establishment of a parasite population including age and sex of the host, immune status and concurrent infections

1.6.1 Age of the host

Growing lambs acquire immunity to gastrointestinal nematode infections at a slower rate than older sheep (Manton, Peacock, Poynter, Silverman & Terry, 1962; Dineen, Gregg & Lascelles, 1978; Douch & Morum, 1993). Generally, immunity to gastrointestinal nematode infection in sheep gradually improves with age over the

first 12 months (Watson & Gill, 1991). This has been reported for sheep infected with *H. contortus* (Manton *et al.*, 1962; Benitez-Usher, Armour, Duncan, Urquhart & Gettinby, 1977), *T. colubriformis* (Gibson & Parfitt, 1972, 1973; Chiejina & Sewell, 1974a, b; Dineen *et al.*, 1978) and *T. circumcincta* (Smith, Jackson, Jackson & Williams, 1985). The relative immunological unresponsiveness of immature lambs has been described by Watson, Colditz, Andrew, Gill & Altmann (1994) and supports other studies which suggest that lambs are unable to mount an effective immunity to nematode infection until they are at least 6 months of age (Waller & Thomas, 1981; Soulsby, 1985). It has been suggested that the unresponsiveness of young lambs may be partly due to the immaturity of gut effector mechanisms rather than the failure to produce parasite-specific antibodies (Gregg, Dineen, Rothwell & Kelly, 1978). The factors involved in the development of age-related immunity are as yet unknown, but have been linked to factors such as puberty, bodyweight and condition rather than chronological age (Abbott & Holmes, 1990).

1.6.2 Sex of the host

The sex of the host may have an important influence on the development and expression of immunity to infection (Barger, 1993). The general findings from ruminant and laboratory studies are that entire males are more susceptible to nematode infection than are females or castrated males. Male sheep have been shown to be more susceptible than females to experimental infections with *Oesophagostomum columbianum* (Dobson, 1964; Bawden, 1969), *T. colubriformis* (Windon & Dineen, 1981), *H. contortus* (Colglazier, Lindahl, Wilson, Whitmore & Wilson, 1968; Adams, 1989) and *T. circumcincta* (Gruner, Mandonnet, Bouix, Vu Tien Khang, Cabaret, Hoste, Kerboeuf & Barnouin, 1994; Stear, Bairden, Duncan, Gettinby, McKellar, Murray & Wallace, 1995). Other studies have found no evidence of sex-related differences in susceptibility to mixed nematode infection (Albers, Gray, Piper, Barker, Le Jambre & Barger, 1987; Woolaston, Barger and Piper, 1990) although the animals used in these latter studies may have been pre-pubertal.

1.6.3 Reproductive status of the host

The increased susceptibility around parturition and lactation to gastrointestinal nematode infection, the periparturient rise, is well documented in

ewes and has been extensively reviewed (Dunsmore, 1965; Connan, 1974,1976; Michel, 1974,1976; Lloyd, 1983), and is also seen in cows, sows, bitches, mice, rats, guinea pigs, rabbits and goats (Lloyd, Amerasinghe & Soulsby, 1983; Barger, 1993; Chartier, Hoste, Bouquet, Malpaus, Pors & Koch, 1998). This temporary loss of immunity is thought to be associated more with lactation than pregnancy or parturition (Connan, 1968a) and can be prevented by removing the suckling stimulus at birth (Connan, 1968a; O'Sullivan & Donald, 1970). This relaxation of immunity provides the large amount of larvae that are a major source of infection for the extremely susceptible lambs (Brunsdon, 1966; Familton, 1991).

There are two different theories as to how this relaxation of immunity manifests itself. Michel (1974, 1976) thought that the rise could be accounted for simply by a suspension of mortality in the adult worms, whereas Connan (1968b) and O'Sullivan & Donald (1970) thought that it was a combination of an increase in the establishment of incoming larvae, the development of larvae that were previously arrested in the mucosal glands, a failure to regulate the fecundity of the adult female worms and an inability to expel the existing populations. Both O'Sullivan & Donald (1973) and Donald, Morley, Waller, Axelsen, Dobson & Donnelly (1982) found a higher level of rejection of larvae of both *T. colubriformis* and *T. circumcincta* in the non-lactating compared to the lactating ewes, supporting the latter argument.

The main cause of this phenomenon is now thought to be under hormonal control although other factors such as poor nutrition, lack of antigenic stimulus and stress caused by lambing have also been thought to be secondary factors (Barger, 1993). Recent work by Donaldson, van Houtert & Sykes (1997, 1998) showed that an increased level of protein in the ewe could significantly reduce the faecal egg count, from 21 days prior to parturition until the end of the study, at 21 days after parturition, and significantly reduce worm burdens, whereas an increase in the energy content of the diet had no such effect.

The precise control mechanisms are still not clear (Barger, 1993). Historically it was thought that prolactin, a pituitary peptide whose main function is the initiation and maintenance of lactation, was primarily responsible, as around the time the faecal egg count rises a similar rise is also seen in the level of plasma prolactin (Dunsmore, 1965; Kann & Martinet, 1975). This has recently been demonstrated in dairy goats with a significant correlation between faecal egg counts

and plasma prolactin levels in the four weeks around parturition (Chartier, Hoste, Bouquet, Malpaus, Pors & Koch, 1998). Also pharmacological elevation of plasma prolactin levels in unbred ewes using injections of diethylstillboestrol (Gibbs, 1967; Salisbury & Arundel, 1970; Blitz & Gibbs, 1972) or acepromazine (Bryant, Connan & Greenwood, 1968; Connan, 1974) increased faecal egg count as did the administration of exogenous prolactin to unbred ewes (Blitz & Gibbs, 1972). Helminth naïve lambs given exogenous prolactin also showed increases in both size and fecundity of adult female *H. contortus* but decreases in their number (Fleming, 1993). However, doubts have been cast on the importance of prolactin as the major contributory factor. In laboratory animals, for example in rats, there is a marked rise in plasma prolactin levels on the afternoon and evening of pro-oestrus and again on the day of oestrus (Neill, 1970), which is not seen in the male rat (Amenomori, Chen & Meites, 1970) but this does not impair the ability of the female rat to expel worms and they develop resistance faster than male rats (Murray, Jarrett & Jennings, 1971). Connan (1974) pointed out that similarly, oestrus in ewes and the corresponding elevated prolactin levels, caused no effect on egg count. Experimental work done by Coop, Mellor, Jackson, Jackson, Flint & Vernon (1990) and Jeffcoate, Fishwick, Bairden, Armour & Holmes (1990) also suggests that prolactin may not be the primary cause of the periparturient rise. Coop and his co-workers found that the peak of the faecal egg count occurred several days before the peak of the plasma prolactin level and in ewes artificially stimulated into milk production there was no periparturient rise seen, despite high levels of plasma prolactin. Jeffcoate *et al.* (1990) used bromocryptine, a prolactin antagonist, to reduce plasma prolactin to baseline levels but there was no reduction in the periparturient rise. Furthermore, there was no effect seen on the lamb growth rates from these ewes, suggesting that lactation was not significantly impaired, which in turn suggests that the role of prolactin in lactation is maybe not as straightforward as first thought. Other hormones have been suggested to be important such as progesterone (Lloyd, 1983) or corticosteroids (Connan, 1974) but the evidence provided by Coop *et al.* (1990) makes this unlikely. Clearly further work needs to be done to establish the precise cause of this phenomenon.

There is increasing evidence to support the theory that there is a differing host response to different species during the periparturient period (Brunsdon, 1970;

O'Sullivan & Donald, 1973; Gibbs & Barger, 1986; Jackson, Jackson & Williams, 1988; Donaldson *et al.*, 1997, 1998; Leathwick, Miller, Vlassof & Sutherland, 1997; Leathwick, Miller, Brown & Sutherland, 1999). Gibbs & Barger (1986) found that although *T. circumcincta* faecal egg counts were increased ten fold in lactating compared to dry ewes and there was establishment of *T. colubriformis*, the lactating ewes were no more susceptible to artificial infection with *H. contortus* than dry ewes. It appears that both pregnant and lactating ewes are more susceptible to *T. circumcincta* than either *T. colubriformis* (Donaldson *et al.*, 1997, 1998; Leathwick *et al.*, 1997) or *T. vitrinus* (Jackson *et al.*, 1988).

It has been shown that lactating ewes have no reduction in their humoral immunity, as shown by their response to horse red blood cells (Jeffcoate *et al.*, 1990), or cell mediated immunity, as shown by their response to vaccination with BCG vaccine (Jeffcoate *et al.*, 1990) . The periparturient rise was prevented when ewes were vaccinated with the protective 'hidden' antigen, H11, from the gut of the adult parasite *Haemonchus contortus*, (Andrews, Hole, Munn & Rolph, 1995). That study also showed a transfer of immunity to the lambs, with anti-H11 antibody in the plasma of the lambs conferring a moderate level of protection against a challenge of *H. contortus* given when the lambs were five weeks old.

1.6.4 Arrested Larval Development (Hypobiosis)

The temporary cessation in the development of nematodes, known as hypobiosis, serves to synchronize events within the host and is thought to coincide with the onset of environmental conditions adverse to the survival of the free-living stages, or as a consequence of host immunity (Gibbs, 1986). The proportion of challenge which undergo inhibition is also governed by the age, acquired immunity and reproductive status of the host (Michel, Lancaster & Hong, 1979). Resumption of development appears to be timed to occur when environmental conditions are suitable for the survival of the free-living stages, the mechanisms of which are not fully understood, involving host reproductive status, immune status, nutrition and stress (Armour, 1980).

In a temperate climate the primary stimulus for the induction of inhibited development of *Ostertagia* spp appears to be declining temperatures in the autumn (Armour & Bruce, 1974). Following ingestion of 'conditioned' free-living stages,

development is arrested at the L_4 stage and the larvae remain in the gastric glands. Significant numbers of established adult worms may also survive within the host (Waller & Thomas, 1978). Type II ostertagiosis disease may occur in yearling calves following their first grazing season and results from the synchronous maturation of hypobiotic larvae ingested during the previous Autumn.

Eysker (1978) concluded that under European conditions the main cause of inhibition was developing host immunity, rather than the effect of environmental stimuli upon the free-living stages.

1.7 Anthelmintic use and development of resistance

1.7.1 Anthelmintics available

There are three main classes of broad spectrum anthelmintics available for use in small ruminants, Class 1 Benzimidazoles and Probenzimidazoles, Class 2 Levamisole and Morantel and Class 3 Avermectins.

The first efficient broad spectrum anthelmintic to be introduced was thiabendazole, launched back in 1962 by Merck, Sharp and Dohme, and since then a wide range of benzimidazoles has been developed. Their mode of action is that they interfere with tubulin binding, so disrupting cell function.

The Class 2 anthelmintics, including levamisole, morantel and pyrantel act as cholinergic agonists, resulting in parasite paralysis.

The Avermectins, Class 3, including ivermectin and moxidectin, irreversibly alter chloride channels in the parasites neuromuscular membranes, which also results in parasite paralysis.

1.7.2 Anthelmintic resistance

In recent years, anthelmintic resistance has been the subject of numerous reviews (Jackson, 1993; Hazelby, Probert & Rowlands, 1994; Prichard, 1994; Barnes, Dobson & Barger, 1995; Conder & Campbell, 1995; Waller, 1997; Gill & Lacey, 1998; Sangster, 1999; Smith, Grenfell, Isham & Cornell, 1999) Anthelmintic resistance is now so widespread that it affects all commercially available anthelmintic classes, across most classes of livestock (Sangster, 1999). The cost of developing a new animal drug was estimated by Animal Pharm to be approximately US$57 million in 1996, although the cost of developing such a drug today for use in

animals destined for food was estimated to be around US$100 million (Witty, 1999). It is therefore unlikely that any major new classes of anthelmintics will be developed in the foreseeable future and this makes the conservation of the existing drugs even more important. The integration of other management strategies such as grazing management and improving the nutritional status of the host can be used in conjunction with a carefully controlled chemotherapeutic regime to control the parasitic burden.

1.7.3 Development of anthelmintic resistance

Due to the rapid development of resistance, it is most likely that the resistant genes are already present in the population. The selection pressure is exerted when the drug is used, so depleting the nematode population of the homozygous susceptible population. This leads to the development of a heterozygous resistant population and eventually to homozygous resistant individuals. The selection pressure is increased with a high frequency of treatments and with a drug efficacy being in the range of between 90-99%.

1.7.4 Detection of anthelmintic resistance

The traditional methods used *in vivo*, either the faecal egg count reduction test (FECRT) or the critical efficacy test (CET), cannot detect low levels of resistance and involve a high cost (Martin, Anderson & Jarrett, 1989). The *in vitro* methods used are mostly of use in the laboratory as research tools, as again they all have drawbacks, mostly involving access to expensive equipment, specialist knowledge and cost. The use of a PCR based technique to determine the whether or not either larval or adult *T. circumcincta* carry genes conferring resistance has recently been described (Elard, Cabaret & Humbert, 1999).

1.7.5 Reversion studies

When the selection pressure of a particular anthelmintic was removed, i.e. that drug was no longer used at all on that nematode population known to be resistant, it was hypothesised that the susceptibility may return. The evidence in the literature (Hall, Ritchie & Kelly, 1982; Borgsteede & Duyn, 1989; Jackson, 1993) seems to suggest that this is not the case and that no reversion occurs.

1.7.6 Fitness of resistant strains

The survival of the resistant parasites depends on them being just as 'fit' in order for them to survive. Recent evidence suggests that this is the case with benzimidazole resistant *T. circumcincta* (Elard, Sauve & Humbert, 1998) and that the pathogenicity and immunogenicity are also no different from a susceptible strain when both a benzimidazole resistant and multiple benzimidazole and ivermectin strain were examined (Barrett, Jackson & Huntley, 1998). This confirms evidence collected in the field that the resistant strains are equally well adapted for survival.

1.8 Alternative strategies for control

As there are unlikely to be any major new classes of anthelmintics on the market within the foreseeable future, the need to conserve the available drugs is very important. This has led to alternative strategies such as altering the nutritional status of the host (Coop & Holmes, 1996), grazing management strategies (Barger, 1997) breeding genetically more resistant animals (Gray, 1997), the use of vaccines (Emery, 1996) and biological control methods (Larsen, Nansen, Gronvold, Wolstrup & Henriksen, 1997) being investigated.

1.8.1 Altering the nutritional status of the host

It has been suggested for many years that improving the plane of nutrition of the host can reduce production losses (e.g. Clunies-Ross & Graham, 1932; Fraser & Robertson, 1933). Since then numerous studies have been carried out to investigate the effect of nutrition on the host response to gastrointestinal nematode infection and have recently been reviewed by Coop & Holmes (1996) and van Houtert & Sykes (1996). Studies have provided evidence strongly suggesting that protein supply is more important than energy in improving the hosts' response (Bown, Poppi & Sykes, 1991; Michael & Bundy, 1992; Coop, Huntley & Smith, 1995; Donaldson *et al.*, 1997, 1998).

It is thought that in the parasitised animal there may be competition for the available protein between the demands of mounting an immune response, repairing the damaged gastrointestinal tract and maintaining growth (Coop & Holmes, 1996).

When the requirement for metabolisable protein (MP) in relation to metabolisable energy (ME) is high, which is the situation in the young growing lamb and the periparturient ewe, this competition may lead to an impairment in the response of the immune system (Donaldson, 1997). If additional protein could be supplied to meet the demands of all of these systems then it is possible that both the resilience and resistance of the lambs could be improved (Coop & Holmes, 1996), where resilience is defined as "the ability to maintain a relatively undepressed production level when infected" and where resistance is defined as "the ability to suppress establishment and/or subsequent development of infection" (Albers, Burgess, Adams, Barker, Le Jambre & Piper, 1984). In addition, if the protein could be 'protected' to avoid the fermentation processes of the rumen, then a larger proportion of the protein ingested would be available for absorption in the small intestine (Coop & Holmes, 1996; Wu & Papas, 1997). Indeed, experiments with dietary supplementation using fishmeal (a rumen by-pass protein) have shown that the immunological responses of the host can be enhanced (Kambara, McFarlane, Abell, McAnulty & Sykes, 1993; van Houtert, Barger, Steel, Windon & Emery, 1995b).

This poses the question, is there a response to protein *per se* or could it be that certain essential amino acids are limiting? In growing lambs, there will be a strong demand for sulphur containing amino acids as a result of tissue deposition and wool growth. In addition, the response of parasitised animals involves considerable increases in mucoproteins which are rich in sulphur bonds. Recently, 'protected' specific amino acids have become available, including the essential sulphur amino acid, methionine. It has been shown that the addition of 'protected' methionine to the diet has increased wool growth (Coetzee, deWet & Burger, 1995), liveweight gain and fibre diameter in lambs (Mata, Masters, Buscall, Street & Schlink, 1995; Mata, Masters, Chamberlain & Young, 1997) although one study found no effect on any production parameters (Baldwin, Horton, Wohlt, Palatini & Emanuele, 1993). It has also been shown to increase liveweight gain and cashmere production in goats (Souri, Galbraith & Scaife, 1998), increase yields and production of milk proteins and fats in dairy cows (Overton, LaCount, Cicela & Clark, 1996; Armentano, Bertics & Ducharme, 1997; Kudrna, Lang & Mlazovska, 1998; Robinson, Chalupa, Sniffen, Julien, Sato, Watanabe, Fulieda & Suzuki, 1998) and both increase growth (Esteve-

Garcia & Llaurado, 1997) and enhance immune responses (Tsiagbe, Cook, Harper & Sunde, 1987) in chickens.

Recent studies (van Houtert, Barger & Steel, 1995a; Datta, Nolan, Rowe, Gray & Crook, 1999) have suggested that the provision of high levels of protein for a short period while the lambs are young can have far reaching effects. In one study Datta *et al.*, (1999) reported a significant effect on liveweight gain, wool production, antibody production to both *H. contortus* and *T. colubriformis* antigenic challenge *in vitro* and faecal egg count for the 69 week period following 9 weeks of supplementary feeding, using crossbred lambs which were 5 months old at the start of the study. The precise economic benefits from such a system still need to be evaluated in order to ascertain whether this would be a practical option in an everyday field situation, but this is an area that deserves further investigation.

1.8.2 Grazing management strategies

The greatest benefits to control are achieved when drug treatment is combined with some form of grazing management whereby treated animals are moved to pastures with lower levels of parasite contamination. However, the results of field studies disagree as to whether this 'treat-and-move' strategy promotes the development of anthelmintic resistance (Martin, Anderson & Jarrett, 1985) or has no greater effect than treating sheep which are set-stocked on the same pasture (Waller, Donald, Dobson, Lacey, Hennessy, Allerton & Prichard, 1989). The premise behind most integrated grazing management programmes is to move susceptible animals off contaminated pastures before the risk of infection becomes too high. Animals are then moved to pastures with much lower levels of contamination. These 'safe' pastures are the result of a period of little or no parasite contamination. As simply resting the pasture is seldom a viable option, the pasture can be used for crop production or to graze resistant adults or individuals of another species. Alternate grazing with cattle and sheep has been proposed for the control of bovine parasitic gastro-enteritis, though a four year study conducted in Scotland found this to be unsuccessful (Bairden, Armour & Duncan, 1995). The most important factors in determining the success of integrated grazing programmes are the host-specificity of the parasite, the longevity of the free-living stages and the duration of pasture resting or alternate use.

1.8.3 Breeding for resistance

The natural variation present both within- (Whitlock, 1958) and between-breeds (Stewart, Miller & Douglas, 1937) causing differing levels of immunological responsiveness, which is known to be under genetic control (Wakelin, 1985) could be exploited, by the use of selective breeding programmes to improve the inherent resistance and resilience of the flock. The majority of this breeding work has been done in Australia and New Zealand where breeding programmes have been established against *H. contortus*, *T. colubriformis* and *T. circumcincta* and this work has been extensively reviewed (Albers *et al.,* 1987; Piper, 1987; Baker, Watson, Bisset, Vlassof & Douch, 1990; Windon, 1990; Gray, 1991; Kloosterman, Parmentier & Ploeger, 1992; Gray, Woolaston & Eaton, 1995; Bisset & Morris, 1996; Woolaston & Baker, 1996; Gray, 1997). Work has also been done using cashmere goats in Scotland (Patterson, 1996). The selection criteria have been based on either resistance, using faecal egg count as a marker, or resilience, which is defined as the ability to perform despite larval challenge, where there is no direct measure, except using the liveweights as a guide. The selection for resistance or resilience alone would not be enough of an incentive for the majority of farmers to adopt this method and the cost of implementing the breeding programme must be less than the associated cost that the positive trait will incur. The majority of the breeding programmes have only produced positive attributes, however one of the New Zealand groups reported an increased incidence of scouring, and so an increase in the soiling of the fleece, sometimes resulting in the animals having to be crutched, and the associated costs that incurs, both in the reduced wool quality and quantity and in the manual labour costs.

1.8.4 Biological control

Various biological control agents have been suggested including nematophagous fungi, earthworms, mites, predatory nematodes, viruses and bacteria (Larsen, 1999).

1.8.4.1 Nematophagous fungi

The nematophagous fungi *Duddingtonia flagrans* is probably the most promising candidate for use in a biological control system and has been very

successful. In extensive studies it has been shown to reduce pasture contamination and faecal contamination when used in sheep (Faedo, Larsen & Waller, 1997; Githiga, Thamsborg, Larsen, Kyvsgaard & Nansen, 1997; Yeates, Waller & King, 1997; Faedo, Barnes, Dobson & Waller, 1998), calves (Nansen, Larsen, Gronvold, Wolstrup, Zorn & Henriksen, 1995), horses (Bird & Herd, 1995; Fernandez, Larsen, Nansen, Gronvold, Henriksen & Wolstrup, 1997) and pigs (Nansen, Larsen, Roepstorff, Gronvold, Wolstrup & Henriksen, 1996; Retkevicius, Larsen, Knudsen, Bach, Nansen, Gronvold, Henriksen & Wolstrup, 1998). In both laboratory and field studies feeding lambs fungal spores has resulted in reductions in larval numbers of both faecal culture and pasture larval counts, one study found that the administration of fungal spores in feed blocks was as effective as a conventional, strategic anthelmintic treatment in controlling parasites (Yeates *et al.*, 1997). Githiga *et al.*, (1997) fed lambs *D. flagrans* spores in barley prior to being turned out onto naturally infected pasture. Cultures were taken from their faeces and in the lambs fed the fungal spores only 1-28% of the predominantly *Ostertagia /Trichostrongylus* larvae developed compared to 60-80% in the untreated controls. Worm-free tracer lambs grazed on the pasture previously grazed by the fungal treated lambs showed a reduction in total worm burden of 86%, compared to the tracer lambs grazing the pasture where the untreated control lambs had previously grazed.

Although this approach would probably not be sufficient as a control measure on its own, the integration of the use of this fungi with other management strategies is a viable option.

1.8.4.2 Other biological control agents

Of the other suggestions put forward as potential candidates there has only really been serious investigation into bacteria, particularly *Bacillus thuringiensis* with reports that the toxin could affect the free living stages of some cattle parasitic nematodes (Ciordia, Porter & Bizzell, 1967) and that eggs and larvae of *T. colubriformis* could be killed by *B. thuringiensis israelensis* toxin (Bone, Bottjer & Gill, 1985, 1986, 1987, 1988). There has been no further development with this work since these publications.

1.8.5 Vaccines

This area has been the focus of much attention lately, although with relatively little success.

After the production of the first commercial vaccine against nematodes, Dictol™ which is effective against the cattle lungworm *Dictyocaulus viviparus* and was obtained by attenuating infective larvae, there were high hopes that vaccines could be prepared in a similar manner for the gastrointestinal nematodes. However, this turned out to be not the case, and although some attenuated larvae can give partial protection, for other species, such as *T. circumcincta*, no protective immunity is conferred when attenuated larvae are used. Therefore, other methods have been sought, including the use of 'natural' antigens or for blood feeders such as *H. contortus* the use of 'hidden' antigens. These are antigens that are normally hidden from the host's immune response and are present on the internal surface of the parasite gut, so coming into contact with host antibodies when a blood meal is taken. The advantage of the natural antigen approach is that immunity is boosted each time larvae are ingested, i.e. when the lambs are out on contaminated pasture. Recently a purified larval surface antigen of *H. contortus* has been used successfully in vaccination trials, as determined by significant reductions in both faecal egg counts and worm burdens, when used with aluminium hydroxide as the adjuvant (Jacobs, Wiltshire, Ashman & Meeusen, 1999).

Recently Emery, McClure, Davey & Bendixsen (1999) immunised new born lambs with a recombinant *T. colubriformis* 17 kDa antigen in incomplete Freund's adjuvant, and demonstrated a significant reduction in faecal egg count and in worm burden. This was shown to be associated exclusively with the production of IgG (1) antibody and offers the real prospect of vaccination before weaning. The trickle infection of 2,000 *T. colubriformis* L_3 three times a week for six weeks following birth, followed by a challenge seven days later and then a *post mortem* ten days after that, also led to a significant reduction in worm burden, lower than those observed in four month old control lambs. Previously it was postulated that vaccination of very young lambs may be difficult due to the lack of innate immunity (Colditz, Watson, Gray & Eady, 1996) but this work suggests that that may not be the case. Further investigation into this area is clearly required.

1.9 Aims

The main aims of this project were to provide data on two different stages of the parasites' life cycle. Firstly, to look at the free living stages of the parasite and investigate the influence of environmental factors on the migration rate of the infective larvae using artificial growth chambers. Once these factors had been established, the rate of ingestion of larvae was to be investigated using oesophageally fistulated lambs. The second component of this project was to look at certain aspects of the parasitic stage. Two main areas were to be studied, the window of susceptibility in the lactating ewe and the influence of the nutritional status of the lamb on acquisition and expression of immunity. The complex relationship between all of these factors is shown in Figure 1.2.

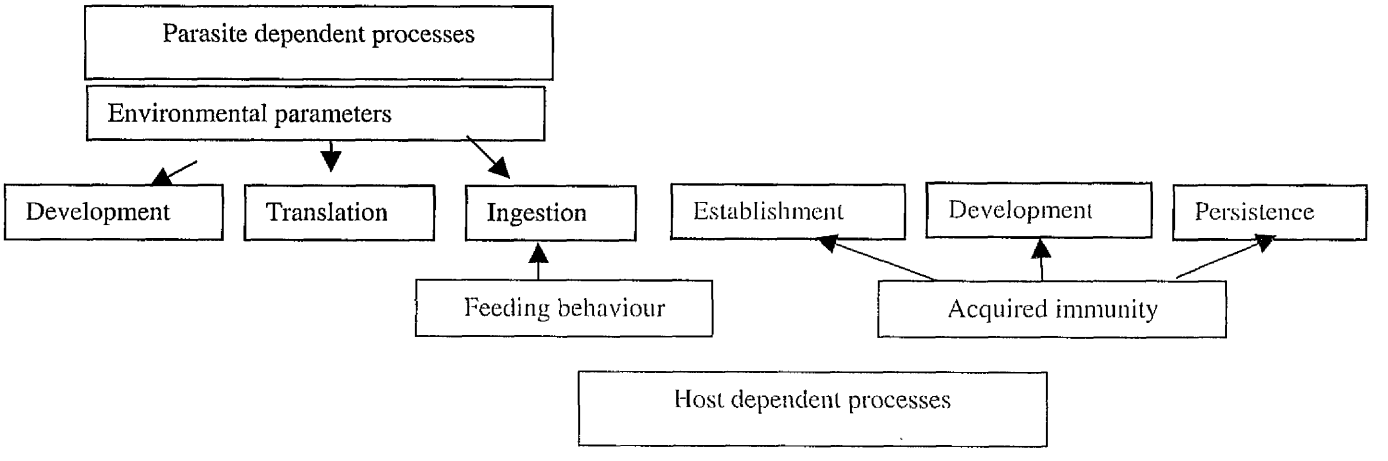


Figure 1.2 *The interaction between the parasite and host dependent processes.*

In order for a parasite to find a host, it must first, develop into a viable infective larvae, second, locate itself onto a piece of grass and third, be ingested by the host. One of the aims of this project was to examine, in depth, the environmental factors influencing this translation onto the grass sward and the migration on the sward. Following on from this, the feeding behaviour of the lamb and how that influences larval intake were also examined in some detail.

The periparturient rise in faecal egg count experienced by the lactating ewe is one of the major sources of contamination for the young lamb just starting to graze. There is very little available data on this window of susceptibility, and this will obviously have a major impact on pasture contamination and lamb productivity.

The addition of dietary supplements, specifically protein, have been shown to be of benefit in the parasitised animal. In order to examine this further, a sulphur containing amino acid protected from degradation in the rumen, methionine, was given to parasitised lambs of two different ages, to look at effects on both resilience and resistance. It has been suggested by previous studies that the supplementation of this protein has been of benefit on resilience when an intestinal parasite is used, and it was thought that a similar effect may be seen using *T. circumcincta*, an abomasal parasite.

The final chapter was used to examine possible consequences of protein supplementation on the host and parasite populations, using a computer generated model. Simulations could be run over a ten year period, and changes in supplementation levels were simulated by using an anthelmintic bolus at varying efficacies, to produce the reduction in egg counts seen in supplemented animals.

The aim of this thesis was to examine several points in this complex host-parasite relationship and to provide data on several key areas within this relationship. A clearer understanding of environmental factors influencing larval migration, for example, would be of benefit when used in conjunction with information on when the periparturinet ewe is most susceptible to infection, to enable the optimum time for anthelmintic use to be determined.

Chapter 2

General Materials and Methods

2.1 Animals

2.1.1 Ewes

Greyface (Scottish Blackface cross Border Leicester) ewes aged between 2 and 5 years bearing twin lambs were dosed with ivermectin (Oramec, MSD Agvet, UK) at the manufacturer's recommended dose rate and then kept housed under conditions designed to avoid accidental infection.

2.1.2 Worm-free lambs

Lambs were born and raised indoors and kept under conditions designed to avoid accidental infection with gastrointestinal nematodes.

2.2 Sward Preparation and use

2.2.1 Preparation and use of the sward from cultivated turf

The pots were prepared using a cultivated turf, Inturf SS3 (Stewart and Co. Seedsmen Limited, UK), which was cut to a 12 cm diameter, placed on top of a pot full of steam sterilised multipurpose compost (Homebase, UK), placed under a 150 watt natural daylight bulb (Sungro-Lite, Mulhearn and Brotchie Ltd., UK) and kept well watered until used. Fourteen pots were required for each experiment and were trimmed to a uniform height of 3 cm. Immediately before the start of the experiment, each pot was watered using a garden sprayer (Homebase, UK) for 10 seconds from a height of 15 cm from the base of the sward. At time 0, each pot was each seeded with 2,000 L_3, using a Finn pipette to place the larvae onto the mat at the base of the sward and placed inside the growth chamber (Model S10H, Conviron Ltd., UK) in randomly assigned places. Two pots were harvested after 15 minutes, removing the top 2 cm and bottom 1 cm separately. The remaining twelve pots were harvested at 1, 2, 3, 4, 6 and 12 hours after seeding. The grass samples were kept at 4°C until counted using the direct count technique as described in 2.3.1.

2.2.2 Planting and use of the sward from seed

The pots were prepared as described by Rees (1950). Briefly, approximately 120 g of steam sterilised multipurpose compost (Homebase, UK) was placed in the base of a 12 cm diameter round plant pot. A further 60 g of compost was mixed with 2.5 g superphosphate (J. Arthur Bowers, UK) and 1 g of ammonium sulphate (J. Arthur Bowers, UK). Half of this fertilised compost was placed on top of the soil in the pot, 4 g of perennial rye grass (*Lolium perenne*) seeds were placed evenly over this and then covered with the remaining compost. The pot was watered, placed in a plastic bag and put in the dark until the start of germination. Once the seedlings had appeared the pots were placed under a 150 watt natural daylight bulb and kept well watered. The experimental protocol was similar to that described in 2.2.1, except only twelve pots were used as no sample was taken at one hour after larval seeding. Since the swards were drying out fairly quickly in the first experiment, as shown by the datalogger during the course of the experiment, a mist was created inside the chamber by holding the nozzle of the sprayer pointing upwards and spraying, at a height of 22 cm above soil level for 20 seconds in three places inside the chamber. This was done hourly for the first eight hours of the experiment. The pots due to be processed were removed before the chamber was sprayed.

2.3 Larval Recovery Methods

2.3.1 Direct count technique (DCT)

A 1.00 mm sieve was stacked on top of a 38 μm sieve and the grass sample was placed on the top. It was washed vigorously for 10 minutes and the washings containing the larvae were collected on the 38 μm sieve. The retentate was washed off the 38 μm sieve into a beaker, stained with helminthological iodine, poured into a 55 mm gridded contact plate (Sterilin, UK) and counted using a stereo microscope at x100 magnification.

2.3.2 Taylor (1939) technique

The sample was soaked overnight in 5 litres of warm tap water (25°C) which contained a small amount of detergent (0.25 ml per 5L, Tween 20, Aldrich Chemical Co., UK).

Following soaking, the herbage was removed in small handfuls which were squeezed to remove as much water as possible and discarded. The sample was then sedimented in a cold room (4°C) overnight and its volume reduced to 2 litres. Following two further sedimentations to 500 ml and 100 ml respectively the sample was poured into seven 15 ml polyallomer, squashable centrifuge tubes (Beckman, USA). After centrifugation (1000 rpm for 2 minutes) the supernatant was removed using a vacuum line and the pellet re-suspended gently in 12 ml saturated sodium chloride solution prior to recentrifugation (1000 rpm for 2 minutes). Using artery forceps, the tube was clamped just below the meniscus and the contents of the upper chamber washed into a 15 ml polyallomer centrifuge tube. The recovered larvae were pooled, washed twice centrifugally using tap water and the sample volume reduced to 0.3 ml. The sample was transferred to a 4 ml disposable polystyrene cuvette (LIP Ltd., Shipley, UK) rinsing over with saturated potassium chloride. The cuvette was then topped up with saturated potassium iodide solution and sealed.

Counts were conducted on a compound microscope using a calibrated eyepiece graticule (Miller square, Graticules Ltd., UK) at ×100 magnification. When samples contained high numbers of larvae the graticule was used. The total number of larvae observed in the large square along two traverses of the cuvette were multiplied by 3 or the total number of larvae observed in the small square along two traverses of the cuvette were multiplied by 9 in order to obtain the total number of infective larvae. For samples with few larvae, all the larvae in the cuvette were counted.

2.3.3 Martin, Beveridge, Pullman & Brown (1990) technique

The sample was placed in a large plastic container and covered with water, detergent (1ml per 2 litres, Tween 20, Aldrich Chemical Co., UK) was added and the sample left to soak for 4 hours. The herbage was removed and washed in two separate volumes of water before being discarded and the washings added to the container and left to sediment overnight. The supernatant was removed using a vacuum line and the remaining contents of the container were poured through a 1 mm coarse sieve into a conical container, fixed with alcohol and left to sediment overnight. The volume of the sediment was recorded, the sediment mixed and a 2 ml sample transferred to a 15 ml centrifuge tube (Beckman, USA) for counting.

Saturated potassium iodide solution was added prior to centrifugation (2 minutes at 2000 r.p.m.). The supernatant was transferred to a 50 ml centrifuge tube (Beckman, USA), the sample made up to 50 mls with tap water, mixed thoroughly and centrifuged (2 minutes at 2000 r.p.m.). The supernatant was removed using a vacuum line and the resulting pellet was transferred to a cuvette. The sample was counted using the method described in 2.3.2.

2.3.4 Heath & Major (1968) technique

The sample of macerated herbage was thoroughly mixed and washed through a series of three sieves, of aperture 1.98, 1.02 and 0.19 mm respectively, using a high pressure spray. The first sieve was removed when the washings occupied half of a 12 litre bucket, the second when the bucket was three quarters full and the rest of the bucket was filled with washings from the final sieve. The washings were then left to stand overnight. The supernatant was removed using a vacuum line, the sediment washed in to a 2 litre cylinder and allowed to settle overnight. The supernatant was removed, and the sediment washed into a 250 ml cylinder. This sample was stored overnight at 4° C.

To facilitate the sedimentation of organic material, gases were removed from the remaining fluid by creating a vacuum above the fluid. The sample was divided equally between four centrifuge tubes, saturated zinc sulphate was added, the tubes were inverted and then centrifuged (15 minutes at 1200 r.p.m.). Four samples of 0.5 ml were collected and counted using the method described in 2.3.2.

2.4 Parasitological Techniques

2.4.1 Larval culture

Faeces from a monospecifically infected donor lamb were collected and placed in plastic trays, covered with a polythene bag with ventilation holes and left for 10 days at 22°C. The trays were then flooded with warm water (25°C) and left to soak for 2 hours. The fluid was decanted and left to sediment at 4°C for 2 hours. After sedimentation the volume was reduced using a vacuum line and the larvae cleaned by Baermannisation through high wet-strength paper (Cleanaroll Ltd.). Larval yield was determined by calculating the number of larvae present in an

aliquot of the larval suspension. Infective larvae were stored in tap water at 4°C and used within 3 weeks of being harvested.

2.4.2 Faecal egg count

Faecal egg counts were performed using a modified flotation technique as described by Christie & Jackson (1982). Rectal faecal samples were taken into a 300 x 250 mm, 100 gauge polythene bag and assigned a score reflecting their consistency. This faecal score ranged from 1 for a sample consisting almost wholly of blood and mucus to a score of 5 for a sample consisting of dry, hard pellets, as shown in Table 2.1.

Table 2.1 *Scoring system used to monitor faecal consistency*

Score	Condition
1	Sample consisting wholly of blood and mucus
2	Sample fluid consisting largely of faecal debris together with some mucus and or blood
2-3	Fluid sample containing no blood or mucus.
3	Sample soft, unformed consisting of faecal debris
3-4	Sample partially formed, obvious pellets within faecal mass.
4	Sample formed consisting of moist pellets.
5	Sample formed consisting of dry hard pellets.

The sample was then weighed and 10 ml of tap water added per gramme of faeces. Each sample was then emulsified using a stomacher (Seward Medical Ltd., UK). A 10 ml sub-sample was then removed and passed over a 1 mm aperture sieve. The retentate was washed with an additional 5 ml of tap water, compressed to recover as much fluid as possible and then discarded. The filtrate was poured into a 15 ml polyallomer, squashable centrifuge tube (Beckman, USA) and centrifuged at 1000 rpm (228 *g*) for 2 minutes.

The supernatant was removed using a vacuum line and the faecal pellet re-suspended gently in 12 ml saturated sodium chloride solution prior to recentrifugation (1000 rpm for 2 minutes). Using artery forceps, the tube was

clamped just below the meniscus and the contents of the upper chamber washed using saturated salt solution into a 4 ml disposable polystyrene cuvette (LIP Ltd., Shipley, UK). The cuvette was topped up with saturated salt solution and sealed. Counts were done on a compound microscope using a calibrated eyepiece graticule (Miller square, Graticules Ltd., UK) at ×40 magnification. The eyepiece graticule was used to count eggs in samples with high egg concentrations When samples contained high numbers of eggs the graticule was used. The total number of eggs observed in the large square along two traverses of the cuvette were multiplied by 3 or the total number of eggs observed in the small square along two traverses of the cuvette were multiplied by 9 in order to obtain the total number of eggs per gramme (EPG) of faeces. For samples with few eggs all the eggs in the cuvette were counted to give EPG.

2.4.3 Egg hatch assay

Faecal material was collected directly from the rectum into a polythene bag and 40 mls of tap water was added. The faecal material was emulsified using a Stomacher (Seward Medical Ltd., UK) then suspended in approximately one litre of tap water. The faecal suspension was washed over a series of stainless steel sieves (1 mm, 500μm, 212μm, 75μm and 38μm) and theretentate collected on the 38 μm sieve, containing the eggs, was poured into 15 ml polyallomer, squashable centrifuge tubes (Beckman, USA) and centrifuged at 1000 rpm (228 *g*) for 2 minutes. The supernatant was removed using a vacuum line and the faecal pellet re-suspended gently in 12 ml saturated sodium chloride solution prior to recentrifugation (1000 rpm for 2 minutes). Using artery forceps, the tube was clamped just below the meniscus and the contents of the upper chamber were washed and rinsed into a 15 ml polyallomer centrifuge tube with tap water. The eggs were washed centrifugally three times in tapwater. The total volume was made up to 10 mls and the number of eggs present in 100 μl were determined. The eggs were re-suspended so that 100 μl contained approximately 100 eggs.

A 1000 ppm stock solution was prepared by dissolving 0.1g of thiabendazole in 20 mls of DMSO and made up to 100 mls with distilled water. This stock solution was used to produce a range of working stock solutions. The range of concentrations that were used in the well for investigating suspected resistant strains were 0, 0.05,

0.1, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, and 1.5 ppm. 100 µl of egg suspension was added to one side of each of duplicate wells in a 24 well (16 mm well diameter) culture plate (Corning, USA), 10 µl of working concentration stock solution was added to the other side of each well and then 1890 µl of distilled water was added. The culture plate was placed in an airtight container with a piece of damp tissue to keep the humidity high and incubated at 25°C for 48 hours. 20µl of helminthological iodine (Appendix I) was added to each well and, using an inverted microscope at a magnification of x100, the numbers of eggs and larvae in each well was counted and the ED_{50} values calculated.

2.4.4 Post-mortem procedures

All animals were stunned with a captive-bolt pistol and exsanguinated. The animal was opened along the ventral midline and ligatures placed at the omasal/abomasal and abomasal/duodenal junctions prior to the removal of the abomasum.

The abomasum was opened along its greater curvature and the contents collected. An entire abomasal fold was removed and fixed for histopathology. The abomasum and its contents were then soaked in approximately 4.5 litres of warm 0.85 % saline solution for 4 hours at 37 °C with regular agitation (Jackson, Jackson & Smith, 1984). Following incubation, the abomasal folds were gently run between the finger and thumb in order to remove the superficial mucosa, the abomasum was discarded and the abomasal contents made up to a volume of 5 litres. A 10% subsample (500 ml) was taken and fixed with 20 ml formalin.

2.4.5 Worm counts

A 100 ml aliquot, representing 2% of the total worm population, was taken from each of the fixed sub-samples and stained with 10-15 ml of helminthological iodine (Appendix). The stained sample was washed over a 38 µm aperture sieve to remove excess iodine before being examined under a stereo microscope using a 55 mm contact plate (Sterilin, UK). Any *Teladorsagia* worms present were recovered and preserved in 2% formalin, before being staged and sexed following the method described by Denham (1969).

2.5 Haematology

2.5.1 Collection of blood and plasma samples.

Animals were bled, at the same time of day in each experiment, by jugular venepuncture into 10 ml vacutainer tubes (Becton Dickinson, UK) containing lithium heparin. The tubes were centrifuged at 3000 rpm (2060 g) for 20 minutes and the plasma carefully removed using a Pasteur pipette into two 2 ml aliquots which were frozen at – 20 °C for subsequent analysis.

2.6 Histology

2.6.1 Tissue preparation

Sections of the abomasum and small intestine were removed immediately after slaughter and fixed in 4% w/v paraformaldehyde in phosphate buffered saline (PBS) pH 7.4 (Appendix) for 6 hours (Newlands, Huntley & Miller, 1984). Tissues were dehydrated through a graded ethanol series, cleared in toluene and embedded in paraffin wax (Miller, Jackson, Newlands & Appleyard, 1983a). Tissue sections were cut to 5 μm using a microtome (Leica Instruments GmbH, Milton Keynes, UK), deparaffinised in xylene and rehydrated prior to staining.

2.6.2 Mast cell and globule leukocyte counts

For histochemical enumeration of mast cells, tissue sections were stained overnight with toluidine blue at pH 0.5 (Enerback, 1966). Stained sections were washed thoroughly in tap water, dehydrated in ethanol, cleared, air dried and permanently mounted with Coverbond mounting medium (American Hospital Supply Corporation, Illinois, USA). Stained cells were counted under a compound microscope with a ×10 eyepiece containing a calibrated graticule and a ×40 objective lens giving a viewing area of 0.08 mm^2. For abomasal sections, the counts were made from the muscularis to the mucosal surface on a minimum of 20 fields from 3 separate sections of the fold, and are expressed as the number of cells 0.2 mm^{-2} abomasal mucosa.

2.7 Oesophageal Fistulation

2.7.1 Surgical procedures

Eight worm-free Suffolk cross Greyface lambs of around 4 months of age were weaned from their dams into individual pens and kept housed in a slatted floor shed for three weeks before being surgically prepared as described by Pfister, Hansen & Malecheck (1990). The lambs were trained on the trays planted with grass from one week after surgery until the start of the experiment, a total of around three weeks. At the start of the experiment the four lambs which had best adapted to grazing from the trays were chosen.

2.7.2 Collection of extrusa

The feeding regime ensured that the lambs were hungry prior to being offered the tray of grass. The oesophageally fistulated lambs' plug was removed and a 75 x 50 cm polythene bag split along one of its lengths was tied just behind the lambs front legs with string. The two loose ends of the bag were tied behind the lambs neck, thus creating a pouch that any ingested material would be collected in. Once the lamb had taken fifty bites from the tray the bag was removed, the contents were weighed and frozen until being processed by the direct count technique as described in 2.3.1.

2.7.3 Measurement of bite depth

A 20 x 40 cm plastic tray seeded with rye grass (*Lolium perenne*) at 920 seeds per tray (Illius, Clark & Hodgson, 1992) was trimmed to either 3 or 6 cm. A grid was then placed on top of the tray and at twenty points, randomly generated by a computer, the sward stick (Bircham, 1981; Barthram, 1986) was lowered into position. The point where the first blade of grass touched the sward stick was measured and recorded. The tray was placed in the pen with the lamb, and the lamb took fifty bites. The tray was removed and the same twenty points were re-measured using the sward stick.

2.8 Biochemical Techniques

2.8.1 Plasma pepsinogen assay

Plasma pepsinogen concentrations were determined by a modification of the method of Mylrea & Hotson (1969) using bovine serum albumin as substrate and a glycine / hydrochloric acid buffer (pH 2.0). Pepsinogen activity was expressed as international units (IU) where 1 international unit equals 1 μmole tyrosine released per minute per litre of plasma at 37°C. Stored plasma samples were removed from –20 °C and allowed to thaw out overnight in a fridge. Glycine buffer (0.1M pH 2, Appendix) and 1% bovine serum albumin (BSA, Appendix) were mixed together in a ratio of 4:1 before dispensing 0.5 ml of this mixture into each eppendorf, to which 0.1 ml of plasma was added (each sample was run in duplicate). These 'test' samples were mixed using a vortex mixer and incubated in a water bath at 37°C for 4 hours. In a further set of duplicate samples (unincubated controls) the 0.1 ml of

plasma was added to the 0.4 ml of 10 % Trichloroacetic acid (TCA, Appendix) and the sample mixed and centrifuged for 3 minutes at 13,000 rpm (11,000 g). Following the 4 hour incubation period, 0.4 ml of 10 % TCA was added to the test samples, mixed and centrifuged as above.

A standard curve for tyrosine was constructed by making up 0μl, 100μl, 250μl, 500μl, 750μl and 1000μl volumes of working standard (1 in 10 dilution) to a total volume of 2 ml using 1 ml of 10 % TCA and distilled water. The appropriate number of rotors were loaded into a Monarch centrifugal semi-automated spectrophotometer (Instrumentation Laboratories, UK) with Folins and Ciocalteau's reagent (1 in 3 dilution) in one reagent boat and 1N NaOH (Appendix) in another. The standards and sample supernatants were then transferred into 0.2 ml sample cups (Instrumentation Laboratories, UK) and their absorbances read at 690 nm.

Plasma pepsinogen concentrations were then calculated using the mean absorbance of the standard curve having a tyrosine concentration of 0.0039 µM (for 75 µl volume used with the Monarch):

$$\frac{\text{Test - Control}}{\text{Mean Std - Blank}} \times \mu\text{M Tyrosine} \times \frac{1000}{\text{Vol plasma (ml)}} \times \frac{1}{\text{Time (mins)}}$$

$$\therefore \quad \frac{\text{Test - Control}}{\text{Mean Std - Blank}} \times 0.0039\ \mu\text{M} \times \frac{1000}{0.0075} \times \frac{1}{240}$$

$$\therefore \quad \frac{\text{Test - Control}}{\text{Mean Std - Blank}} \times 2.166 = \text{U (}\mu\text{M Tyrosine/min/litre at 37 °C)}$$

2.8.2 Plasma albumin

Plasma albumin levels were determined using a standard kit, IL test™ Albumin (Instrumentation Laboratories, UK) which uses the selective binding of bromocresol green to albumin. A 50 µl plasma sample was placed in a 0.2 ml sample

cup (Instrumentation Laboratories, UK) and the absorbances read at 620 nm using a Monarch 2000 centrifugal semi-automated spectrophotometer.

2.8.3 Plasma total protein

Plasma total protein levels were determined using a standard kit, MPR3 (Boehringer Mannheim, Germany) which uses the Biuret method, where the proteins form a coloured complex with cupric ions in an alkaline medium. The plasma sample was prepared as in the plasma albumin test and the absorbance was read at 546 nm. This test was run concurrently with the plasma albumin test.

2.9 Statistical Analysis

All statistical analyses were performed using Minitab statistical software, version 10.2. Arithmetic means are given with ± 1 standard deviation (SD) or standard error of the mean (SEM) unless stated otherwise. Where data were skewed or had unequal variances they were transformed by $\log_{10}$ or $\log_{10}(\chi+1)$. The transformation for particular data sets and the tests used are detailed in each chapter.

Chapter 3

Investigation of different environmental factors on larval migration using growth chambers

3.1 Introduction

The ability to predict the occurrence of peak larval contamination on the pasture is valuable in allowing the most effective use of anthelmintics. This has the potential to avoid unnecessary dosing, which can lead to a more rapid selection of anthelmintic resistance. There are obvious economic benefits of using less drug whilst minimising production losses.

The development of infective larvae from eggs deposited on the pasture depends a range of environmental factors, including temperature, relative humidity, rain, sunlight and wind (Stromberg, 1997). The factors influencing *T. circumcincta* development have been investigated by both field studies (Gibson & Everett, 1976; Boag & Thomas, 1977; Callinan, 1978) and under laboratory conditions by Pandey *et al.* (1989, 1993), looking at both development and survival of eggs and infective larvae at different temperatures and relative humidities. The optimum temperature for development was found to be 16°C, with the relative humidities of 35 and 50% providing a longer survival time than either 75 or 95%. The larval development rates and survival rates do not necessarily have the same optimum conditions. Higher temperatures may produce quicker development rates but there may be a depletion of lipid reserves leading to a higher mortality rate (Urquhart *et al.*, 1991).

There is evidence to suggest that infective larvae develop differently when subjected to different environmental conditions. Rossanigo & Gruner (1996) showed that under optimal developmental conditions *T. circumcincta* and *T. colubriformis* infective larvae grew longer, mainly due to an increased length of intestinal cells, and that these longer larvae migrated faster out of an agar block and exsheathed faster. In the case of *T. circumcincta* there was also a significantly lower establishment rate in lambs with the shorter infective larvae, although the adult worms showed no significant difference in size or fecundity.

The factors influencing the migration of larvae on the sward are more difficult to measure, due to the added errors of sampling and recovery methods. There are two different components influencing the migration rates, they are the initial migration from the soil onto the base of the sward and the migration up and down the sward. The larvae are capable of migrating downwards into the soil as well as onto the sward, as shown by studies on the cattle parasites *O. ostertagi* (Fincher & Stewart, 1979; Al Saqur *et al.*, 1982; Krecek & Murrell, 1988) and *Cooperia*

oncophora (Fincher & Stewart, 1979) and in the sheep parasites *Teladorsagia* spp. and *Trichostrongylus* spp. (Furman, 1944; Crofton, 1948; Sturrock, 1961; Callinan & Westcott, 1986). The soil is thought to be an important reservoir where the infective larvae can over winter then emerge back onto the pasture when the weather becomes more favourable (Al Saqur *et al.*, 1982).

The major factors influencing the migration rates are the same environmental factors as those influencing the development and survival of the eggs and larvae. The larvae move on the sward in a moisture film, using the surface tension of the film to move vertically (Stromberg, 1997; Niezen *et al.*, 1998c). If this dries out then the larvae either retreat with the edge of the moisture film or are left stranded on the sward, leaving them very vulnerable to desiccation. The moisture film position depends on the density of the sward, the height of the sward and the relative humidity of the microclimate surrounding the sward (Niezen *et al.*, 1998c).

The largest influences of temperature and light intensity may be their effect on the moisture content of the sward, as it is difficult to separate out these factors individually. It is most likely that a combination of all these factors influence migration rates.

There is conflicting evidence as to whether there is a species difference in migration rates under the same environmental conditions. Some authors report no significant difference between *Trichostrongylus* spp. and *Teladorsagia* spp. (Callinan & Westcott, 1986; Niezen *et al.*, 1998c) while others have reported differences between *H. contortus*, *Trichostrongylus* spp. and *Teladorsagia* spp. (Rogers, 1940) and differences between *H. contortus* and *H. placei* (Krecek *et al.*, 1991, 1992, 1995). It would be expected that parasites with different geographical distributions would react differently to temperature, for example. This seems to be supported by Rogers (1940) who reported reduced migration rates for *H. contortus* on wet swards.

There is also evidence suggesting that migration rate may be influenced by sward species (Crofton, 1948; Silangwa & Todd, 1964; Moss & Vlassof, 1993; Scales *et al.*, 1995; Niezen *et al.*, 1998c) but it is not clear what these differences in migration rate are attributable to. It has been shown that the external morphology of the leaf can influence the existence and thickness of water films (Wallace, 1959), although the effect this may have on infective larvae has not been investigated. It is

likely that different plant species create different micro-climates and this, along with distinct leaf morphologies, may explain the different migration rates.

There is little data on sward density effects, both Kauzal (1941) and Silangwa and Todd (1964) recovered greater numbers of larvae from a 'dense' sward than from a 'sparse' sward, although no figures were given as to density of seeds that had been sown. Both authors speculated that the denser the sward the greater the probability of the larvae 'finding' a blade.

Numerous methods for recovering infective larvae from large quantities of herbage, i.e. between 250-500 grams have been published (Taylor, 1939; Dinaburg, 1942; Durie, 1959; Sturrock, 1961; Donald, 1967; Heath & Major, 1968; Lancaster, 1970; Smeal & Hendy, 1972; Jørgensen, 1975; Mwegoha & Jørgensen, 1977; Young & Trajstman, 1980; Bairden, Duncan & Armour, 1981; Cheijina, 1982; Gettinby, McKellar, Bairden, Theodoridis & Whitlaw, 1985; Martin *et al.*, 1990; Krecek *et al.*, 1991; Eysker & Kooyman, 1993; Fine, Hartman, Krecek & Groeneveld, 1993; Aumont, Frauli, Simon, Pouillot, Diaw & Mandonnet, 1996). Most methods are based on washing the larvae from the herbage, although Jørgensen (1975) described a technique utilising the migration of lungworm larvae from an agar block.

The recovery rates for these methods range from 28-100% and the time, equipment, expense and expertise needed also vary widely. The effects of storage time of the sample has been investigated by Fine *et al.* (1993), using *H. contortus* infective larvae. Their findings indicate that recovery rates decline over time and this decline is sharper with a higher concentration of larvae on the herbage.

In order to maximise the numbers of larvae recovered from the very small (2-10 grams) herbage samples provided by the pots, a more efficient method was required. The initial part of this trial was to establish the recovery rates from the standard method routinely used in the laboratory for field studies (Taylor, 1939), a more recently published method (Martin *et al.*, 1990) and a newly developed direct count technique (DCT) for small herbage samples.

This second component of the trial was designed to investigate the effects of temperature, relative humidity and light intensity upon migration of *T. circumcincta* infective larvae. The studies were conducted in a growth chamber which enabled manipulation of environmental factors. A data logger was used to record

micrometeorological variations. The study also investigated migration rates on swards of different maturities, a young sward and a mature, tillered sward.

3.2 Materials and Methods

3.2.1 Comparison of three different larval recovery techniques from small amounts of herbage

The direct count technique (DCT), Taylor (1939) technique and the Martin *et al.* (1990) technique are described in Chapter 2. Grass samples of 10g, free from larval contamination, were collected, seeded with approximately 2,500 *T. circumcincta* infective larvae and processed using the three different techniques.

3.2.2 Growth chamber experiments

3.2.2.1 Preparation of the infective larvae

T. circumcincta larvae were cultured as described in Chapter 2 and a suspension containing approximately 1,200 *T. circumcincta* L_3/ ml was prepared. The swards were prepared, the pots were seeded with 2,000 L_3, the swards were harvested and counted using the direct count technique as described in Chapter 2. In experiments 2 and 3 the dry weight of the sward was calculated, by drying the sward in an oven at 60 °C for 48 hours.

3.2.2.2 Experimental design

The experimental design for the growth chamber experiments are shown in Table 3.1. All experiments were run at between 75 and 85% relative humidity (RH). A data logger (Model CR10) was attached to a control pot placed within the chamber and used throughout the experiments to monitor moisture levels on the sward, light intensity and temperature within the chamber.

3.2.2.2.1 Experiment 1 Temperature study using cultivated swards

In experiments 1.1, 1.2 and 1.3, the effects of 3 different temperatures, 20°C, 15 °C and 10°C respectively, on larval migration were investigated using the swards from the cultivated turf. All of the experiments were run at a light intensity of $65WM^{-2}$.

3.2.2.2.2 Experiment 2 Temperature study using 'sown' swards

In experiments 2.1, 2.2 and 2.3, the effects of the same 3 temperatures were investigated using the swards grown from seed. All of the experiments were run at a light intensity of $65WM^{-2}$.

3.2.2.2.3 Experiment 3 Illumination study using 'sown' swards

In experiments 3.1, 3.2 and 3.3, the effects of 3 different levels of light intensity, $65WM^{-2}$, 50 WM^{-2} and 32 WM^{-2} respectively, were investigated using the swards grown from seed. All of the experiments were run at 15°C.

Table 3.1 *Experimental design for the growth chamber experiments*

Experiment number	Temperature (^0C)	Light intensity (WM^{-2})	Sward type
1.1	10	65	Cultivated
1.2	15	65	Cultivated
1.3	20	65	Cultivated
2.1	10	65	Seed
2.2	15	65	Seed
2.3	20	65	Seed
3.1	15	32	Seed
3.2	15	50	Seed
3.3	15	65	Seed

3.2.2.3 Statistical analysis

The data was log transformed (x+1) then analysed using REML (Genstat). In experiments 2 and 3 the dry weight was used as a co-variate.

3.3 Results

3.3.1 Comparison of three different larval recovery techniques from small herbage samples

The mean recovery rates (% ± SEM) from the10g grass samples for the Taylor (1939), Martin *et al.* (1990) and DCT were 49 ± 7, 51 ± 9 and 92 ± 4 respectively.

3.3.2 Growth chamber experiments

3.3.2.1 Experiment 1 Temperature study using cultivated swards

Two pots free from larval contamination were processed in the same manner as the artificially infected swards. No infective larvae were recovered.

The average numbers of larvae recovered from the two duplicate pots and the section of sward which they were recovered are shown in Figure 3.1. The proportion of larvae that migrated ranged from 4 -29 % of the total number seeded onto the pot, as shown in Figure 3.2. There was no significant difference between the numbers of larvae recovered from the time 15 pot for each of the three temperatures.

Larval migration at 10 and 15°C followed a very similar pattern, with very little movement of larvae on the swards until 12 hours post seeding. There was a completely different pattern of activity with the 20°C pots, with immediate activity of the larvae being recorded.

The effect of the pot was found to be not significant showing very little inter-pot variation. There were significant time ($p< 0.05$) and section effects ($p< 0.001$) and the time section interaction was statistically significant ($p< 0.001$), where section defines either the top or bottom portion of the sward. There were significantly more larvae at the base of the grass stems than at the top. When the 3 temperatures are looked at as a whole, there was only one significant fluctuation between 0 and 1 hours.

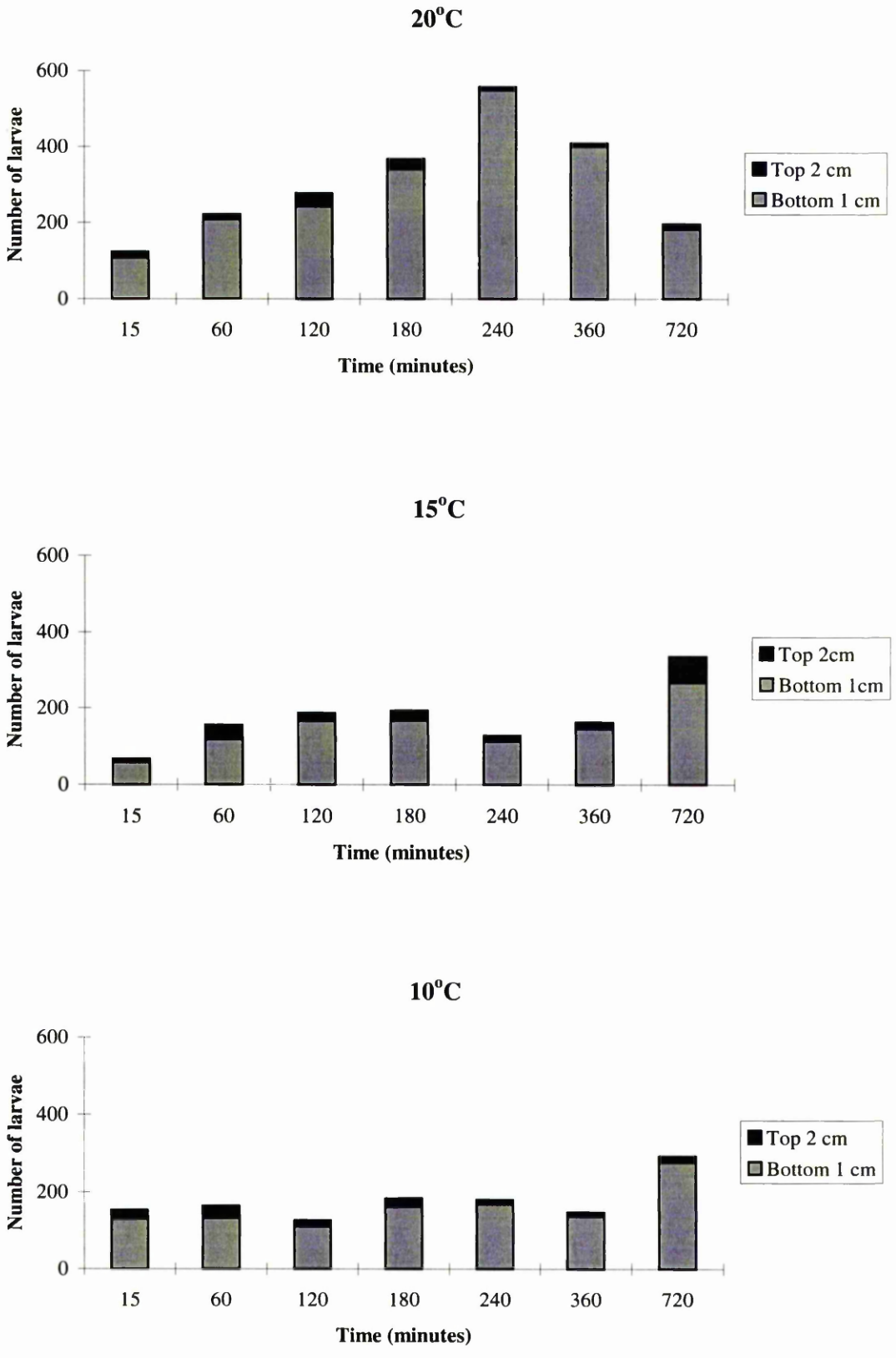


Figure 3.1 *Average numbers of larvae recovered at three different temperatures from a sward prepared from cultivated turf*

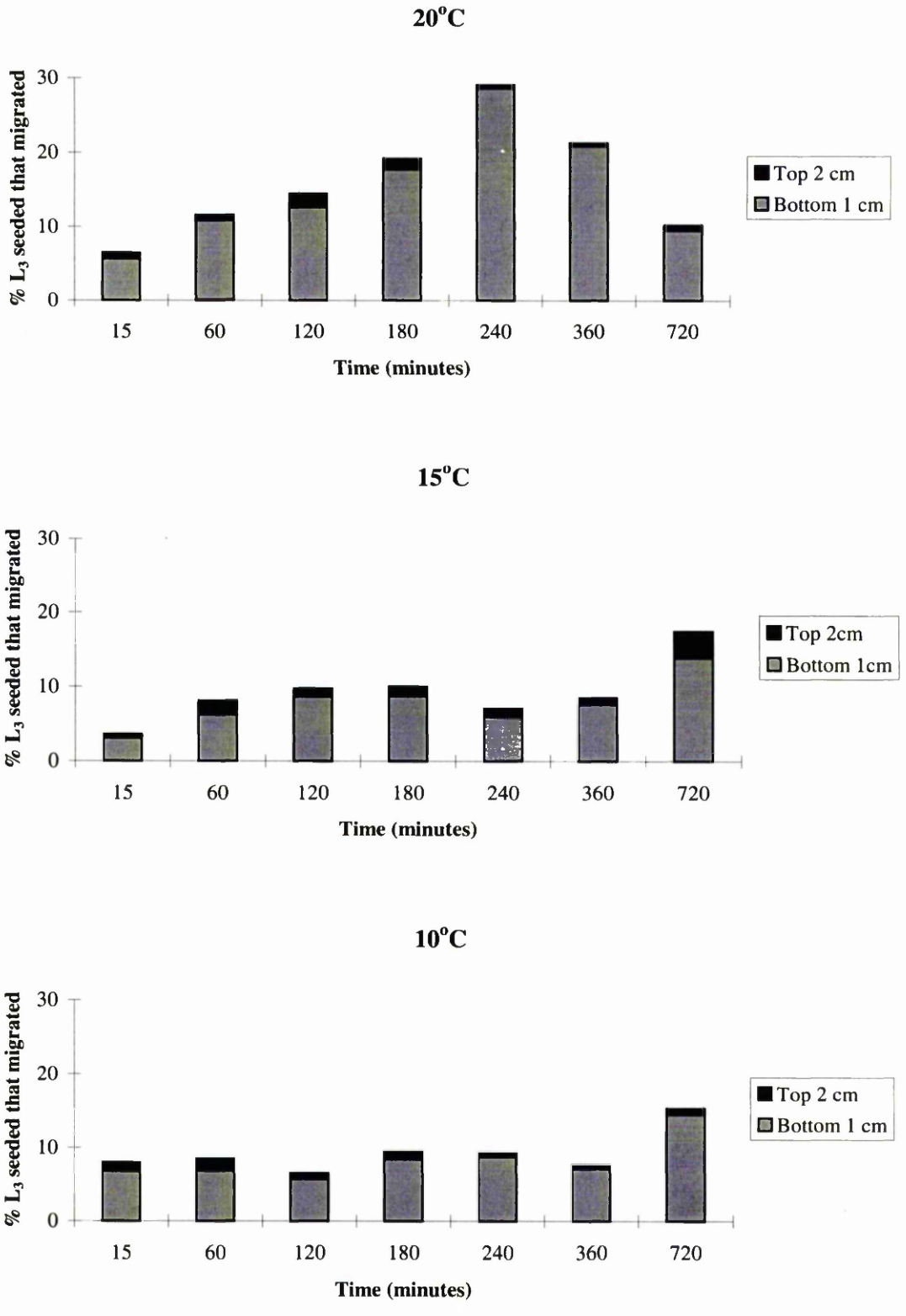


Figure 3.2 *Percentage of larvae that migrated on the sward prepared from cultivated turf at three different temperatures*

3.3.2.2 Experiment 2 Temperature study using 'sown' swards

The average numbers of larvae recovered per gram of dry herbage from the duplicate pots are shown in Figure 3.3. The repeat of experiment 1, using the sward grown from seed showed a very large difference in migration rate, with only between 0.05 - 4 % of the total number of larvae seeded recovered, as shown in Figure 3.4. The greatest migration rate was seen at 15° C in the pots processed after 12 hours. There were very similar patterns of migration in the 20°C pots, with no clear trend as to when optimum migration was occurring. The migration at 15°C was very similar for all the samples until 12 hours when larval counts increased 6 fold. The peak migration at 10°C occurred 4 hours after larval seeding with a gradual rise and decline before and after this period. The same pattern was observed in the proportion of larvae recovered from the top and bottom of the sward at this temperature.

The dry weight was found to be a significant covariate ($p< 0.05$) and time ($p<0.05$), temperature ($p< 0.01$), section ($p< 0.001$) and the time temperature interaction ($p<0.01$) were also significant. There were significantly more larvae recovered from the 15°C pots than from the other two temperatures and between times 6 and 12, larval numbers on the 15°C pots increased, whereas for the other two temperatures the reverse was true.

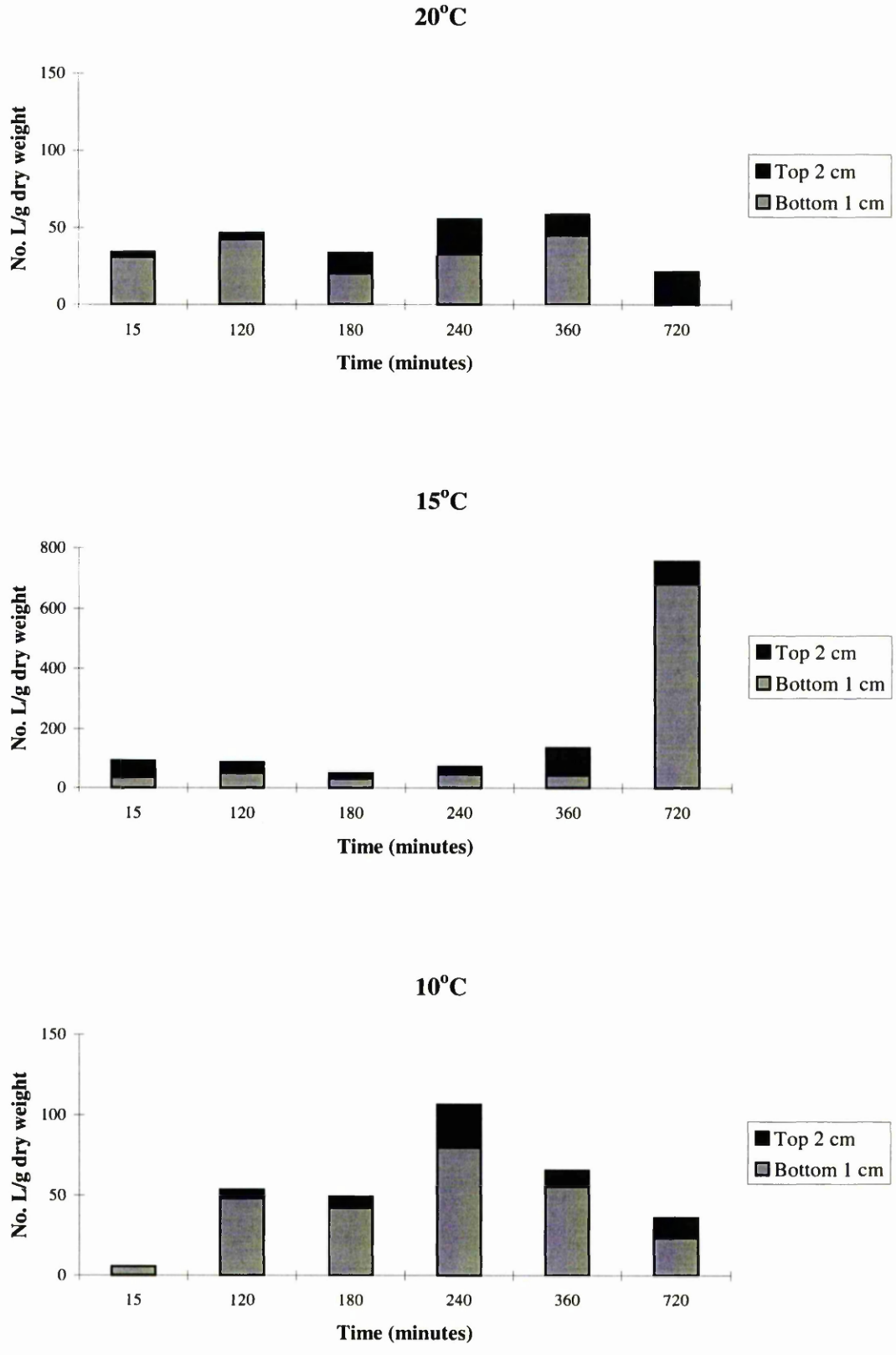


Figure 3.3 *Average numbers of larvae per gram dry weight herbage recovered at three different temperatures from a sward planted from seed*

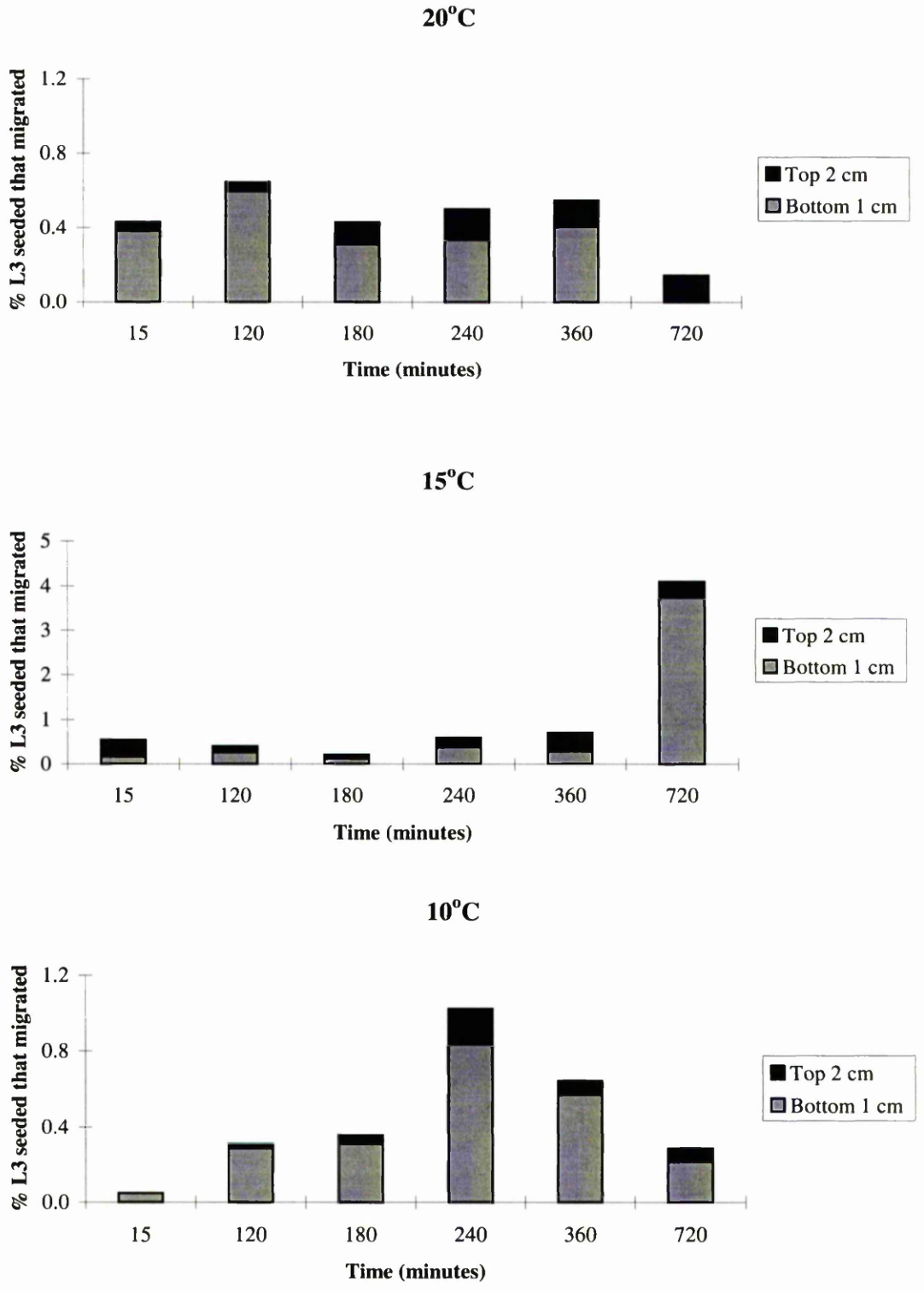


Figure 3.4 *Percentage of larvae that migrated on the sward planted from seed at three different temperatures*

3.3.2.3 Experiment 3 Illumination study using 'sown' swards

The average numbers of larvae recovered from the duplicate pots are shown in Figure 3.5. The migration rates were again very low, with only between 0.1 - 2 % of the seeded larvae recovered, as shown in Figure 3.6. The highest recovery rate was from 32 WM^{-2} processed after 12 hours. There were greater numbers of larvae recovered from the top part of the sward at 65 WM^{-2}. The greatest migration was at 50 WM^{-2} from the pots processed after 6 hours.

As in the Experiment 2, dry weight was a statistically significant covariate, with time ($p< 0.001$), light ($p< 0.05$) and section ($p<0.001$) also significant. The time by light interaction was not significant ($p= 0.074$). As in the previous two experiments, significantly more larvae were recovered from the base of the sward than from the top section. There is a statistically significant increase in larval counts between times 15 and 120 and between times 120 and 180. The larval count for the 65 WM^{-2} pots were significantly less than the other two illuminations. The count at time 15 for the 50 WM^{-2} pots was greater then the other two illuminations but this was not statistically significant.

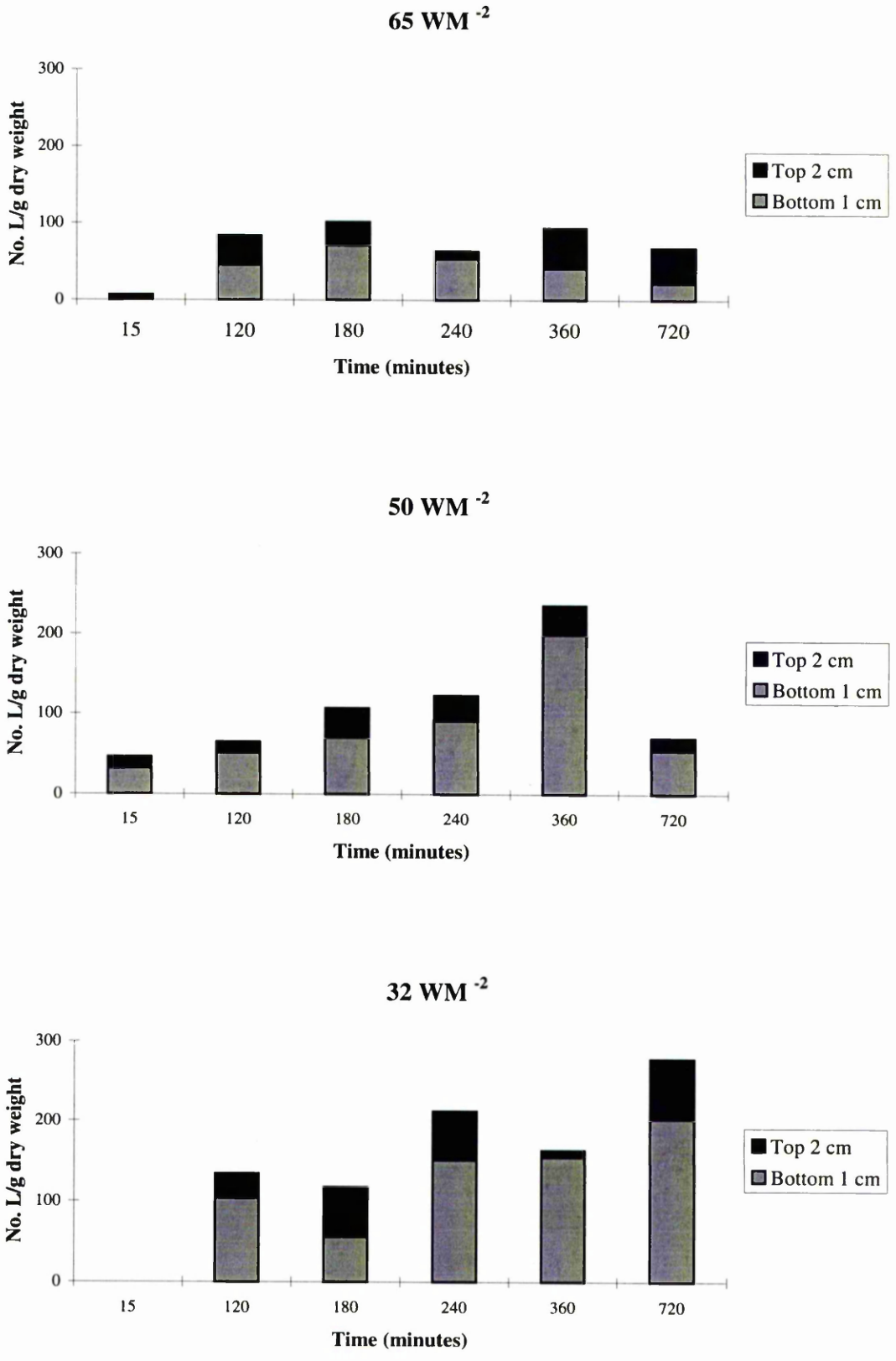


Figure 3.5 *Average numbers of larvae per gram dry weight herbage recovered at three different light intensities from a sward planted from seed*

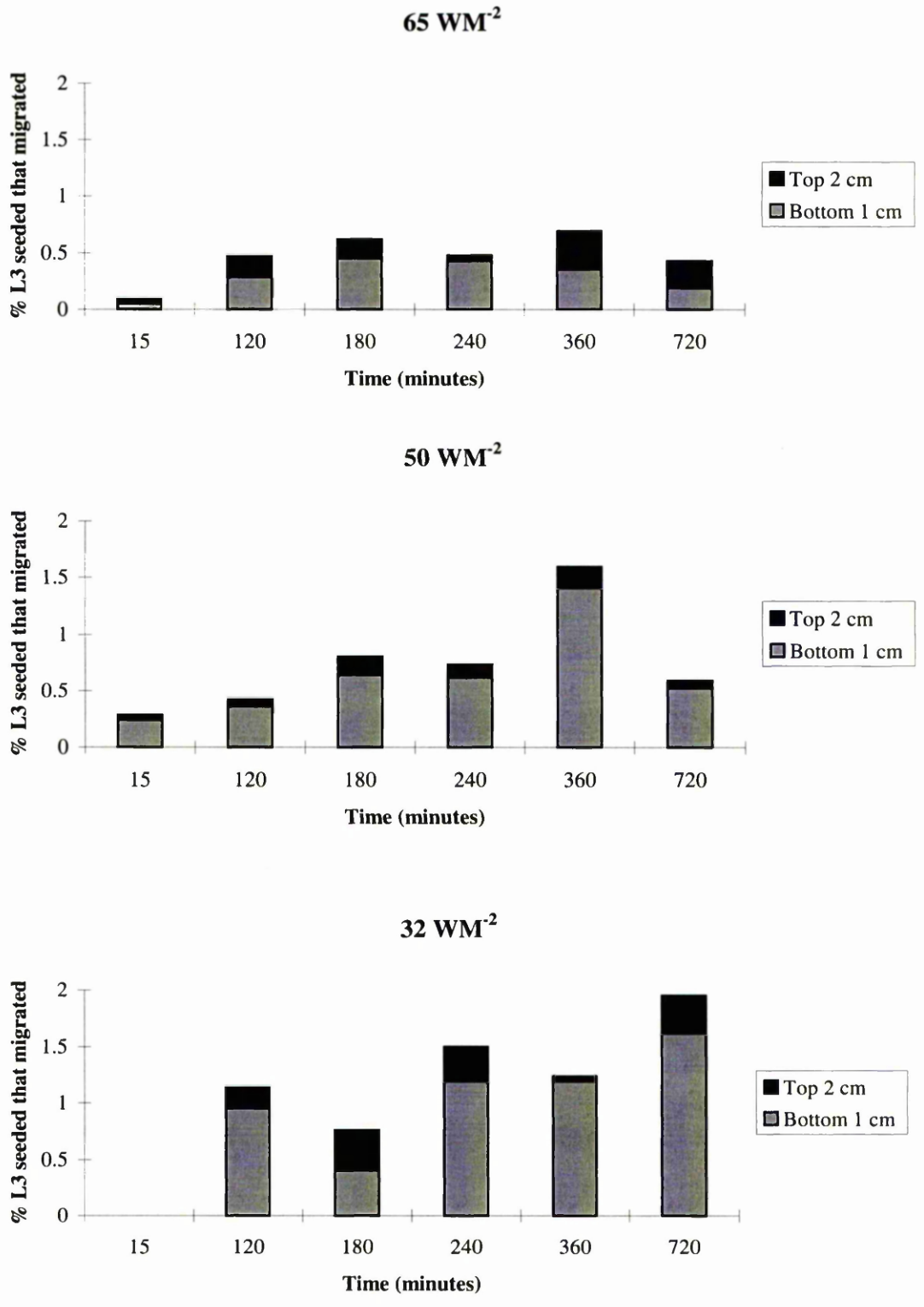


Figure 3.6 *Percentage of larvae that migrated on the sward planted from seed at three different light intensities*

3.3.2.4 Comparison of experiment 2.2 and experiment 3.3

Experiment 2.2 and experiment 3.3 were run under the same environmental conditions, that is at 15°C and with 65 WM^{-2} of light. When these two sets of pots were compared directly, dry weight was not found to be a significant covariate and neither was section. Both time and time by class interaction were significant, where class records the experiment from where the data came from. There was a clear upward trend in both experiments, but this fluctuation was different for the two experiments. The light intensity experiment was performed two weeks after the temperature experiment using the same batch of larvae, so it is possible that the difference in larval age may, in some part, explain these different migration rates.

3.4 Discussion

The difference in the recovery rates between the three methods tested reflect the difficulty in retrieving larvae from small herbage samples. A direct comparison between different workers' results is therefore almost impossible, unless recovery rates for their methods are stated. The DCT offers a useful method for examining small herbage samples infected with different levels of larval contamination. The role of pasture sampling is often not to give a quantitative result but to provide information on the fluctuation of pasture contamination over the grazing season. There is often a balance needed between recovery rates achieved and time taken and expense, in a busy laboratory environment. Expertise is also required when it comes to reading the sample and being able to distinguish between infective larvae and free living nematodes, plant parasitic nematodes are also present in many samples. The majority of the methods published are very similar in outline, with variations occurring in only minor steps and are based on the washing of the larvae from the swards. The DCT is fairly time consuming and is most effective with small herbage samples, but it is cheap, requires no specialist equipment and provides high recovery rates. It is now routinely used in the laboratory as the method of choice for small herbage samples. Care must be taken not to contaminate the herbage samples with soil as this affects the recovery rates, making the samples very difficult to read.

There were larvae present on all of the swards at time 15, except for experiment 3.1 using 32 WM^{-2}, which suggests that the initial migration may be very rapid. The numbers of larvae on the swards at this time were not significantly

different between experiments suggesting that this migration may be independent of external stimuli. The majority of the larvae were recovered from the bottom 1cm of the sward, the few numbers of larvae recovered from the top part of the sward may be attributable to contamination from the withdrawal of the Finn pipette, the tip of which might still have a few larvae attached. Two of the cultivated turf pots were processed as described for the artificially infected swards, to check for larval contamination, and no infective larvae were recovered. The swards grown from seeds were grown in steam sterilised soil and the seeds themselves were kept in a dry environment making survival of any contaminant larvae extremely unlikely. The unique appearance of the infective larvae makes them easily distinguishable from other nematodes that may be present in the soil.

There was a well developed mat at the base of the mature sward but as the sward grown from seed was so young no mat had developed. This may have made a difference to the migration rates as the larvae may have been placed on the mat and so further up the sward at the start of Experiment 1. This may in part explain the difference in migration rates between the two types of sward. Also the young grass had not tillered, unlike the mature cultivated turf, creating pockets where moisture could gather and so altering the micro-environment. The mature turf is more representative of the field situation and so may have more relevance. Subsequent studies were done using a mature tillered sward, as described in Chapter 4.

The direct count technique recovers on average around 80-90% of larvae seeded and as the same technique was used in all 3 experiments, it is unlikely to be the major cause of the large difference in the migration rates.

Previously, Silangwa & Todd (1964) reported that on average less than 2 % of the larvae seeded were recovered from their experimental swards, a similar figure to that of Callinan & Westcott (1986) who recovered approximately 2.5 % of the larvae seeded. Rees (1950) using *H. contortus* seeded pots in field conditions recovered between 0-38 % of the larvae seeded on the sward over a period of 24 hours. The different recovery methods used by the authors will obviously have a bearing on the difference in migration rates measured. Also some authors (Krecek, *et al.,* 1991; Moss & Vlassoff, 1993; Barutzki & Gothe, 1998) used faeces placed directly on the plots, so making it unfeasible to make direct comparisons due to the added differences in hatching and development rates. Field studies have estimated

that less than 1% of eggs become available as infective larvae on herbage (Boag & Thomas, 1975).

The optimum migration time varied, from 4 hours at 20°C to 12 hours at 10°C and 15°C for the mature sward. The young sward, in contrast, showed the greatest migration at 6, 12 and 4 hours at 20, 15 and 10°C respectively. The greatest migration was at the longest time at the lowest illumination. All of the temperature variable experiments were run at 65 WM^{-2} which actually returned the lowest numbers of larvae recovered. As this experiment was done last there was no way of knowing this prior to running the previous two experiments. The proportion of larvae recovered from the top and bottom of the sward in the illumination experiments at 50 WM^{-2} showed no change over the time course. The other two illuminations showed a greater variation but there was no perceivable pattern.

Previously, Niezen *et al.* (1998c) and Niezen, Miller, Robertson, Wilson & Mackay (1998d) reported a greater larval density on the top part of the sward using eight different sward types but in all of these experiments the majority of the larvae were on the bottom part of the sward, although for Experiment 1 it is difficult to make assumptions as no dry weights were taken making it impossible to calculate larval density. Interestingly, at the highest illumination in Experiment 3 a greater larval density was recorded on the top part of the sward.

The light intensities used were low when compared to a typically dull day, which has a light intensity of approximately 150 $W M^{-2}$. This finding is in agreement with Rees (1950) who found the maximum migration occurred at very low light intensities of between 1- 40 WM^{-2} at dusk and dawn, using *H. contortus* larvae and rye grass.

The differences in activity in Experiment 1 are consistent with observations of *T. circumcincta* larvae becoming hyperactive at higher temperatures but as a consequence mortality is higher due to the depleted lipid reserves (Urquhart *et al.*, 1991). Obviously in order for the larvae to be ingested, migration onto the sward is essential but a balance between this essential movement and excess movement which shortens the life span of the larvae is required to ensure the success of the species.

In summary, the very low migration rates make it difficult to draw definitive conclusions from these experiments. It seems likely that the complex interaction between all the environmental conditions, combined with the further influence of

sward type and height, act together to influence the migration rate of *T. circumcincta* infective larvae.

Chapter 4

The use of oesophageally fistulated lambs to investigate larval intake and bite depth.

4.1 Introduction

To investigate the epidemiology of a nematode species with a free living larval stage it is important to be able to estimate the level of pasture contamination. There are numerous methods published for the estimation of both number and species of larvae present on the pasture, where the pasture has been sampled by either plucking or cutting (Taylor, 1939; Dinaburg, 1942; Crofton, 1954; Parfitt, 1955; Michel & Parfitt, 1955; Durie, 1959; Sturrock, 1961; Donald, 1967; Lancaster, 1970; Smeal & Hendy, 1972; Mwegoha & Jorgensen, 1977; Young & Trajstman, 1980; Cheijina, 1982; Martin *et al.*, 1990; Eysker & Kooyman, 1993). This method provides an estimate of the larval concentration on the herbage at a given point in time and is extremely useful in looking at fluctuating levels of larval contamination over a grazing season. It also has the advantage of being cheap to perform, requiring no specialist equipment and utilising cheap reagents.

Tracer animals that are usually worm naïve, although some workers have used previously exposed, anthelmintic treated animals (Gruner, Cabaret, Sauve & Pailhories, 1986), provide different information. They are put out to graze on the pasture for a period of time, kept indoors for 2-3 weeks and then slaughtered to assess the worm burden (Waller *et al.*, 1981; Gruner & Cabaret, 1985). They are very useful for providing data on the level of ingestion of larval stages and the level of inhibition experienced by the animal.

These two methods have been compared previously using both sheep (Waller, Dobson, Donald & Thomas, 1981) and cattle (Cabaret, Raynaud & Lestang, 1982) and have provided evidence suggesting that the number of larvae recovered from pasture can be correlated to the tracer animals' worm burden.

The use of oesophageally fistulated lambs to assess larval intake was first described by Heath, Southcott & May (1970). Since it allows animals to graze naturally, it is an effective way to examine intake and to look at exactly what the lambs are ingesting and what is happening at a single point in time. A previous study in Scotland comparing manually collected samples and oesophageally fistulated lambs has been done previously by Gettinby *et al.*, (1985), who demonstrated a greater recovery of larvae from the fistulated lambs than from the manual sampling.

The methods used to recover the larvae from the masticated herbage are necessarily different from those used for pasture samples due to the finer particulate

matter and opaque washings from split chloroplasts (Heath & Major, 1968). Methods described in the literature for the counting of larvae from the masticated herbage samples tend to be based on that of Heath & Major (1968), which involves washing and sedimentation of the sample then the formation of a vacuum above the sample to encourage the organic material to settle with a final flotation to concentrate the larvae. This concentration technique provides clear samples that are easy to read and the authors reported recovery rates of 67-93%. It is however, time consuming and processing large numbers of samples at one time is impractical.

Preliminary experiments showed that the direct count technique could be used with approximately the same recovery rates as the manually collected samples, with relatively clear samples.

The collection of the samples manually from the trays using scissors, was to enable a direct comparison to be made between numbers of larvae per gram dry weight recovered from the two methods.

4.2 Materials and methods

4.2.1 Comparison of two different larval recovery techniques from masticated herbage

The direct count technique (DCT) and the Heath & Major (1968) technique are described in Chapter 2, in sections 2.3.1 and 2.3.4 respectively. Masticated samples, free from larval contamination, were collected from oesophageally fistulated sheep, seeded with known numbers of *T. circumcincta* infective larvae and processed using the two different techniques.

4.2.2 Surgical preparation and training of lambs

The worm-free lambs were surgically prepared and trained using the swards as described in Chapter 2.7.1.

4.2.3 Preparation and seeding of swards

The trays of grass were grown and prepared as described in Chapter 2.7.3. The trays were each seeded with approximately 200,000 *T. circumcincta* L_3, using a Finn pipette. Prior to the tray being offered to the lamb, the height of the sward was measured using a sward stick (Bircham, 1981) at 20 points chosen at random and

marked out on a grid, as described in Chapter 2.7.3. These same 20 points were re-measured after the lamb had taken its 50 bites.

4.2.4 Experimental design

Each of the four fistulated lambs received a tray at 4 and 24 hours after seeding, in the order shown in Table 4.1.

Two trays were processed manually at both 4 and 24 hours after seeding, with tray 1 being processed at the same time as tray 2 was offered to the first fistulate and tray 6 being processed at the same time as tray 5 was offered to the fourth fistulate. The fistulate samples were weighed and then immediately frozen. The fresh weights of the samples from the manually collected trays were recorded and these samples were kept at 4 ^{o}C until being processed. Both sets of samples were counted using the direct count technique as described in Chapter 2. A fifth of each of the samples were counted, and the remainder of the sample was dried, in order to get a dry matter estimate. The numbers of larvae per gram dry matter were estimated and used in a direct comparison between the two sampling methods.

Table 4.1 *The feeding regime for the four fistulated lambs*

Day	Tray	Time	Sward height	Lamb no.	Day	Tray	Time	Sward height	Lamb no.
1	2	4	6	1	6	2	24	6	2
1	3	4	6	2	6	3	24	6	3
1	4	4	6	3	6	4	24	6	4
1	5	4	6	4	6	5	24	6	1
2	2	24	3	2	6	2	4	3	2
2	3	24	3	3	6	3	4	3	3
2	4	24	3	4	6	4	4	3	4
2	5	24	3	1	6	5	4	3	1
2	2	4	6	2	7	2	24	6	3
2	3	4	6	3	7	3	24	6	4
2	4	4	6	4	7	4	24	6	1
2	5	4	6	1	7	5	24	6	2
3	2	24	3	3	7	2	4	3	3
3	3	24	3	4	7	3	4	3	4
3	4	24	3	1	7	4	4	3	1
3	5	24	3	2	7	5	4	3	2
3	2	4	6	3	8	2	24	6	4
3	3	4	6	4	8	3	24	6	1
3	4	4	6	1	8	4	24	6	2
3	5	4	6	2	8	5	24	6	3
4	2	24	3	4	8	2	4	3	4
4	3	24	3	1	8	3	4	3	1
4	4	24	3	2	8	4	4	3	2
4	5	24	3	3	8	5	4	3	3
4	2	4	6	4	9	2	24	6	1
4	3	4	6	1	9	3	24	6	2
4	4	4	6	2	9	4	24	6	3
4	5	4	6	3	9	5	24	6	4
5	2	24	3	1					

5	3	24	3	2					
5	4	24	3	3					
5	5	24	3	4					
5	2	4	3	1					
5	3	4	3	2					
5	4	4	3	3					
5	5	4	3	4					

4.2.5 Statistical analysis

The raw data were analysed using analysis of covariance (Minitab v 10.2).

4.3 Results

4.3.1 Comparison of two different larval recovery techniques from masticated herbage

The results from the comparison study are shown in Table 4.2. The recovery rates varied according to the number of larvae seeded on the sample.

Table 4.2 *Mean percentage recoveries for the two different techniques used to recover larvae from masticated herbage*

No. larvae seeded	DCT technique	Heath & Major (1968) technique
500	64.2%	37.6%
1000	79.7%	49.3%
5000	84.1%	65%

4.3.2 Clinical observations

In general, the lambs adapted well to the surgical procedure and there were no surgical complications. A total of 5 samples were not collected or discarded, as shown in Table 4.3. At the start of the trial lamb 1 refused to eat from the offered

trays on a total of 3 separate occasions and lamb 4 refused once, although these were the only problems with the lambs eating from the trays and they generally adapted well. The fifth sample to be discarded was contaminated by regurgitated rumen material.

Table 4.3 *Samples withdrawn from the analysis due to the reasons stated below*

Day	Tray	Time	Sward height	Lamb no.	Comment
1	5	4	6	4	No bites taken
3	4	24	3	1	Only 13 bites taken
3	4	4	6	1	No bites taken
4	3	24	3	1	No bites taken
5	3	24	3	2	Rumen contents regurgitated

4.3.3 Larvae recovered from fistulated lambs

Figures 4.1, 4.2, 4.3 and 4.4 show the respective mean recoveries (larvae per gram dry weight (L/G DW) ± S.E.M.) from the 3 and 6 cm swards processed after 4 and 24 hours.

The 3 cm sward processed after 24 hours had significantly fewer larvae per gram dry weight recovered than either the 3 cm, 4 hour treatment ($p<0.05$) or the 6 cm, 24 hour treatment ($p<0.05$). There were no significant differences between the numbers of larvae recovered from each of the individual lambs in any of the treatments.

The results from the means ± SEM of all four of the lambs for the four different treatments are shown in Figure 4.5.

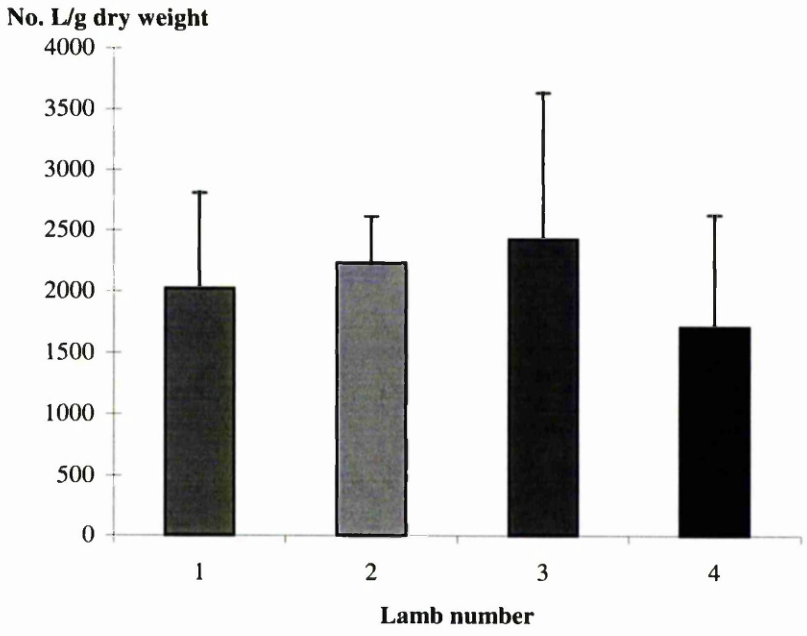


Figure 4.1 *The mean numbers ± SEM of larvae recovered per gram dry weight from the 3 cm sward processed after 4 hours given to each of the four oesophageally fistulated lambs.*

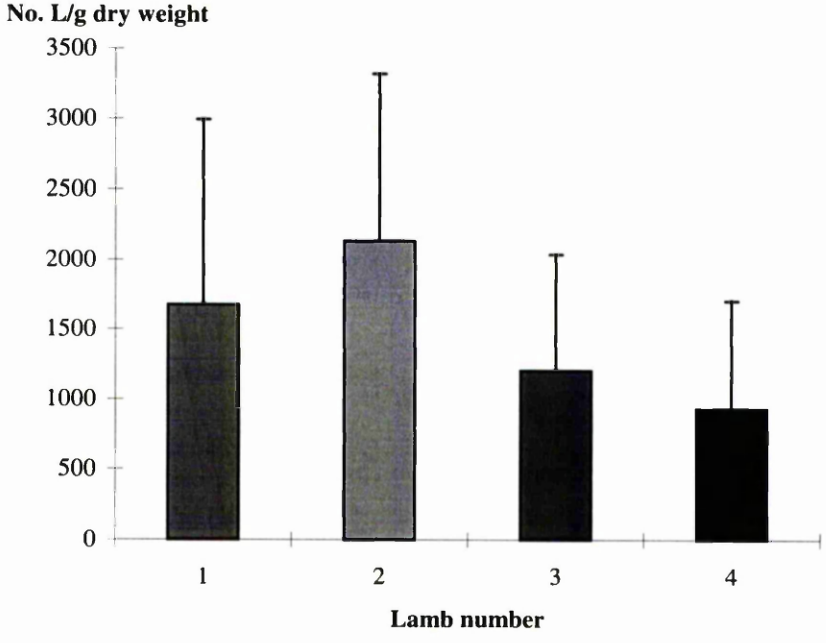


Figure 4.2 *The mean numbers ± SEM of larvae recovered per gram dry weight from the 6 cm sward processed after 4 hours given to each of the four oesophageally fistulated lambs.*

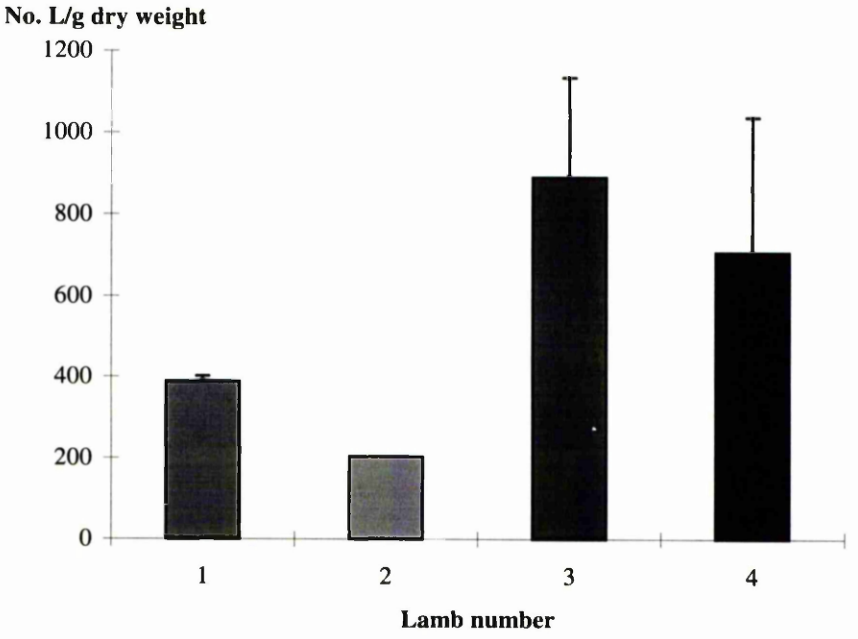


Figure 4.3 *The mean number ± SEM of larvae recovered per gram dry weight from the 3 cm sward processed after 24 hours given to each of the four oesophageally fistulated lambs*

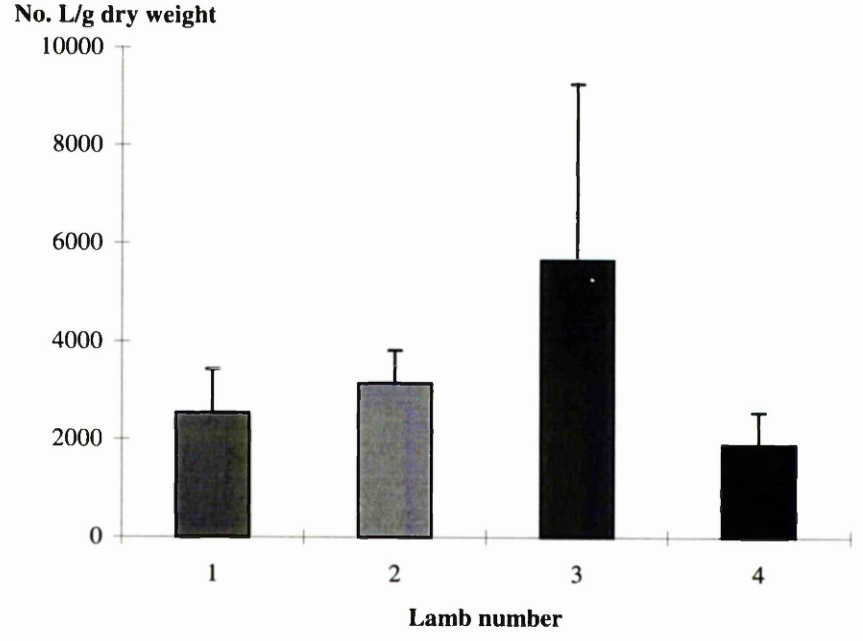


Figure 4.4 *The mean number ± SEM of larvae recovered per gram dry weight from the 6 cm sward processed after 24 hours given to each of the four oesophageally fistulated lambs.*

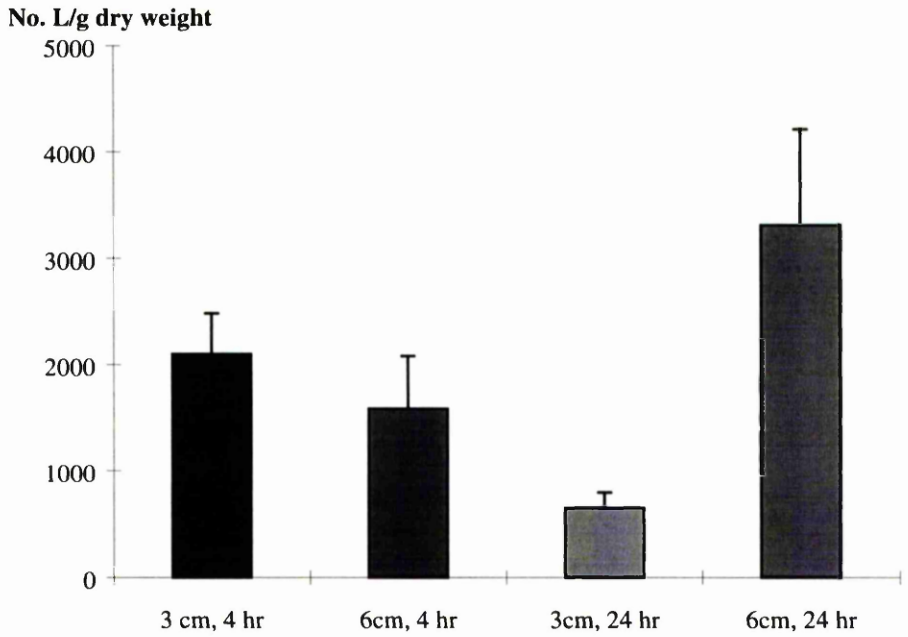


Figure 4.5. *The mean number ± SEM of larvae recovered per gram dry weight from the four fistulated lambs for all four of the treatments.*

4.3.4 Larvae recovered from manually collected trays

The results for the 3 cm and 6 cm swards manually collected and processed after 4 hours are shown in Figures 4.6 and 4.7 respectively. The results for the 3 cm and 6 cm swards manually collected after 24 hours are shown in Figures 4.8 and 4.9 respectively.

There were no significant differences between trays 1 and 6 for any of the four treatments. There were also no significant differences between the numbers of larvae recovered from the top and bottom sections of the sward, in any of the treatments.

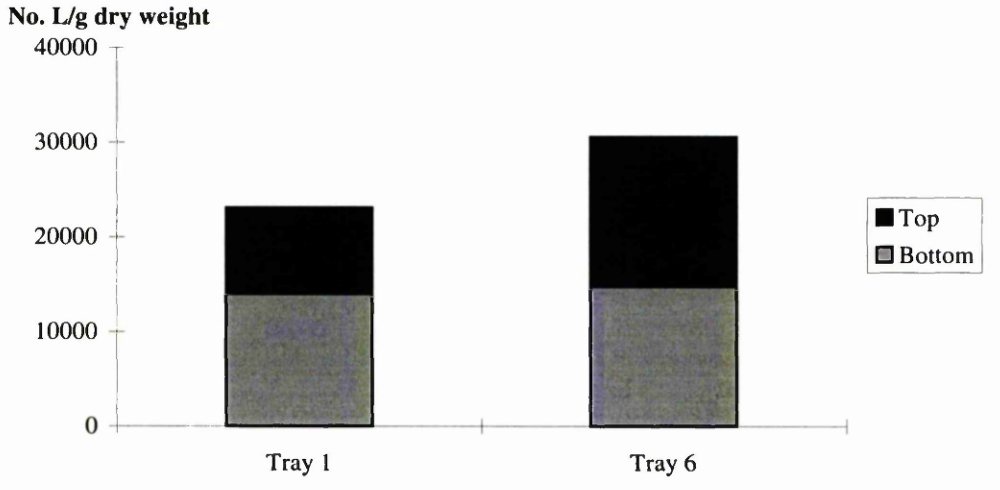


Figure 4.6. *The mean number of larvae recovered per gram dry weight from the 3 cm sward processed after 4 hours and manually collected from trays 1 and 6.*

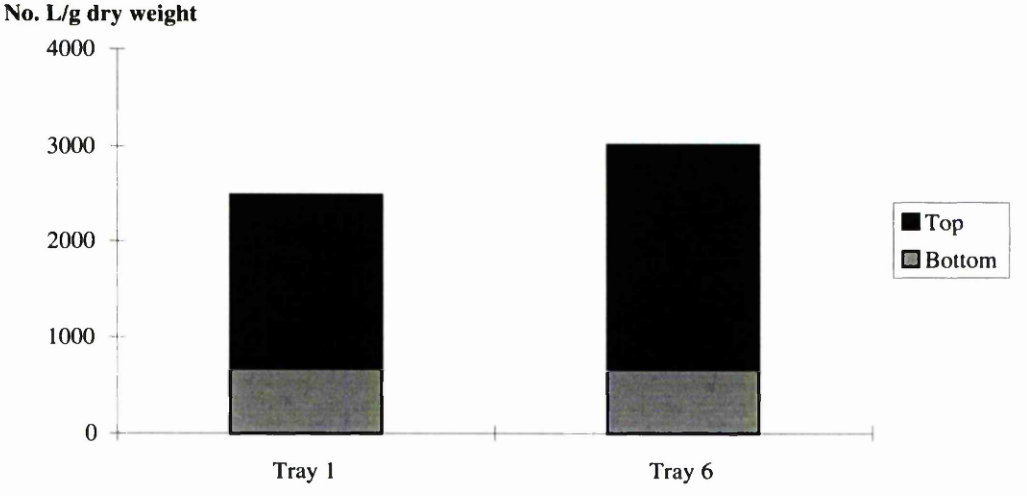


Figure 4.7. *The mean number of larvae recovered per gram dry weight from the 6 cm sward processed after 4 hours and manually collected from trays 1 and 6.*

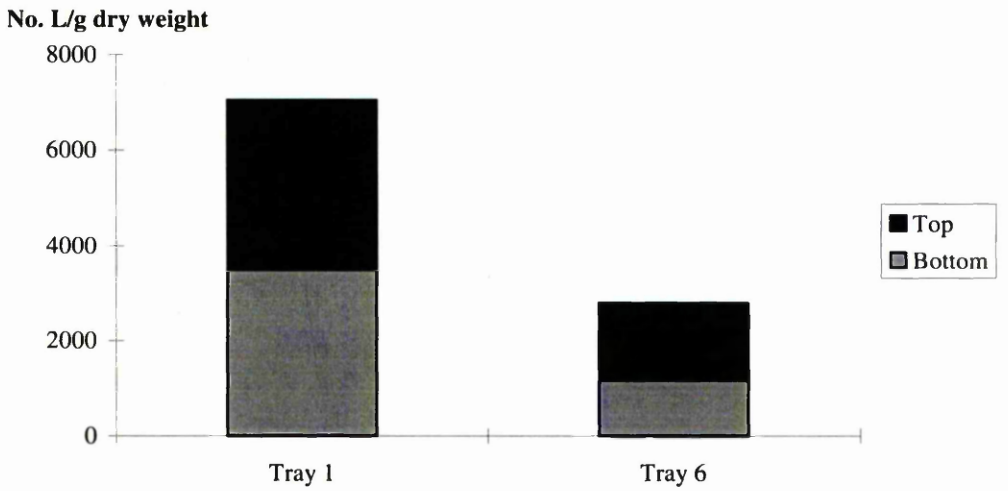


Figure 4.8. *The mean number of larvae recovered per gram dry weight from the 3 cm sward processed after 24 hours and manually collected from trays 1 and 6.*

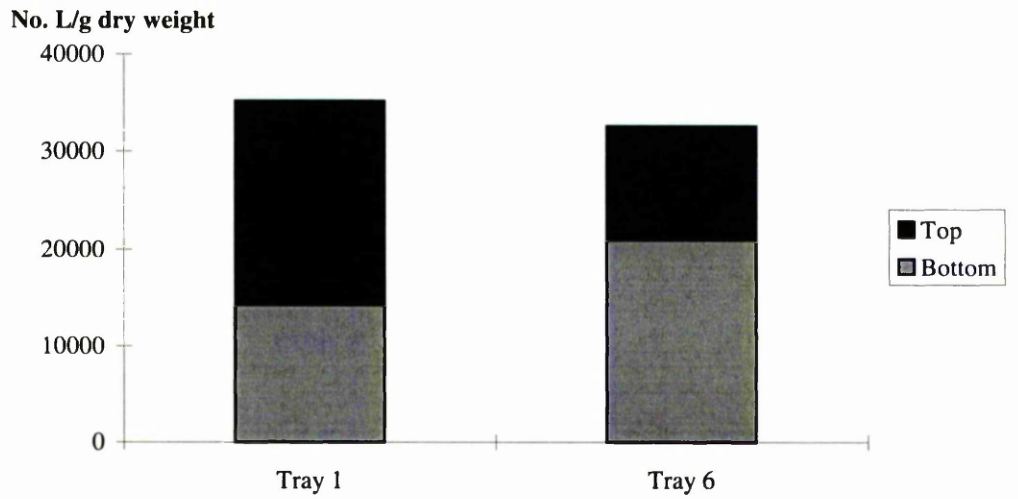


Figure 4.9. *The mean number of larvae recovered per gram dry weight from the 6 cm sward processed after 24 hours and manually collected from trays 1 and 6.*

The results for the mean ± SEM of both the manually collected trays for all four of the treatments are shown in Figure 4.10. The statistically significant differences between the treatments are shown in Table 4.4.

Table 4.4 *Statistically significant differences between the four treatments for the manually collected trays*

Comparator			
3cm, 4 hour	6 cm, 4 hour	3 cm, 24 hour	6 cm, 24 hour
6 cm, 4 hour *** 3 cm, 24 hour ***	-	-	6 cm, 4 hour ** 3cm, 24 hour **

- no significant difference

** significantly lower number of larvae per gram dry weight recovered than comparator $p<0.01$, *** $p<0.001$

The migration rates from the manually collected trays ranged from 6-28% of the total number of larvae seeded, which is very similar to the range seen in the previous migration experiments using a mature, tillered turf.

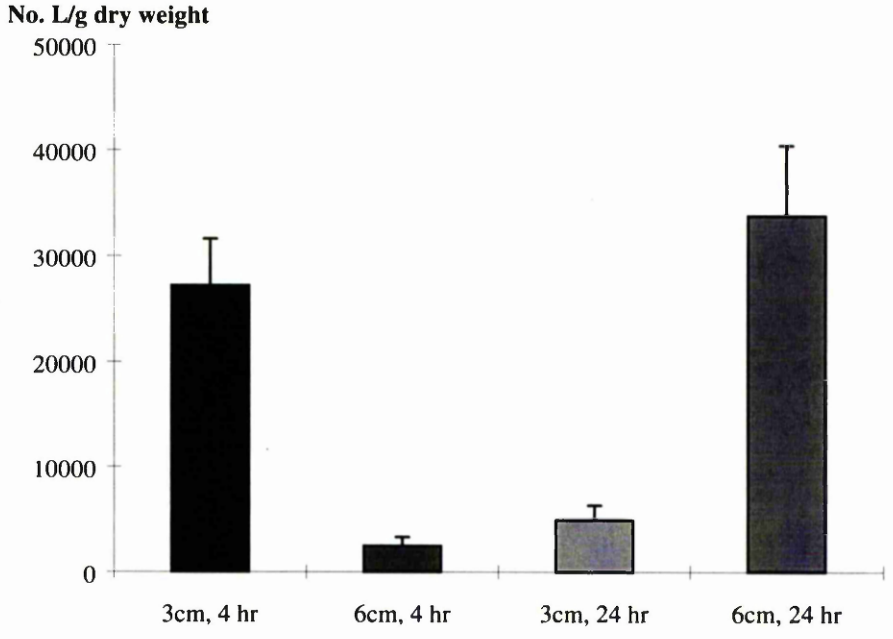


Figure 4.10. *The mean number ± SEM of larvae recovered per gram dry weight from both of the manually collected trays for all four of the treatments.*

4.3.5 Comparison between fistulated lambs and manually collected trays

A direct comparison between the manually collected tray and the fistulated lambs, using the means of both trays and all four lambs, are shown in Figures 4.11, 4.12, 4.13 and 4.14 for the 3cm, 4 hour, 6 cm, 4 hour, 3 cm, 24 hour and 6 cm, 24 hour treatments respectively. In all of the treatments there were greater numbers of larvae recovered from the manually collected trays than from the fistulates and this was significant for the 3cm, 4 hour treatment ($p<0.001$), the 3 cm, 24 hour treatment ($p<0.01$) and the 6 cm, 24 hour treatment ($p<0.001$). There was a ten fold difference in this last case.

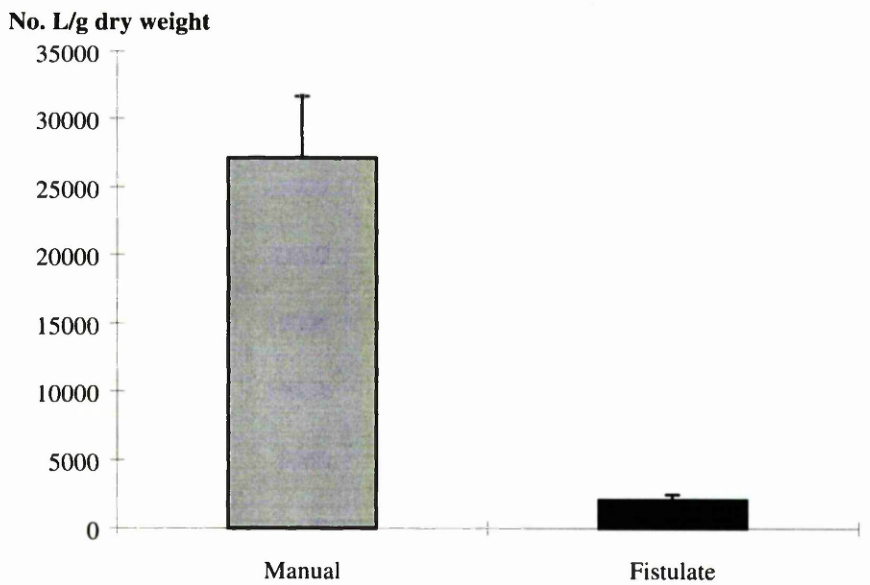


Figure 4.11. *The mean number ± SEM of larvae recovered per gram dry weight from the 3 cm sward processed after 4 hours from both the manually collected trays and the fistulated lambs.*

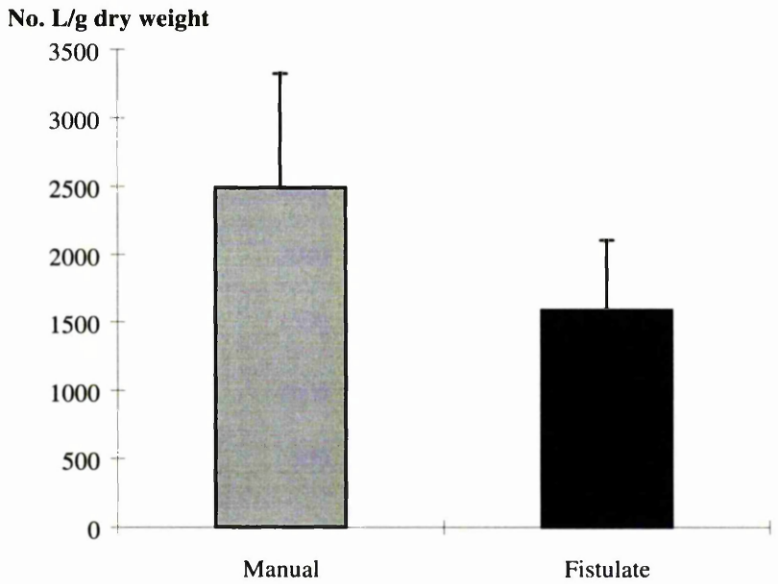


Figure 4.12. *The mean number ± SEM of larvae recovered per gram dry weight from the 6 cm sward processed after 4 hours from both the manually collected trays and the fistulated lambs.*

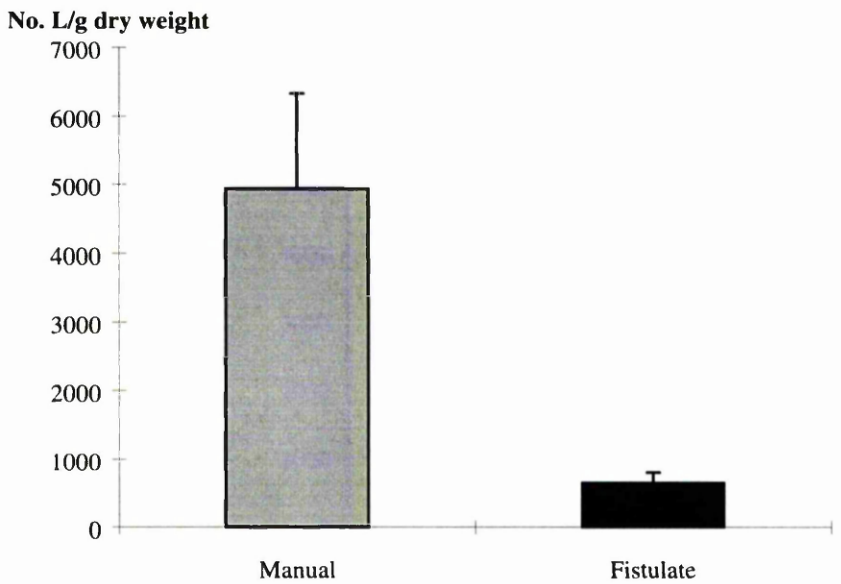


Figure 4.13. *The mean number ± SEM of larvae recovered per gram dry weight from the 3 cm sward processed after 24 hours from both the manually collected trays and the fistulated lambs.*

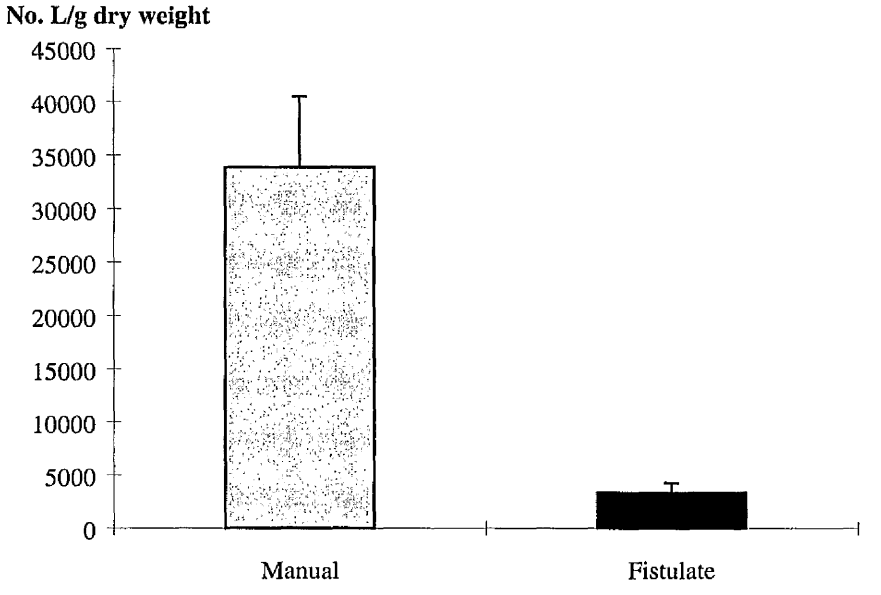


Figure 4.14. *The mean number ± SEM of larvae recovered per gram dry weight from the 6 cm sward processed after 24 hours from both the manually collected trays and the fistulated lambs.*

4.3.6 Bite depth

The bite depth data provided measures at only 20 points on the whole of the tray, therefore it is not possible to compare this with the biomass obtained, as not every bite taken will necessarily fall within these 20 points. Where a bite was recorded, the mean bite depth is shown. Also the proportion of the sward which is bitten horizontally is shown.

The mean bite depth taken by the four individual lambs from the 3 cm sward processed after 4 and 24 hours are shown in Figures 4.15 and 4.16 respectively, with the data from the 6 cm sward processed after 4 and 24 hours are shown in Figures 4.17 and 4.18 respectively. There were no significant differences between the individual lambs in either the 3cm, 4 hour or the 3 cm, 24 hour treatments. However, the 6 cm, 4 hour sward showed several significant differences, as shown in Table 4.5. The only significant difference in the 6 cm, 24 hour treatment was between lambs 1 and 2, with lamb 2 recording significantly ($p<0.05$) deeper bite depths.

Table 4.5 *Statistically significant differences between bite depths recorded for the four fistulated lambs grazing the 6 cm sward 4 hours after seeding*

Comparator			
Lamb 1	Lamb 2	Lamb 3	Lamb 4
Lamb 2* Lamb 3**	-	-	Lamb 3*

- no significant difference

* significantly deeper bite depth than comparator $p<0.05$, ** $p<0.01$

There was no significant difference in the bite depth between the 2 time treatments using the 3 cm sward, however there was a significant difference ($p<0.001$) between the 2 time treatments using the 6 cm sward.

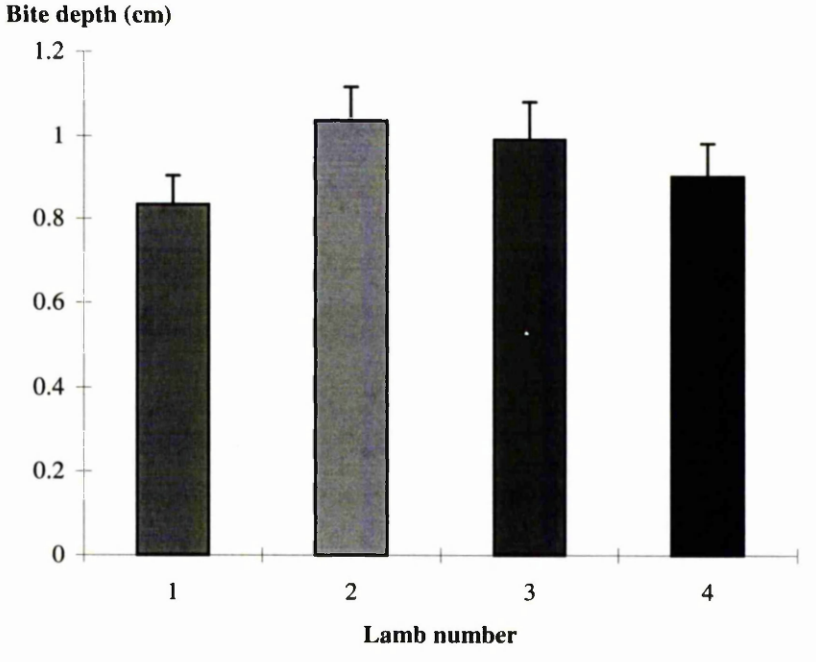


Figure 4.15. *Mean ± SEM depth of bites taken from the 3 cm sward processed after 4 hours given to each of the four oesophageally fistulated lambs.*

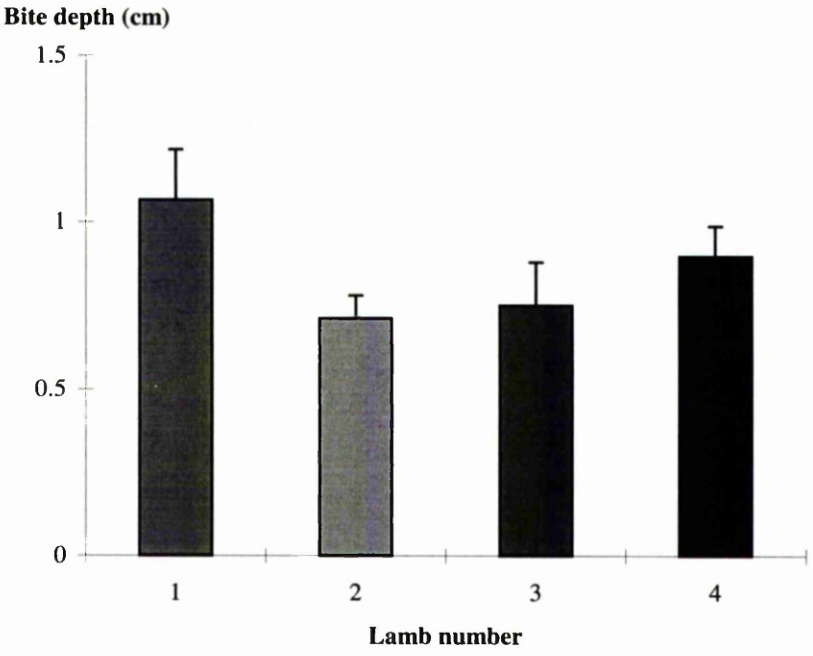


Figure 4.16. *Mean ± SEM depth of bites taken from the 3 cm sward processed after 24 hours given to each of the four oesophageally fistulated lambs.*

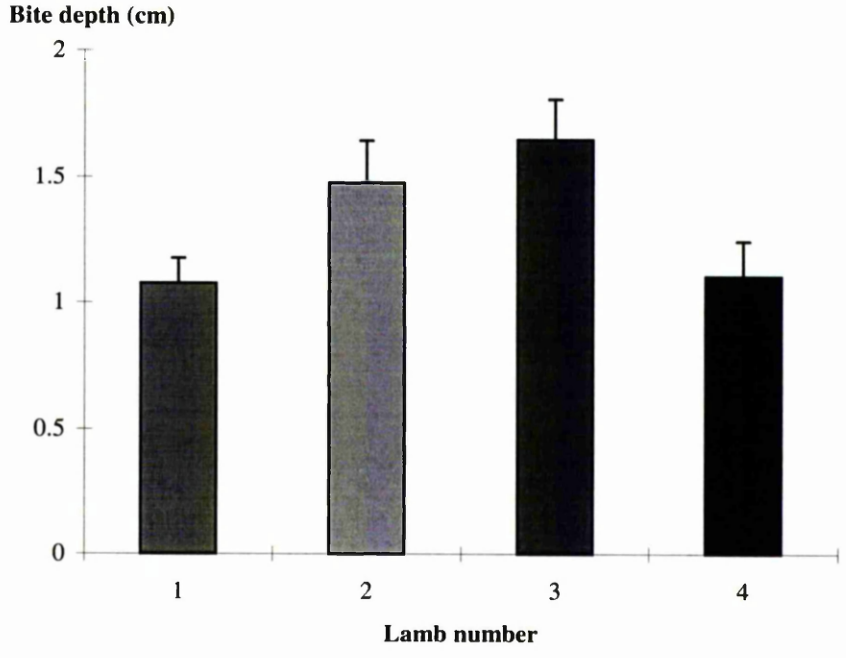


Figure 4.17. *Mean ± SEM depth of bites taken from the 6 cm sward processed after 4 hours given to each of the four oesophageally fistulated lambs.*

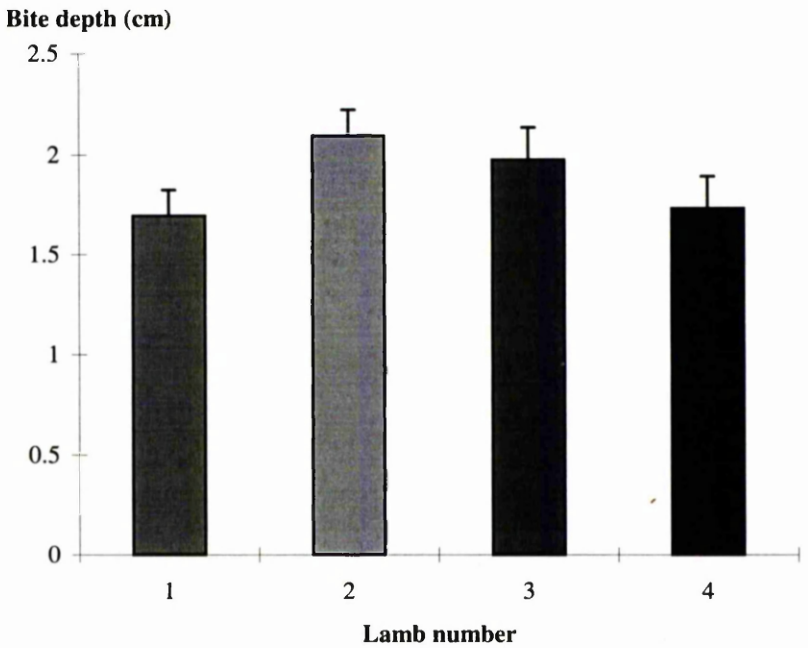


Figure 4.18. *Mean ± SEM depth of bites taken from the 6 cm sward processed after 24 hours given to each of the four oesophageally fistulated lambs.*

4.3.7 Bite proportion

The data showing the proportion of the sward bitten for the 3 cm sward 4 and 24 hours after seeding are shown in Figures 4.19 and 4.20 respectively, for the four fistulated lambs. There were no differences between the lambs on the 4 hour treatment, the only difference with the 24 hour treatment was between lambs 1 and 2, with lamb 1 biting significantly ($p>0.05$) more of the sward. The 6cm, 4 hour treatment bite proportion is shown in Figure 4.21 and the 6 cm, 24 hour in Figure 4.22. As with the bite depth data, there were significant differences with the 6cm, 4 hour treatment, as shown in Table 4.6.

Table 4.6 *Statistically significant differences between proportion of swards bitten for the four fistulated lambs grazing the 6 cm sward 4 hours after seeding*

Comparator			
Lamb 1	Lamb 2	Lamb 3	Lamb 4
Lamb 2* Lamb 3**	-	-	Lamb 3*

- no significant difference

* significantly greater proportion of the sward bitten than comparator $p<0.05$, ** $p<0.01$

As with the bite depth data, the difference between the two time treatments using the bite proportion data showed only a significant difference ($p<0.001$) in the 6 cm sward.

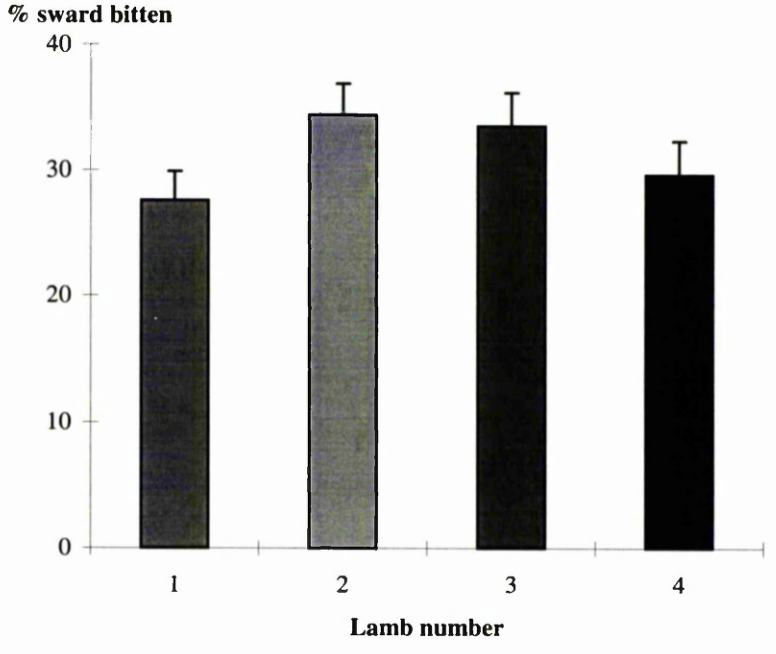


Figure 4.19. *Mean ± SEM percentage of the 3 cm sward bitten and processed after 4 hours given to each of the four oesophageally fistulated lambs.*

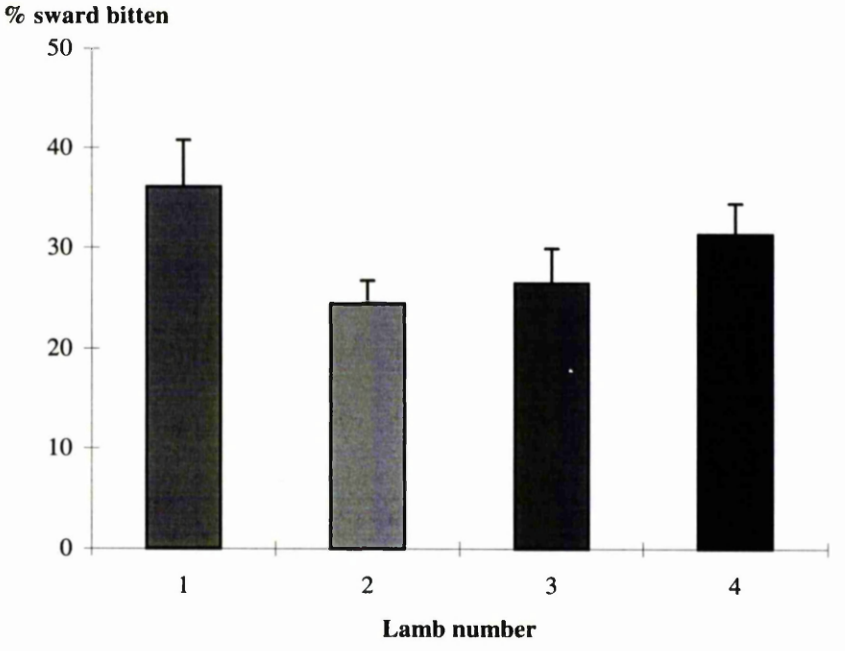


Figure 4.20. *Mean ± SEM percentage of the 3 cm sward bitten and processed after 24 hours given to each of the four oesophageally fistulated lambs.*

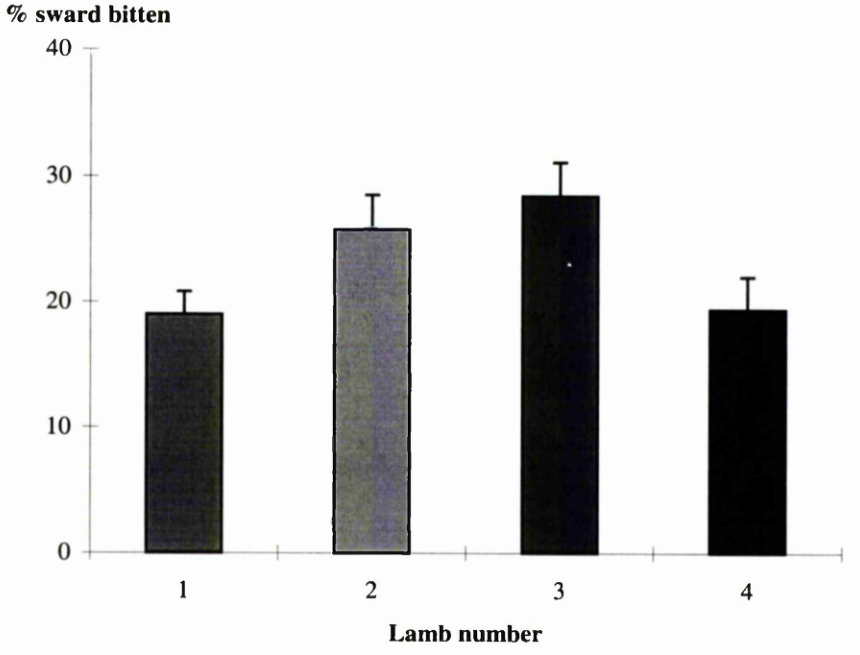


Figure 4.21. *Mean ± SEM percentage of the 6 cm sward bitten and processed after 4 hours given to each of the four oesophageally fistulated lambs.*

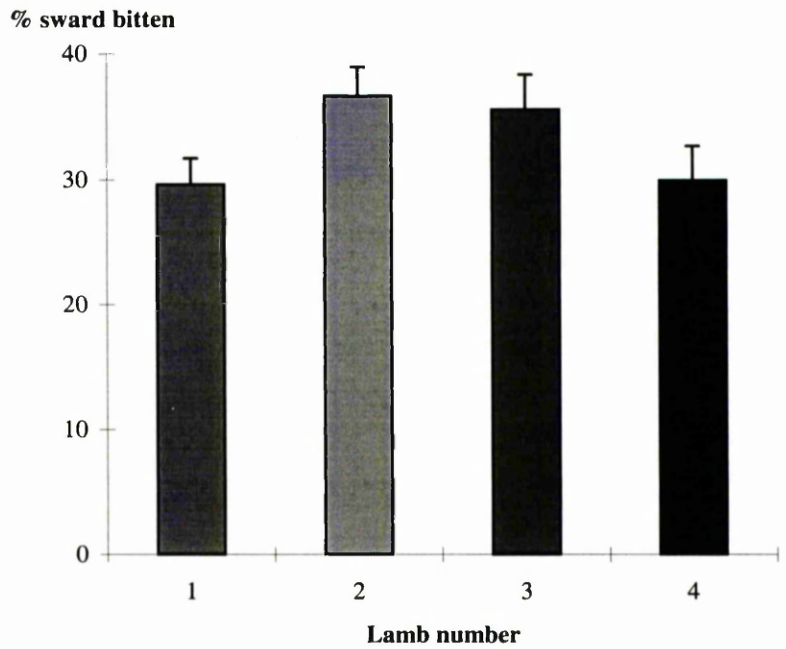


Figure 4.22. *Mean ± SEM percentage of the 6 cm sward bitten and processed after 24 hours given to each of the four oesophageally fistulated lambs.*

4.4 Discussion

All of the lambs were raised together under the same experimental conditions, treated the same throughout the experiment and were all worm naïve animals, as it has been shown that parasitism affects grazing behaviour (Hutchings *et al.*, 1998).

The difference in the lambs' feeding habits on the 6 cm sward, mainly that lambs 1 and 4 were not biting as deeply as lambs 2 and 3, could explain the difference in the two time treatments, as the data within these treatments were different. This difference in grazing habits was not seen in the 3 cm sward, suggesting a difference in behaviour when faced with different height swards, in agreement with Gong, Hodgson, Lambert & Gordon (1996a,b) and Gong, Lambert & Hodgson (1996). There was no difference, however, in the number of larvae ingested per gram dry weight for the four lambs, suggesting that this difference in grazing behaviour is not a major factor in determining numbers of larvae consumed. The majority of the larvae were found on the bottom part of the sward, which could explain why the difference in bite depth on the 6 cm sward resulted in no difference

in larval intake. The difference in the bite depth between the lambs was not deep enough to enter the portion of the sward that contained the greatest number of infective larvae.

The proportion of the sward bitten by the lambs was very similar in both of the 3 cm and 6 cm swards, for all 4 of the lambs. This is in agreement with Woodward (1998) who found bite depth to be proportional to sward height.

There were always greater numbers of larvae recovered from the manually collected trays than from the fistulates, when expressed as numbers of larvae recovered per gram dry weight. This difference was significant for all except the 6 cm sward processed after 4 hours. These findings contrast with those of Gettinby *et al.* (1985) and Heath *et al.* (1970) who reported higher recoveries from fistulate collected samples. Gettinby and co-workers (1985) found between a three- and nine-fold difference between the numbers recovered from fistulae and manually collected samples and Heath *et al.*(1970) found 20-30% greater recovery from masticated herbage than from manually collected herbage. On the other hand, Cabaret *et al.* (1986) found less larvae recovered from the fistulated ewes than the manual collection in the field situation, as did Michel & Parfitt (1955) when investigating *D. viviparus* infection in cattle. The studies done by Gettinby *et al.* (1985) and Cabaret *et al.* (1986) were performed under field conditions, using Scottish Blackface and Merino ewes respectively, so selective grazing behaviour of the ewes may be one factor partly explaining the difference in that study. Cabaret *et al.* (1982) found a greater correlation between tracer worm burdens and manual pasture sampling, when the pasture sampling method was not uniform, but rather a random pattern of 'near to' and 'far from' faecal pats. However, in this experiment conducted on artificial swards, there was no opportunity for selective grazing behaviour to play any part in influencing larval intake.

The recovery rates from the trays were similar to those seen in the first growth chamber experiment (Chapter 3), suggesting that the migration observed on the trays on this occasion was not artificially high. Since the recovery rate for both methods was also very similar, this could not account for the large difference.

The recovery of masticated herbage from fistulated lambs has been estimated to be between 46-80% (Hamilton, McManus & May, 1960; McManus, 1961; Heath *et al.*, 1970) using a similar surgical preparation to the one used in this study. This

will not have a large effect on the numbers of larvae recovered per gram dry weight, however, if it is assumed that the larvae are evenly distributed on the sward and that the extrusa collected is representative of the herbage being ingested. Heath *et al.* (1970) also washed out the mouths and oesophagi of the fistulated sheep and examined the washings for the presence of larvae. Larvae were recovered on only four of the nine occasions and accounted for less than 2% of the larvae on the herbage.

This work provided important information on grazing behaviour and the numbers of larvae being ingested by the lambs. Previous studies using oesophageally fistulated sheep (Heath *et al.*, 1970; Gettinby *et al.*, 1985; Cabaret *et al.*, 1986) have been conducted under field conditions. A more controlled environment was used to limit the effect of other variables involved, such as changing light intensity, wind speed and precipitation. The oesophageal fistulation method has the advantage over the tracer method of not being influenced by the hosts' immune status or by density dependant factors in the level of intake and establishment. The hosts' grazing behaviour will affect the results but as has been suggested by this experiment, this behavioural difference between the lambs did not effect the numbers of larvae recovered. The disadvantages are the expensive and specialist surgery and after care of the fistulated animals.

In order to provide more accurate and valuable information on these factors affecting larval intake by the grazing host, improvements need to be made in several basic areas.

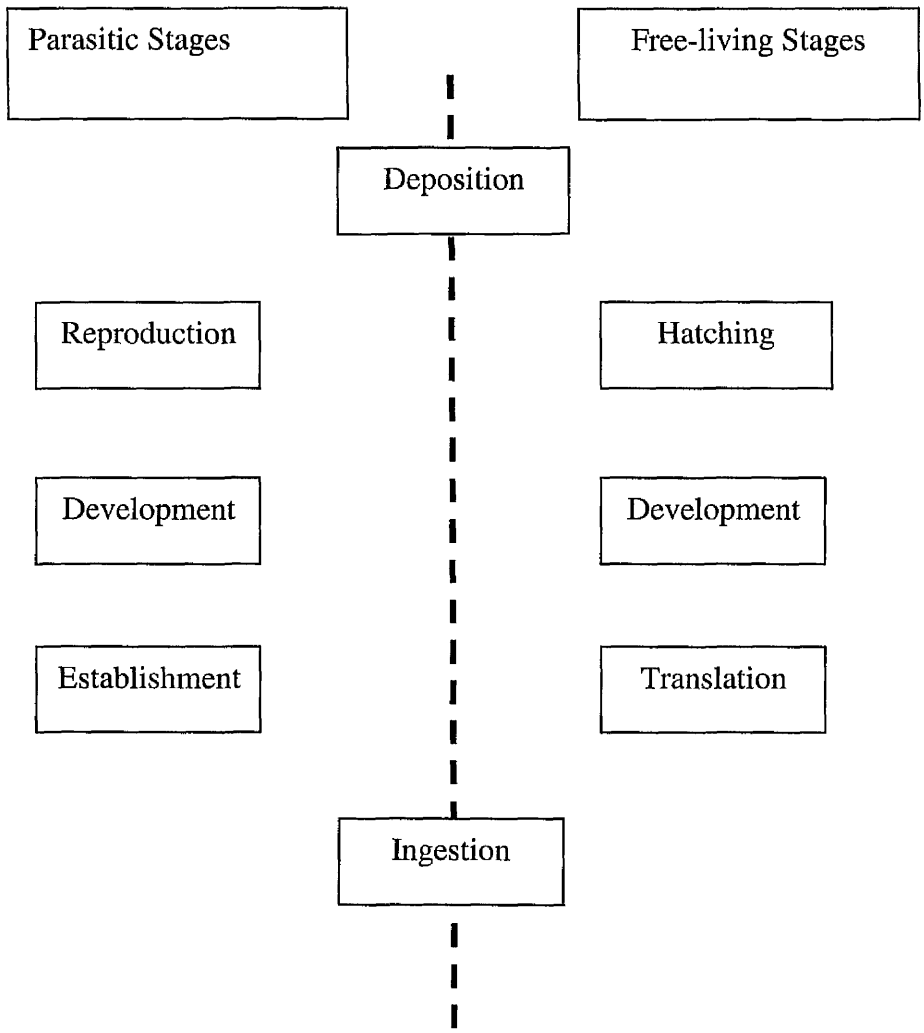
The sward stick that was used to measure sward height was inaccurate and very easy to misread. It was developed by the Hill Farming Research Organisation (Bircham, 1981; Barthram, 1986) and has been used extensively in grazing ecology studies for sheep, goats and cattle (e.g. Bircham & Hodgson, 1984; Lhuillier, 1987; Hutchings, 1991; Chestnutt, 1992; Binnie & Chestnutt, 1994; Rook, Huckle & Penning, 1994; Murphy, Silman & Barreto, 1995; Penning, Newman, Parsons, Harvey & Orr, 1997; Bakker, Gordon & Milne, 1998; Virkajarvi, 1999). For these type of studies the level of accuracy provided is acceptable and often a change in sward height in the magnitude of centimetres is sufficient, unlike these studies where changes in the region of millimetres were required.

Ideally as the bite taken is a three dimensional shape, the entire profile of the bite could be measured. The equipment required to perform such a task is not freely available and is out of the price range of a small study such as this one. As this is such an important part of the study, greater accuracy in the measuring equipment would have been ideal. The measurement of twenty positions on the grid was limited by time constraints, ideally a larger number of positions with this inaccurate method would have been preferable.

The equipment used to measure the sward height is inaccurate and very easy to misread and ideally, the whole area that has been offered to the animal to graze would be measurable. If this were possible, then the biomass values obtained could be directly related to bite size, providing an estimate of the proportion of ingesta collected. In that way, the data obtained on the position of the larvae on the sward obtained from the manually collected samples could be related to which part of the sward the animal is actually ingesting. This in turn would provide information on the most likely conditions when the largest number of larvae would be ingested and so allow control strategies to be most effectively implemented.

In order to improve our understanding of the complex interactions and relationships that exist to bring about larval ingestion by the host, more studies of this type are required. Figure 4.23 shows some of the key processes affecting the life cycle of the parasite, which shows all the different stages where parasite-host interaction leads to changes in this complex relationship. Further studies on the exact portion of the sward being ingested and what level of larval infection that carries are important. There is increasing evidence regarding the effect parasitism has on both grazing behaviour and diet selection (Israf , Coop, Jackson & Jackson, 1996a; Israf, Coop, Stevenson, Jones, Jackson, Jackson, MacKellar & Huntley, 1996b; Hutchings *et al.*, 1998; 1999). The avoidance of faecal material while grazing is affected by the parasitic state of the host (Hutchings *et al.*, 1998). Parasitised animals will preferentially graze the higher protein containing clover rather than ryegrass alone, in order to compensate for the negative effects of parasitism. These factors clearly require further characterisation.

Figure 4.23 *Some of the key processes affecting the life cycle of gastrointestinal nematodes*



Chapter 5

Susceptibility of lactating ewes to infection with *Teladorsagia circumcincta*

5.1 Introduction.

The increased susceptibility around parturition and lactation to gastrointestinal nematode infection is well documented in ewes and has been extensively reviewed (Dunsmore, 1965; Connan. 1974, 1976; Michel, 1974,1976; Lloyd, 1983), and is also seen in cows, sows, bitches, mice, rats, guinea pigs, rabbits and goats (Barger, 1993). This temporary loss of immunity is thought to be associated more with lactation than pregnancy or parturition (Connan, 1968b) and can be prevented by removing the suckling stimulus at birth (Connan, 1968b; O'Sullivan & Donald, 1970).

There are two different theories as to how this relaxation of immunity manifests itself. Michel (1974, 1976) thought that the rise could be accounted for simply by a suspension of mortality in the adult worms, whereas Connan (1968a) and O'Sullivan & Donald (1970) thought that it was a combination of an increase in the establishment of incoming larvae, the development of larvae that were previously arrested in the mucosal glands, a failure to regulate the fecundity of adult female worms and an inability to expel existing populations. Both O'Sullivan & Donald (1973) and Donald *et al.* (1982) found a higher level of rejection of larvae of both *T. colubriformis* and *T. circumcincta* in non-lactating compared to the lactating ewes, supporting the latter argument.

The main cause of this phenomenon is now thought to be under hormonal control although other factors such as poor nutrition, lack of antigenic stimulus and stress caused by lambing are also thought to be secondary factors (Barger, 1993). Recent work by Donaldson *et al.* (1997) showed that an increased level of protein in the ewe could significantly reduce faecal egg count, from 21 days prior to parturition until the end of the study, at 21 days after parturition, and significantly reduce worm burdens, whereas an increase in the energy content of the diet had no such effect.

The precise control mechanisms are still not clear (Barger, 1993). Historically it was thought that prolactin, a pituitary peptide whose main function is the initiation and maintenance of lactation, was primarily responsible, but recent experimental evidence suggests that prolactin may not be the primary cause of the periparturient rise (Coop *et al.,* 1990; Jeffcoate *et al.,* 1990).

There is increasing evidence to support the theory that there is a differing host response to different species during the periparturient period (Brunsdon, 1970;

O'Sullivan & Donald, 1973; Gibbs & Barger, 1986; Jackson *et al.,* 1988; Donaldson *et al.,* 1997; Leathwick *et al.,* 1997). Gibbs & Barger (1986) found that although *T. circumcincta* faecal egg counts were increased ten fold in lactating compared to dry ewes and there was establishment of *T. colubriformis*, the lactating ewes were no more susceptible to artificial infection with *H. contortus* than dry ewes. It appears that both pregnant and lactating ewes are more susceptible to *T. circumcincta* than either *T. colubriformis* (Donaldson *et al.,* 1997; Leathwick *et al.,* 1997) or *T. vitrinus* (Jackson *et al.,* 1988).

The period of susceptibility in the post-parturient ewe to *T. circumcincta* has not been fully established and that was the primary aim of this experiment.

5.2 Materials and Methods.

5.2.1 Experimental design

A total of twenty four lactating Greyface (Scottish Blackface x Border Leicester) ewes with twin lambs were divided into three groups (Groups 1-3, n=8) and along with 8 barren ewes (Group 4) and 8 worm naïve hoggs (Group 5), they were given a single dose of 10,000 *T. circumcincta* infective larvae (L_3). Group 1 received their dose 7 days *post partum* (DPP), group 2, 28 DPP and group 3, 56 DPP. All of the animals were treated with ivermectin (Oramec, MSD Agvet, UK) at the recommended dose rate (2.5 ml per 10 kg liveweight) 14 days before being given the larval challenge. The animals were group housed within their groups and offered concentrates (ESCA nuts) and hay *ad lib.*

Faecal samples were taken directly from the rectum twice weekly and processed using the method described in Chapter 2. Blood samples were collected once a week and plasma pepsinogen, albumin and total protein levels were determined as described in Chapter 2.

5.2.2 Statistical Analysis.

The egg count data were $\log_{10}(x + 1)$ transformed before analysis. The pepsinogen, total protein and albumin data were analysed using the raw data. The data analysis were performed using analysis of covariance, with one covariate unless stated otherwise (Minitab v 10.2).

5.3 Results

5.3.1 Parasitological data

5.3.1.1 Faecal consistency

The group mean faecal consistency scores are shown in Figure 5.1. Table 5.1 shows the significant differences between the groups,

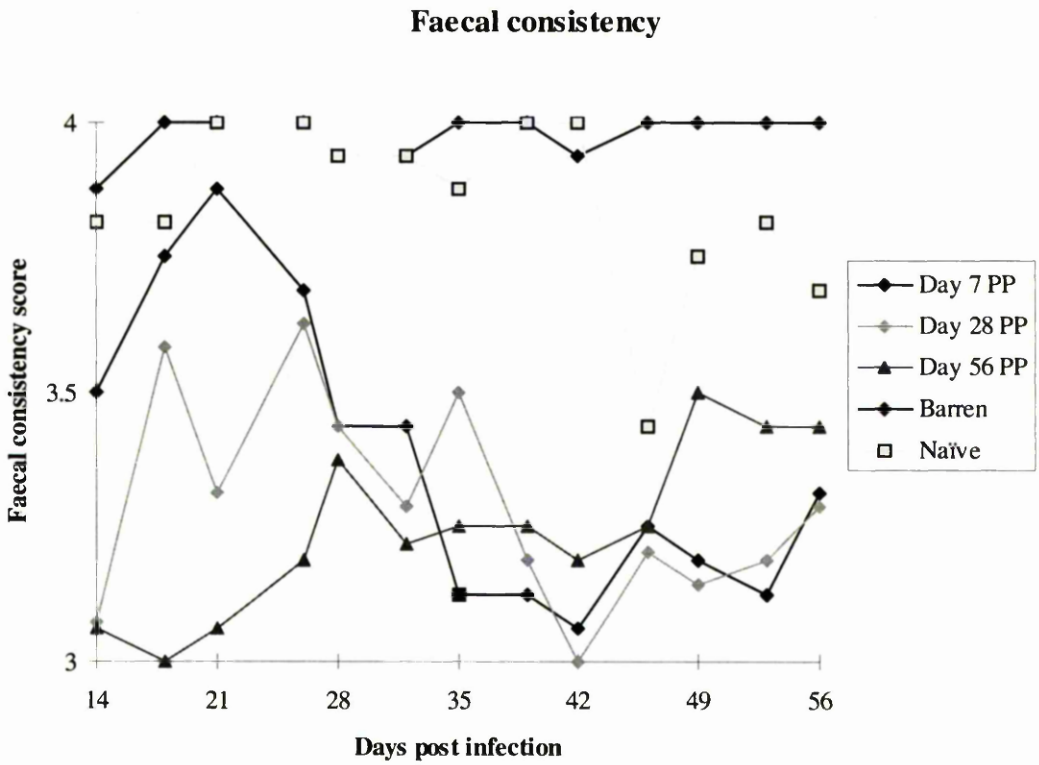


Figure 5.1 *Group mean faecal consistency scores.*

Table 5.1 *Statistically significant differences between the group mean faecal consistency scores.*

	Comparator				
Day P.I.	Day 7 PP	Day 28 PP	Day 56 PP	Barren	Naïve
14	Day 56 PP***	-	-	Day 28 PP*** Day 56 PP***	Day 28 PP*** Day 56 PP***
18	Day 56 PP***	Day 56 PP***	-	Day 28 PP*** Day 56 PP***	Day 56 PP***
21	Day 28 PP*** Day 56 PP***	-	-	Day 28 PP*** Day 56 PP***	Day 28 PP*** Day 56 PP***
28	-	-	-	Day 7 PP** Day 28 PP** Day 56 PP**	Day 7 PP** Day 28 PP** Day 56 PP**
32	-	-	-	Day 7 PP* Day 28 PP* Day 56 PP*	Day 7 PP* Day 28 PP* Day 56 PP*
35	-	-	-	Day 7 PP* Day 28 PP* Day 56 PP*	Day 7 PP* Day 56 PP*
39	-	-	-	Day 7 PP*** Day 28 PP*** Day 56 PP***	Day 7 PP*** Day 28 PP*** Day 56 PP***
42	-	-	-	Day 7 PP*** Day 28 PP*** Day 56 PP***	Day 7 PP*** Day 28 PP*** Day 56 PP***
46	-	-	-	Day 7 PP** Day 28 PP** Day 56 PP** Naïve**	-
49	-	-	-	Day 7 PP** Day 28 PP** Day 56 PP**	Day 7 PP** Day 28 PP**
53	-	-	-	Day 7 PP* Day 28 PP* Day 56 PP*	Day 7 PP* Day 28 PP*

- no significant difference

* significantly lower faecal consistency score than comparator $p<0.05$, ** $p<0.01$, *** $p<0.001$

5.3.1.2 Faecal egg counts

The group means for the faecal egg counts (e.p.g.) can be seen in Figure 5.2 and in Table 5.2 ± S.D.

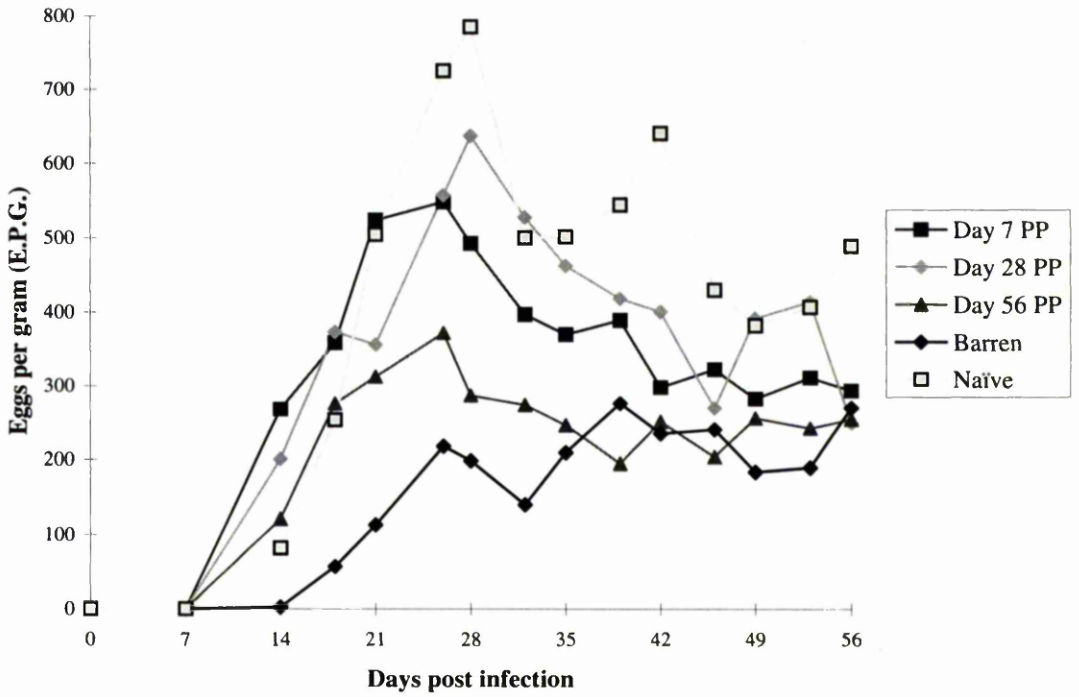


Figure 5.2 *Group mean faecal egg counts.*

Table 5.2. *Group mean faecal egg counts ± S.D. (e.p.g.)*

Day P.I.	Group 1	Group 2	Group 3	Group 4	Group 5
14	268 ± 61.3	201 ± 83.6	122 ± 91.0	3 ± 6.3	82 ± 94.2
18	359 ± 148.5	374 ± 93.3	276 ± 118.8	57 ± 141.7	253 ± 151.4
21	524 ± 166.8	357 ± 53.3	313 ± 152.6	113 ± 208.5	505 ± 216.0
26	549 ± 205.9	558 ± 174.6	372 ± 224.1	218 ± 254.0	726 ± 410.7
28	493 ± 154.1	637 ± 174.4	287 ± 100.5	198 ± 202.0	785 ± 223.7
32	397 ± 198.3	528 ± 137.6	274 ± 179.7	141 ± 116.6	501 ± 188.2
35	370 ± 143.5	462 ± 198.1	246 ± 136.0	209 ± 174.4	502 ± 149.0
39	389 ± 116.4	419 ± 104.6	195 ± 89.2	276 ± 264.0	545 ± 199.8
42	298 ± 120.6	401 ± 151.9	251 ± 114.9	235 ± 230.2	640 ± 217.7
46	323 ± 71.1	270 ± 36.0	204 ± 93.4	240 ± 215.0	430 ± 122.8
49	282 ± 103.3	392 ± 96.5	256 ± 143.6	184 ± 172.2	383 ± 224.9
53	312 ± 210.8	414 ± 120.2	242 ± 100.7	190 ± 177.3	407 ± 166.0
56	294 ± 101.1	249 ± 131.8	254 ± 128.9	270 ± 320.5	489 ± 183.2

There were significant differences between the group mean egg counts on days 14, 18 and 56, as shown in Table 5.3.

Table 5.3. *Statistically significant differences between the group mean faecal egg counts.*

	Comparator				
Day P.I.	Day 7 PP	Day 28 PP	Day 56 PP	Barren	Naïve
14	Barren** Naïve**	Barren** Naïve**	Barren**	-	Barren**
18	Barren**	Barren**	Barren**	-	Barren**
56	Barren*	-	-	Day 56 PP*	Day 7 PP* Day 28 PP* Day 56 PP* Barren*

- no significant difference

* significantly lower faecal egg count than comparator $p<0.05$, ** $p<0.01$

The group mean egg counts for between days 14 to 56 are shown in Table 5.4.

Table 5.4. *Group mean egg counts for between 14 and 56 days post infection.*

	Mean egg count between day 14 and 56
Day 7 PP	$375^{a,b}$
Day 28 PP	$415^{a,b}$
Day 56 PP	$251^{a,c}$
Barren	180^{c}
Naïve	479^{b}

Different superscripts denote significant differences between groups ($p<0.01$).

5.3.2 Blood biochemistry data

5.3.2.1 Plasma albumin.

The group mean plasma albumin concentrations (g/l) are shown ± S.D. in Table 5.5 and in Figure 5.3.

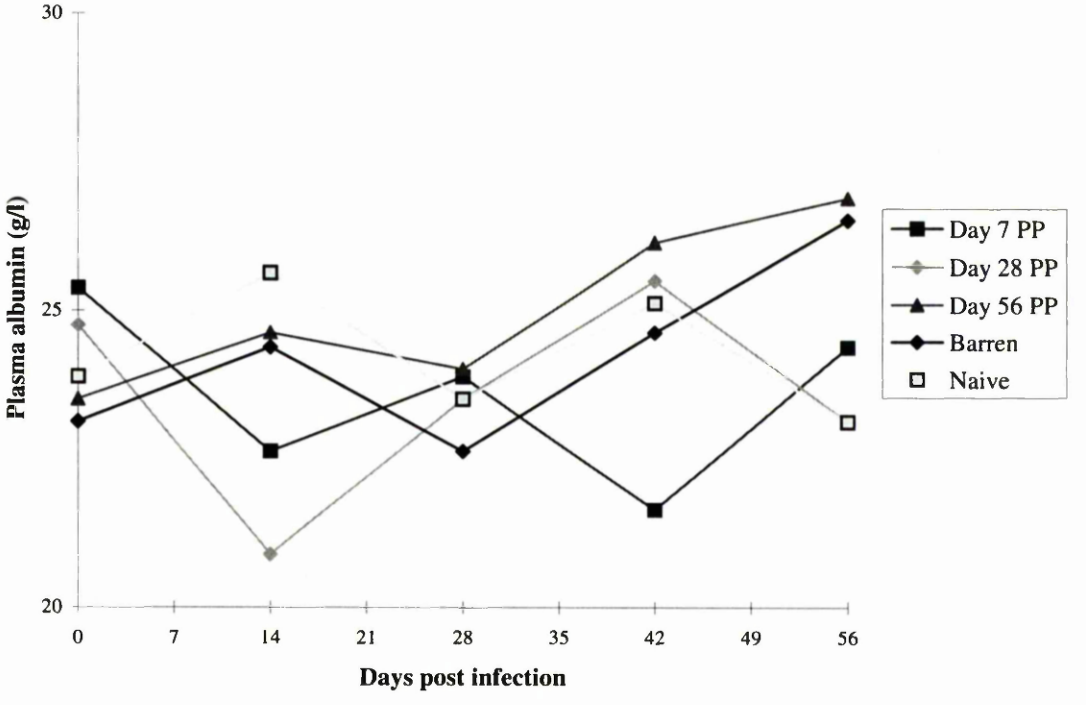


Figure 5.3 *Group mean plasma albumin concentrations.*

Table 5.5. *Group mean plasma albumin concentrations ± S.D. (g/l).*

Day P.I.	Day 7 PP	Day 28 PP	Day 56 PP	Barren	Naïve
0	25.38 ± 3.25	24.75 ± 3.06	23.50 ± 3.66	23.13 ± 6.94	23.88 ± 2.59
14	22.63 ± 2.45	20.88 ± 3.44	24.63 ± 2.77	24.38 ± 5.85	25.63 ± 2.77
28	23.88 ± 2.70	23.50 ± 2.56	24.00 ± 2.62	22.63 ± 4.31	23.50 ± 2.33
42	21.63 ± 2.92	25.50 ± 3.02	26.13 ± 3.44	24.63 ± 4.57	25.13 ± 3.98
56	24.38 ± 3.70	23.13 ± 3.00	26.88 ± 4.09	26.50 ± 7.01	23.13 ± 3.36

There were significant differences on days 14 and 42 as shown in Table 5.6.

Table 5.6. *Statistically significant differences between the group mean plasma albumin concentrations.*

	Comparator				
Day P.I.	Day 7 PP	Day 28 PP	Day 56 PP	Barren	Naïve
14	-	-	-	-	Day 28 PP**
42	-	-	Day 7 PP*	-	-

- no significant difference

* significantly lower plasma albumin concentration than comparator $p<0.05$,

** $p<0.01$

5.3.2.2 Plasma total protein.

The group mean plasma total protein concentrations (g/l) are shown ± S.D. in Table 5.7 and in Figure 5.4.

Table 5.7. *Group mean plasma total protein concentrations ± S.D. (g/l)*

Day P.I.	Group 1	Group 2	Group 3	Group 4	Group 5
0	45.50 ± 7.45	43.13 ± 5.72	51.00 ± 9.15	65.00 ± 13.03	50.25 ± 2.55
14	36.75 ± 5.31	33.88 ± 7.34	54.25 ± 7.36	66.50 ± 13.19	41.63 ± 9.83
28	44.25 ± 11.82	36.38 ± 7.56	38.88 ± 7.88	58.13 ± 18.75	37.25 ± 9.78
42	30.50 ± 8.05	40.38 ± 6.09	40.88 ± 7.34	59.75 ± 16.23	43.63 ± 10.24
56	41.13 ± 8.20	36.63 ± 6.48	39.25 ± 13.07	62.88 ± 11.64	49.75 ± 4.46

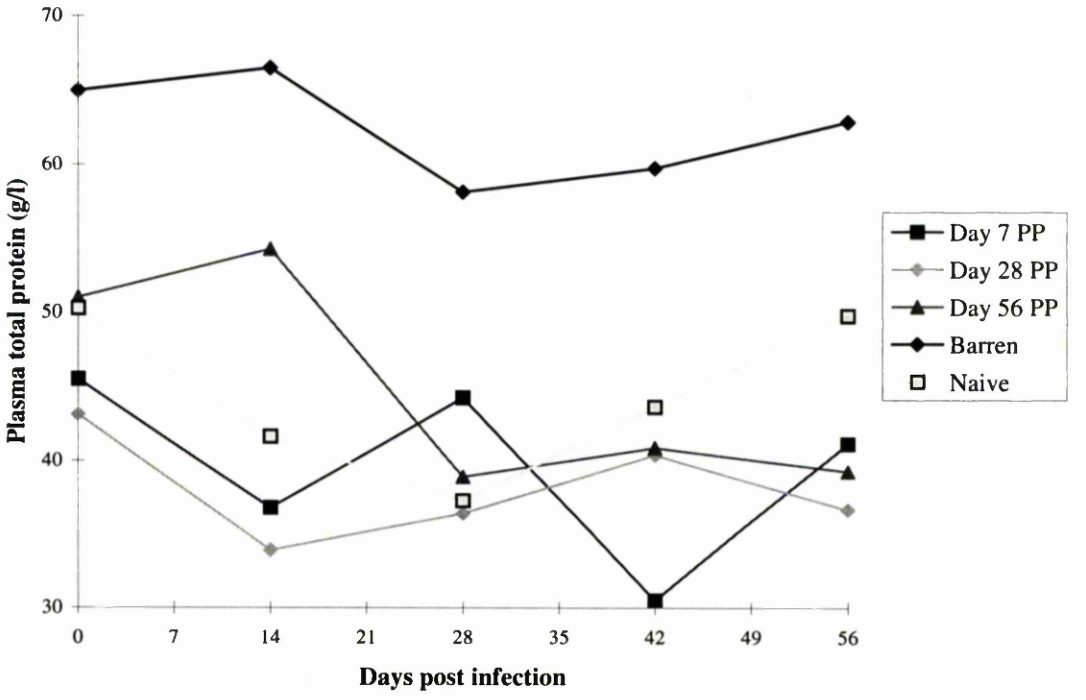


Figure 5.4 *Group mean plasma total protein concentrations.*

There were significant differences on days 0, 14, 42 and 56, as shown in Table 5.8.

Table 5.8. *Statistically significant differences in group plasma total protein concentrations.*

	Comparator				
Day P.I.	Day 7 PP	Day 28 PP	Day 56 PP	Barren	Naïve
0	-	-	-	Day 7 PP* Day 28 PP* Day 56 PP* Naïve*	-
14	-	-	Day 7 PP* Day 28 PP*	Day 7 PP* Day 28 PP* Naïve*	-
42	-	-	-	Day 7 PP* Day 28 PP* Day 56 PP* Naïve*	Day 7 PP*
56	-	-	-	Day 7 PP* Day 28 PP* Day 56 PP* Naïve*	Day 28 PP*

- no significant difference

* significantly lower plasma total protein concentration than comparator $p<0.01$

5.3.2.3 Plasma pepsinogen

The group mean plasma pepsinogen concentrations (U) are shown ± S.D. in Table 5.9 and Figure 5.5, where U = μmoles tyrosine/min/litre at 37°C.

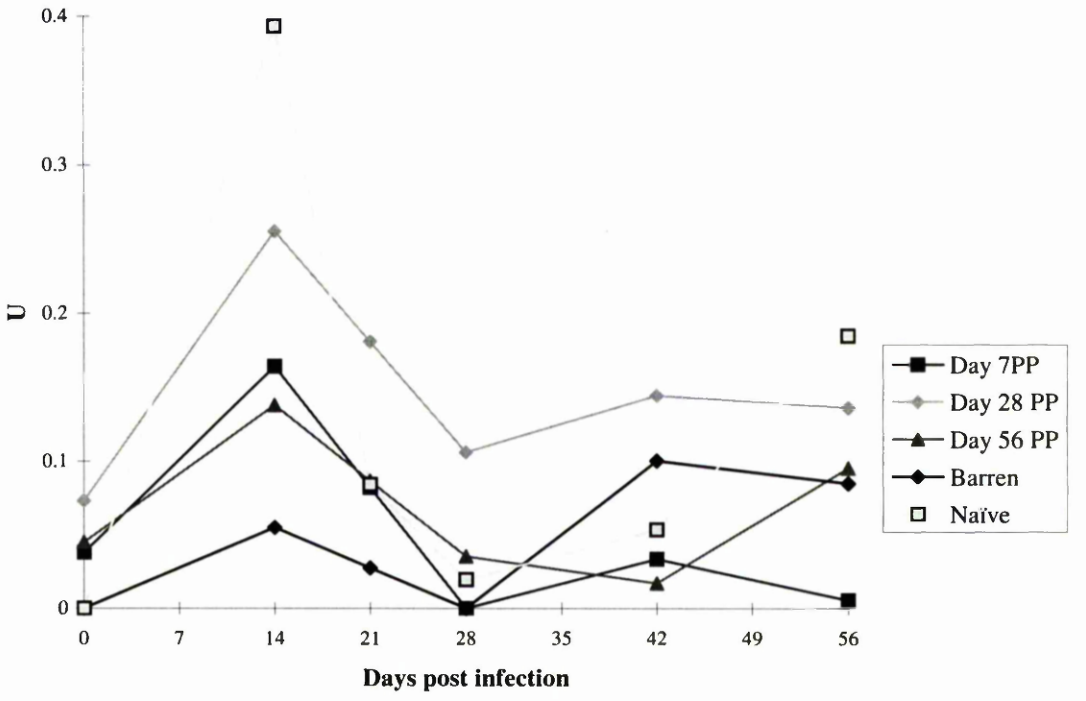


Figure 5.5 *Group mean plasma pepsinogen concentrations.*

Table 5.9. Group mean plasma pepsinogen concentrations (U) ± S.D.

Day P.I.	Group 1	Group 2	Group 3	Group 4	Group 5
0	0.038 ± 0.28	0.073 ± 0.20	0.045 ± 0.13	0.000 ± 0.15	0.000 ± 0.10
14	0.164 ± 0.12	0.255 ± 0.26	0.138 ± 0.21	0.055 ± 0.10	0.393 ± 0.21
28	0.000 ± 0.12	0.106 ± 0.21	0.035 ± 0.10	0.000 ± 0.18	0.019 ± 0.06
42	0.033 ± 0.15	0.144 ± 0.21	0.017 ± 0.08	0.100 ± 0.18	0.053 ± 0.16
56	0.006 ± 0.05	0.136 ± 0.29	0.095 ± 0.08	0.085 ± 0.14	0.185 ± 0.16

On day 14, group 5 had a significantly ($p<0.01$) elevated plasma pepsinogen concentration, compared to all of the other groups.

5.4 Discussion.

Both groups 1 and 2 showed a similar pattern in egg output with the group mean egg count between days 14 and 56 being 374 and 415 and peak values of 549 e.p.g. on day 26 and 637 e.p.g. on day 28 for groups 1 and 2 respectively. The mean

egg count for group 3 between days 14 and 56 was 253 e.p.g., which is similar to the mean egg count of 180 e.p.g seen in the barren ewes over the same period. The barren ewes, in contrast to other studies (Dunsmore, 1965; Arundel & Ford, 1969; O'Sullivan & Donald, 1970; Gibbs & Barger, 1986; Coop *et al.,* 1990) showed a marked rise in faecal egg count, with a peak value of 276 e.p.g. on day 39 post infection, although this figure was both lower and delayed when compared to the peak values in the other groups. Group 5, the worm naïve hoggs, showed a rapid rise in egg count followed by a prolonged high level of egg excretion with a mean egg count between days 14 and 56 of 479 e.p.g. It would be expected that if the egg counts had been followed for a longer period of time, there would have been a gradual decline in the egg count as the animals acquired and expressed immunity.

The prepatent period for *T. circumcincta* is usually between 18-21 days (Armour *et al.,* 1966; Smith, 1988) however it can be seen that egg counts were observed in all groups on day 14. The laboratory strain of *T. circumcincta* that was used in this study has been passaged many times through donor lambs. Each time it is passaged, the donor lamb is faecal sampled daily and when a positive egg count is recorded the faeces are collected and the larvae harvested ten days later. In this way a selection pressure has been applied, selecting for the worms that produce eggs earlier. In this way, the prepatent period of the laboratory strain appears to have been reduced to around 14 days.

The pattern observed in the plasma pepsinogen levels in all 5 groups was similar, with a peak occurring at day 14 followed by a gradual decline. This timing coincides with the emergence of the larvae from the gastric glands in the abomasal mucosa at around 7-10 days post infection. Group 5 showed the highest peak of around 0.4 U. Other studies have found the normal range for pepsinogen values in uninfected sheep to be between 0 and 0.454 U (Lawton *et al.*, 1996) showing that the figures obtained are well within the observed range for uninfected sheep.

A level above 1 U is considered to be a sign of clinical damage although in immune sheep an elevated level of plasma pepsinogen has been seen when no clinical disease was present, i.e. no nematode eggs in the faeces. This is thought to be due to a hypersensitivity reaction in the abomasal mucosa caused by an immune reaction to larvae (Jeffcoate, Wedrychowicz, Fishwick, Dunlop, Duncan & Holmes, 1992).

The plasma albumin levels showed no real trends for any of the groups to be different from the others, which is not unexpected as the dose given was not enough to cause clinical disease. The same is true of the plasma total protein values except for group 4 which had a consistently higher value throughout the study.

These results seem to indicate that the greatest period of susceptibility to *T. circumcincta* in the post-parturient ewe is between 7 and 28 days *post partum.* A study using a similar experimental design but with the intestinal parasite *T. vitrinus* found that the ewes dosed 28 DPP were the most susceptible, with a similar egg output pattern to that seen in the naïve control animals (Jackson, 1989).

Chapter 6

The effect of protein and methionine supplementation on the response of lambs to *Teladorsagia circumcincta*

6.1 Introduction

Significant production losses are caused by gastrointestinal parasites in ruminants of economic importance, particularly in the young, grazing lamb but there can also be effects in periparturient animals where milk production is reduced (Leyva, Henderson & Sykes, 1982; Sykes, 1994). The control strategies that are relied upon at present are primarily based on chemotherapy and/or grazing management (van Houtert, 1997; Barger, 1997). However, the continuing emergence of anthelmintic resistance (e.g. Jackson, 1993; McKenna, 1995; Waller, 1997) and concern about pesticide residues in the environment (Sykes, McFarlane & Familton, 1992; McKellar, 1997) has led to other strategies being investigated. These include sub-unit vaccines (Emery, 1996), breeding for resistant genotypes (Gray, 1997), biological control using nematophagous fungi (Larsen *et al.,* 1997) and manipulation of the hosts' nutritional status (Coop & Holmes, 1996).

It has been thought for many years that improving the plane of nutrition of the host can reduce production losses (e.g. Clunies-Ross & Graham, 1932; Fraser & Robertson, 1933). Since then numerous studies have been carried out to investigate the effect of nutrition on the host response to gastrointestinal nematode infection (see Coop & Holmes, 1996; van Houtert & Sykes, 1996). These studies have provided evidence strongly suggesting that protein supply is more important than energy in improving the hosts' response (Bown *et al.,* 1991; Michael & Bundy, 1992; Coop *et al.*, 1995; Donaldson *et al.,* 1997). It is thought that in the parasitised animal there may be competition for the available protein between the demands of mounting an immune response, repairing the damaged gastrointestinal tract and maintaining growth (Coop & Holmes, 1996). When the requirement for metabolisable protein (MP) in relation to metabolisable energy (ME) is high, which is the situation in the young growing lamb and the periparturient ewe, this competition may lead to an impairment in the response of the immune system (Donaldson, 1997). If additional protein could be supplied to meet the demands of all of these systems, then it is possible that both the resilience and resistance of the lambs could be improved (Coop & Holmes, 1996). In addition, if the protein could be 'protected' partly to avoid the fermentation processes of the rumen, then a larger proportion of the protein ingested would be available for absorption in the small intestine (Coop & Holmes, 1996; Wu & Papas, 1997). Indeed, experiments with

dietary supplementation using fishmeal (a rumen by-pass protein) have shown that the immunological responses of the host can be enhanced (Kambara *et al.*, 1993; van Houtert *et al.*, 1995b). This poses the question, is there a response to protein *per se* or could it be that certain essential amino acids are limiting? In growing lambs, there will be a strong demand for sulphur containing amino acids as a result of tissue deposition and wool growth. In addition, the response of parasitised animals involves considerable increases in mucoproteins which are rich in sulphur bonds. Recently, 'protected' specific amino acids have become available, including the essential sulphur amino acid, methionine. It has been shown that the addition of 'protected' methionine to the diet has increased wool growth (Coetzee *et al.*, 1995), liveweight gain and fibre diameter in lambs (Mata *et al.*, 1995; Mata *et al.*, 1998). However, one study found no effect on any production parameters (Baldwin *et al.*, 1993) and detrimental effects, such as increased egg counts in ewes (Wheeler, Williams & Southcott, 1988) and increased egg counts and decreased milk yields in dairy goats (Bouquet, Hoste, Chartier, Coutineau, Koch & Pors (1997) have also been demonstrated. It has also been shown to increase liveweight gain and cashmere production in goats (Souri *et al.*, 1998), increase yields and production of milk proteins and fats in dairy cows (Overton *et al.*, 1996; Armentano *et al.*, 1997; Kudrna *et al.*, 1998; Robinson *et al.*, 1998) and both increase growth (Esteve-Garcia & Llaurado, 1997) and enhance immune responses (Tsiagbe *et al.*, 1987) in chickens. The effect of 'protected' methionine on resilience and resistance in young, growing lambs was investigated in the following experiments. The influence of methionine was investigated in the rapidly growing lamb and in older, maturer, hoggs.

6.2 Materials and methods

6.2.1 Experimental design

Two experiments were conducted, each using 48 Suffolk x Greyface lambs and an identical experimental design. The lambs had been reared indoors from birth and kept under conditions designed to avoid accidental infection with parasites. For experiment 1 the lambs were 3 ½ months old and for experiment 2 the lambs were 5 ½ months old at the start of the experiment.

Lambs were divided into 6 groups (n=8) to provide uniformity of sex and liveweight, and individually housed. Groups 1 and 2 were offered a basal ration

(MP) containing approximately 120g crude protein (CP) kg^{-1} drymatter (DM), groups 3 and 4 were offered a high protein ration (HP) containing approximately 180g CP kg^{-1} DM and groups 5 and 6 were offered the basal ration supplemented with 'protected' methionine (MPM, Smartamine™, Rhone-Poulenc Animal Nutrition, France) at 2g methionine kg^{-1}DM. Groups 1 (MPI), 3 (HPI) and 5 (MPMI) received 2,000 *T. circumcincta* L_3 five days per week for a total of 56 days and were offered their ration *ad libitum* and groups 2 (MPC), 4 (HPC) and 6 (MPMC) remained uninfected as controls and were pair fed. On day 56 all 48 animals then received anthelmintic, fenbendazole (Panacur, Hoechst, UK) at double the recommended dose rate (10mgs kg^{-1} liveweight), to remove the primary trickle infection, 10 days later all of the lambs were given a single challenge dose of 50,000 *T. circumcincta* L_3 and killed 10 days post challenge.

Faecal consistency scores, faecal egg counts, blood samples and liveweights were taken weekly, as described in Chapter 2. At *post-mortem* the abomasum was removed and selected folds were taken for enumeration of mast cells, globule leukocytes and tissue eosinophils. The abomasum was processed for worm recovery and the number, sex and stage of development of the worm burden estimated as described in Chapter 2. Pepsinogen, albumin and total protein concentrations in the plasma were estimated as described previously in Chapter 2.

6.2.2 Statistical analysis

The faecal egg counts and pepsinogen concentrations were log transformed prior to analysis, $\log_{10}(\chi+1)$, as were the worm burdens and liveweights, $\log_{10}(\chi)$. The rest of the data were analysed using the raw data. The data were analysed using analysis of co-variance with either 1 or 2 co-variates (Minitab, version 10.2).

6.3 Results

6.3.1 Clinical observations

One infected lamb from experiment 1 (group 1) displayed a protracted severe anorexia and was withdrawn from the experiment together with its pair-fed control. One lamb in experiment 2 (group 2) developed urinary calculi on day 66, was removed from the experiment and thus provided no worm burden data.

6.3.2 Food intake and liveweights

The mean food intakes for the infected groups from experiments 1 and 2 are shown in Figure 6.1. The only significant difference in food intake was in the continuously infected young lambs on the moderate protein diet (MPI) when appetite was depressed between weeks 4 and 5 ($p<0.05$). There was a slightly different intake pattern in the two experiments, with all 3 groups of the older lambs showing a more marked decline from week 5 onwards whereas in the high protein and methionine supplemented groups of the young lambs, food intake remained relatively steady after an initial increase between weeks 1 and 3. Figures 6.2 and 6.3 show the average daily growth rates and the average cumulative weight gains respectively, for the infected and control lambs in both experiments. The young lambs had a higher rate of growth throughout the experiment, which was significant in the high protein controls ($p< 0.01$) and methionine continuously challenged ($p<0.05$) and control ($p<0.05$) groups. The exception was the continuously infected moderate protein group, where the young lambs grew slower than the older lambs. There was no significant dietary effect, although there was a trend for the lambs on the high protein ration to have an increased growth rate. The cumulative weight gains of the older lambs on the high protein diet were around 15% higher than the other two groups and similarly in the young lambs there was an increase of around 30% compared to the moderate protein and 10% compared to the methionine groups.

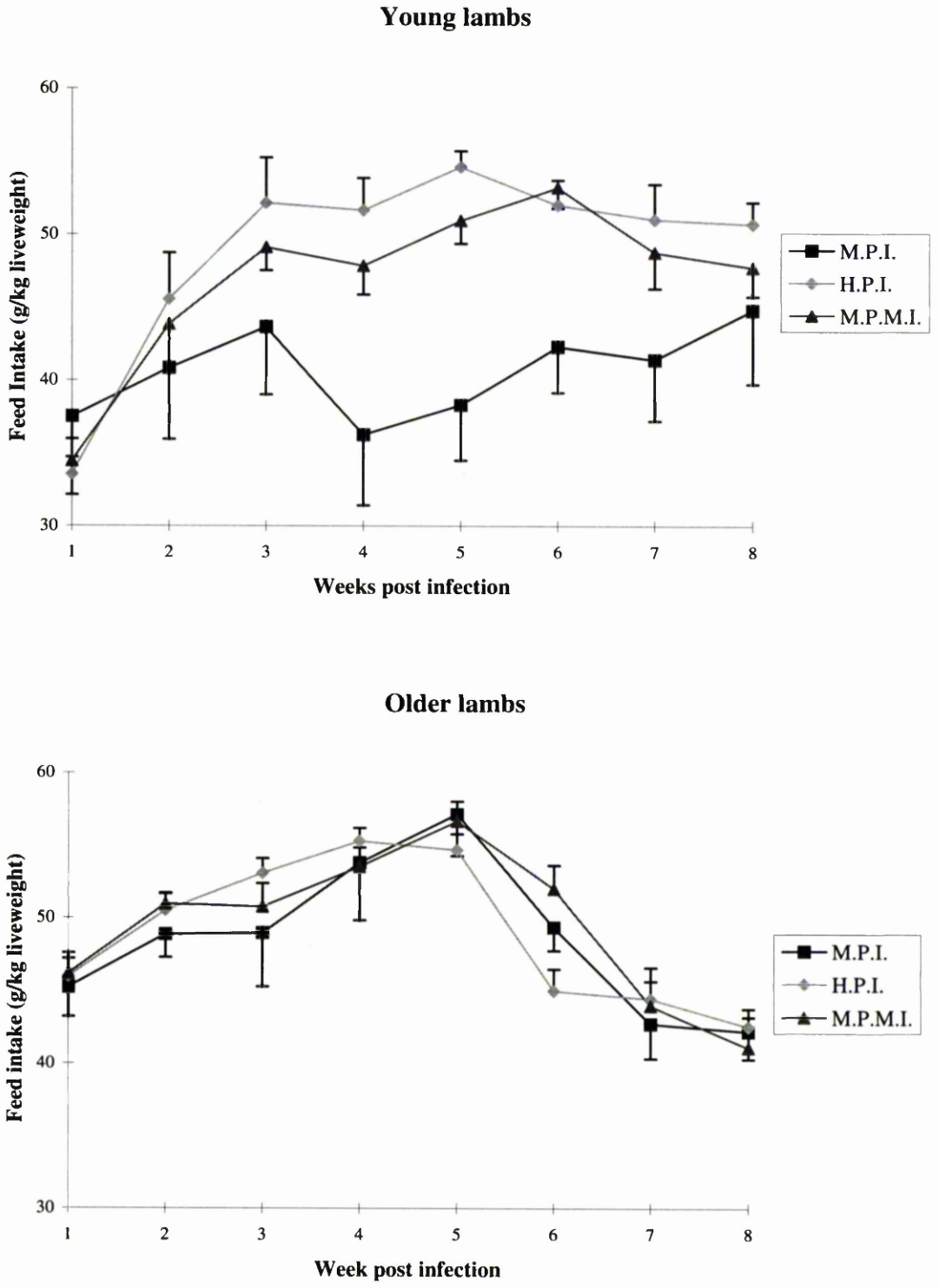


Figure 6.1 *Mean daily feed intake (g/kg liveweight) ± SEM for the continuously challenged lambs in experiments 1 and 2*

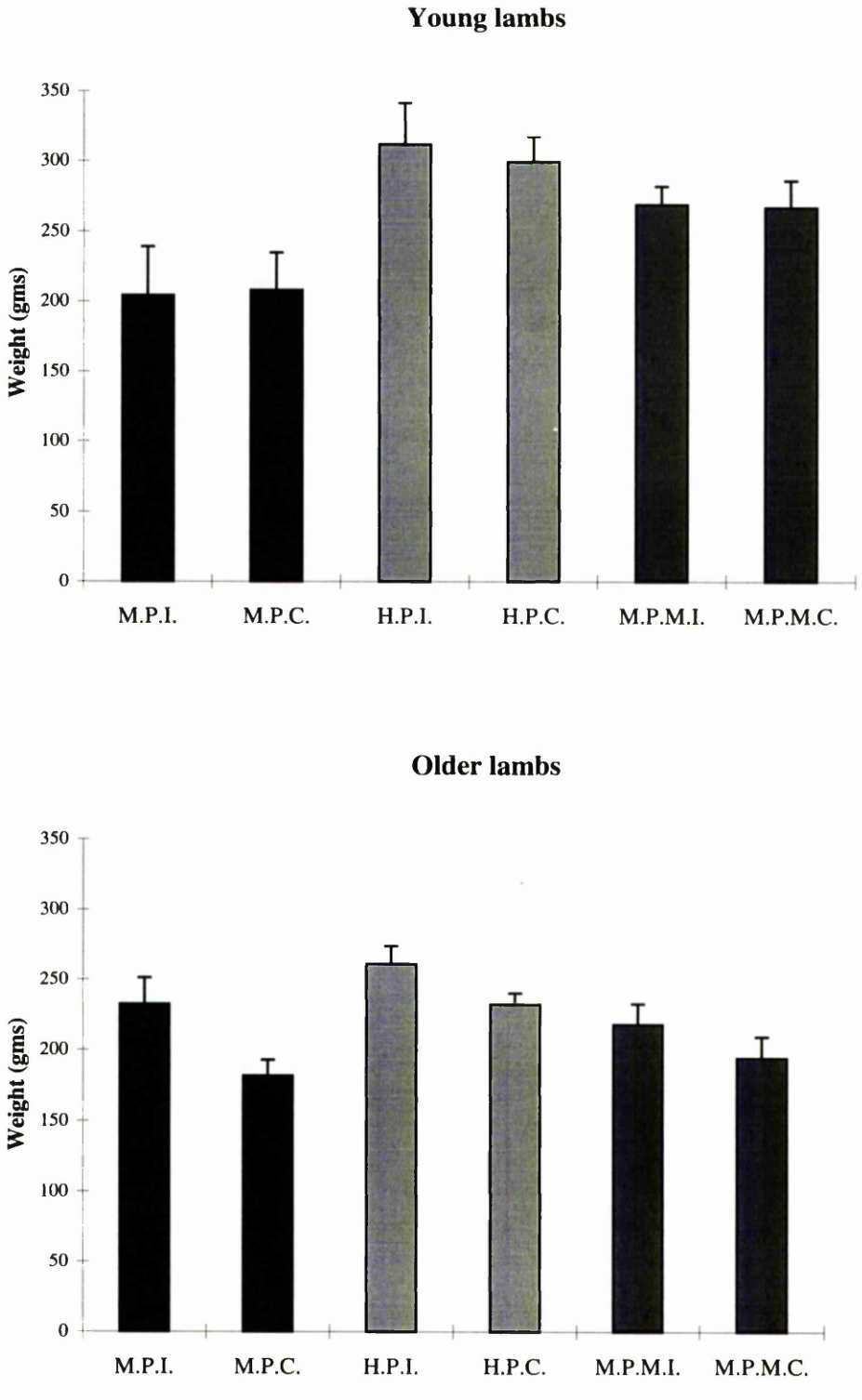


Figure 6.2 *Mean daily weight gains ± SEM for the continuously infected and uninfected lambs in experiments 1 and 2*

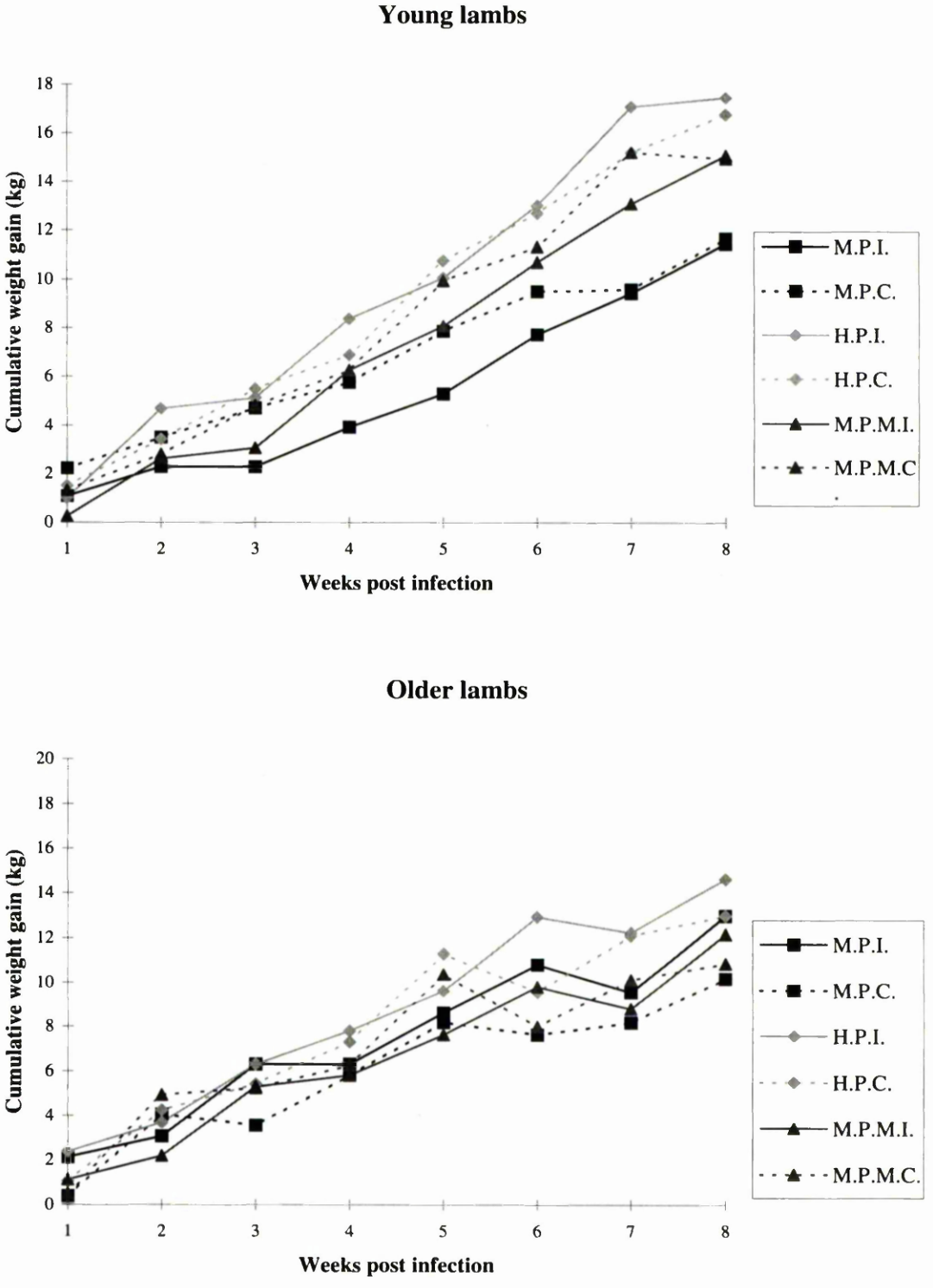


Figure 6.3 *Mean cumulative weight gains for the continuously infected and uninfected lambs in experiments 1 and 2*

6.3.3 Parasitological findings

6.3.3.1 Faecal consistency

Figure 6.4 shows the average group faecal consistency scores. All of the lambs in both experiments showed a decline in consistency score until around 6 weeks after infection began, when there followed a steady increase. There were no significant differences between the dietary groups in either experiment and no differences due to age.

6.3.3.2 Faecal egg counts

Figure 6.5 shows the mean faecal egg counts for the infected lambs in both experiments. The uninfected lambs had zero egg counts throughout the experiment. There was no significant effect on total mean faecal egg count between days 21 and 56 attributable to methionine supplementation or to additional non-rumen degradable protein (NRDP), but in the younger lambs, the moderate protein group (MPI) had a significantly higher egg count at week 6 ($p<0.05$).

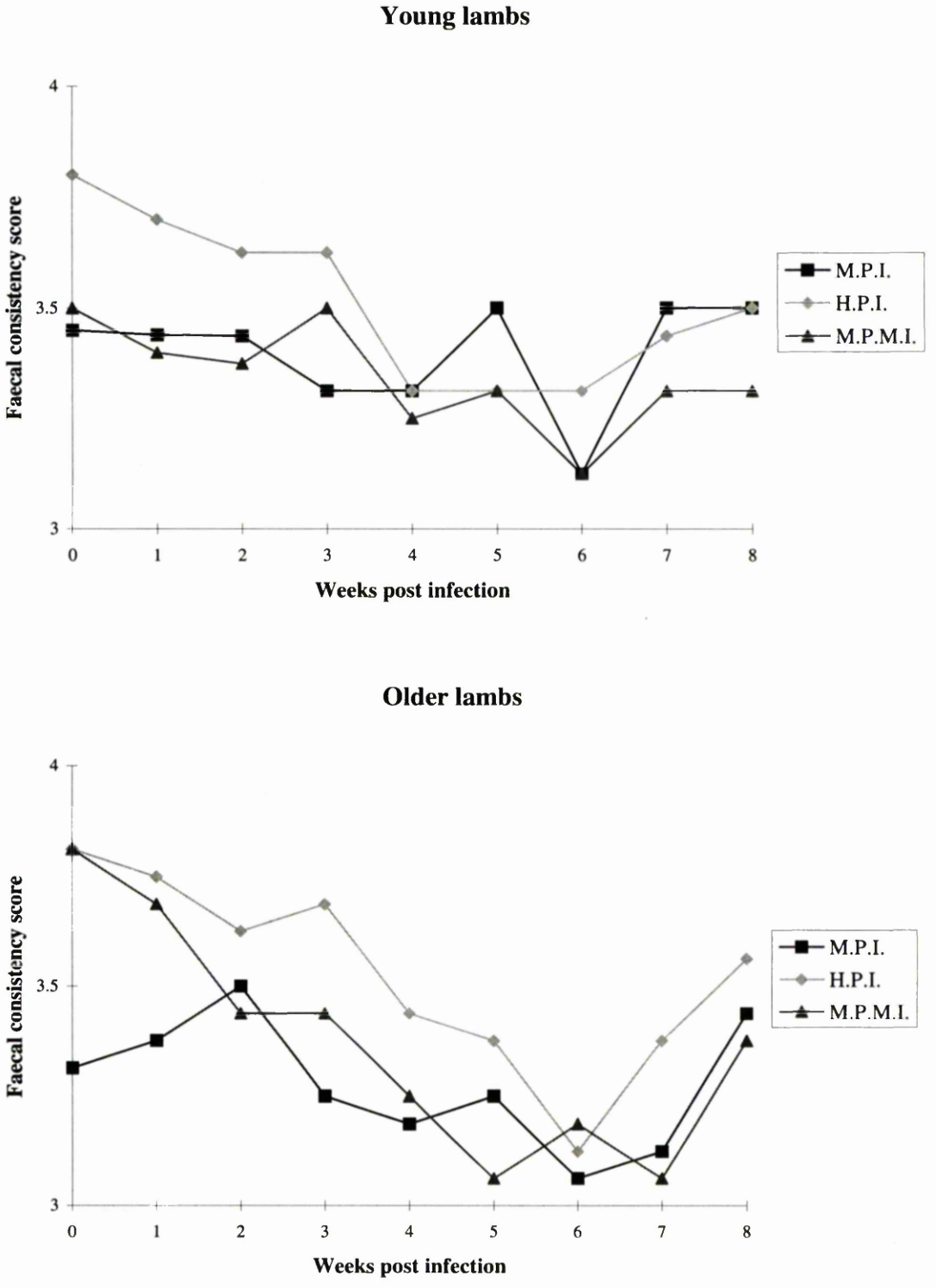


Figure 6.4 *Mean faecal consistency scores for the continuously infected lambs in experiments 1 and 2*

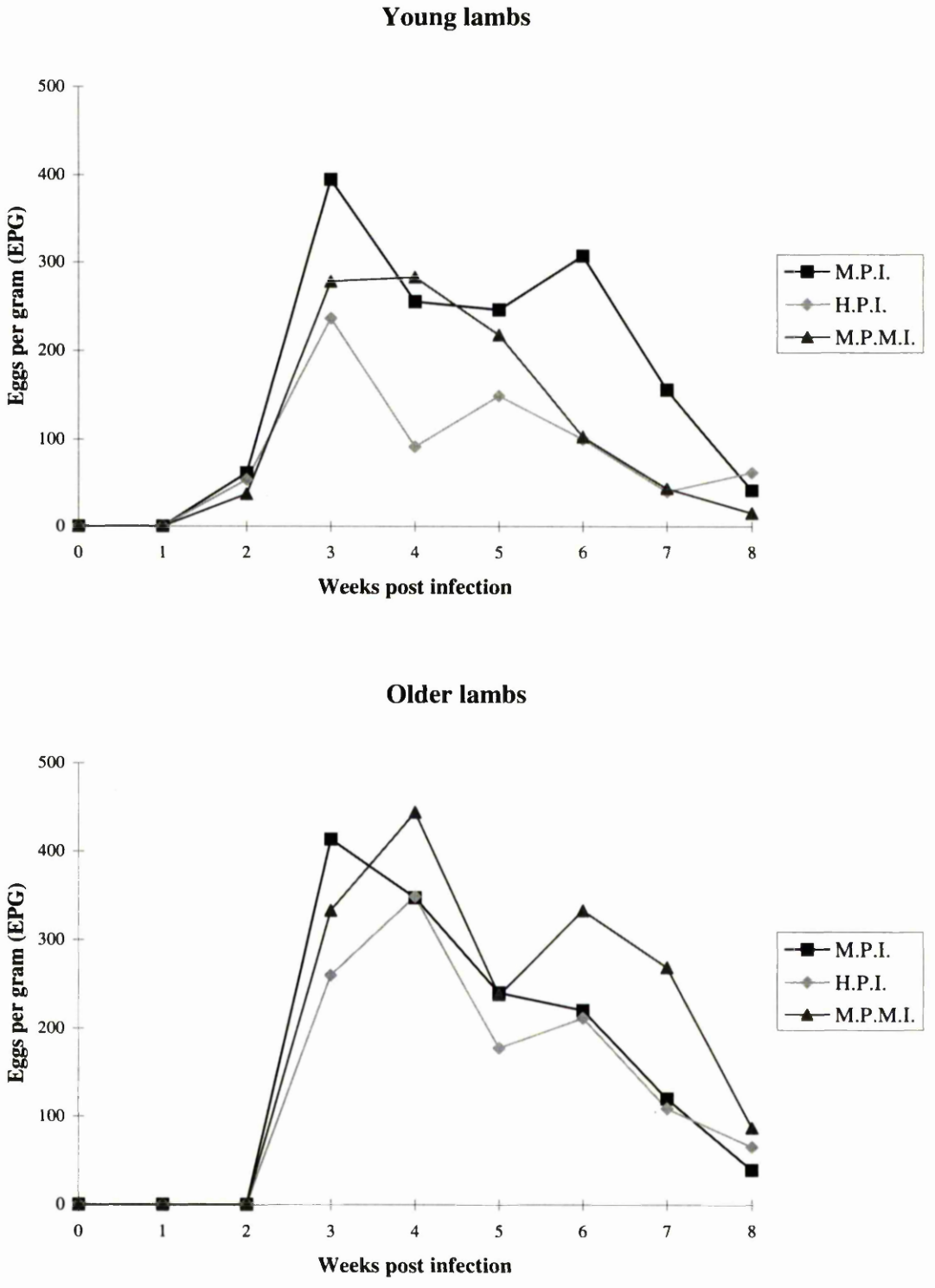


Figure 6.5 *Mean faecal egg counts for the continuously infected lambs in experiments 1 and 2*

6.3.4 Serology

Figure 6.6 shows the group mean plasma pepsinogen concentrations for the continuously challenged lambs in both studies. Although there were no significant effects attributable to supplementation or lamb age there was a tendency for lambs in the high protein groups to have lower plasma pepsinogen concentrations. The plasma albumin concentrations are shown in Figure 6.7. In both experiments there were no significant differences between the continuously challenged and uninfected animals and the concentrations were within the normal range of 20-43 g/l. The group mean plasma total protein concentrations are shown in Figure 6.8 for the continuously challenged lambs. Neither of these parameters showed any dietary or age effects.

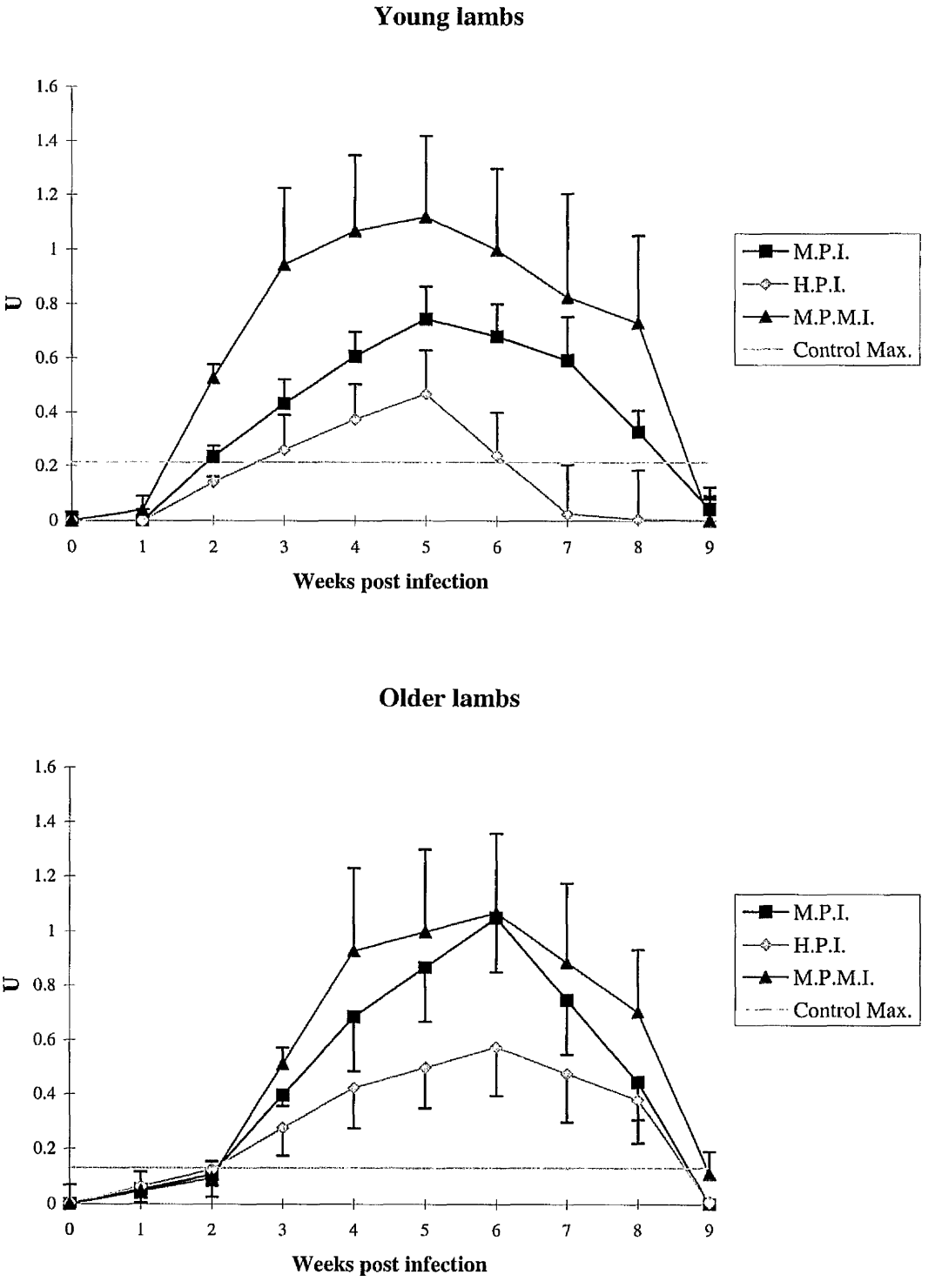


Figure 6.6 *Mean plasma pepsinogen concentrations ± SEM (where U= μmoles tyrosine/ min/ litre at 37°C) for the continuously challenged and uninfected lambs in experiments 1 and 2*

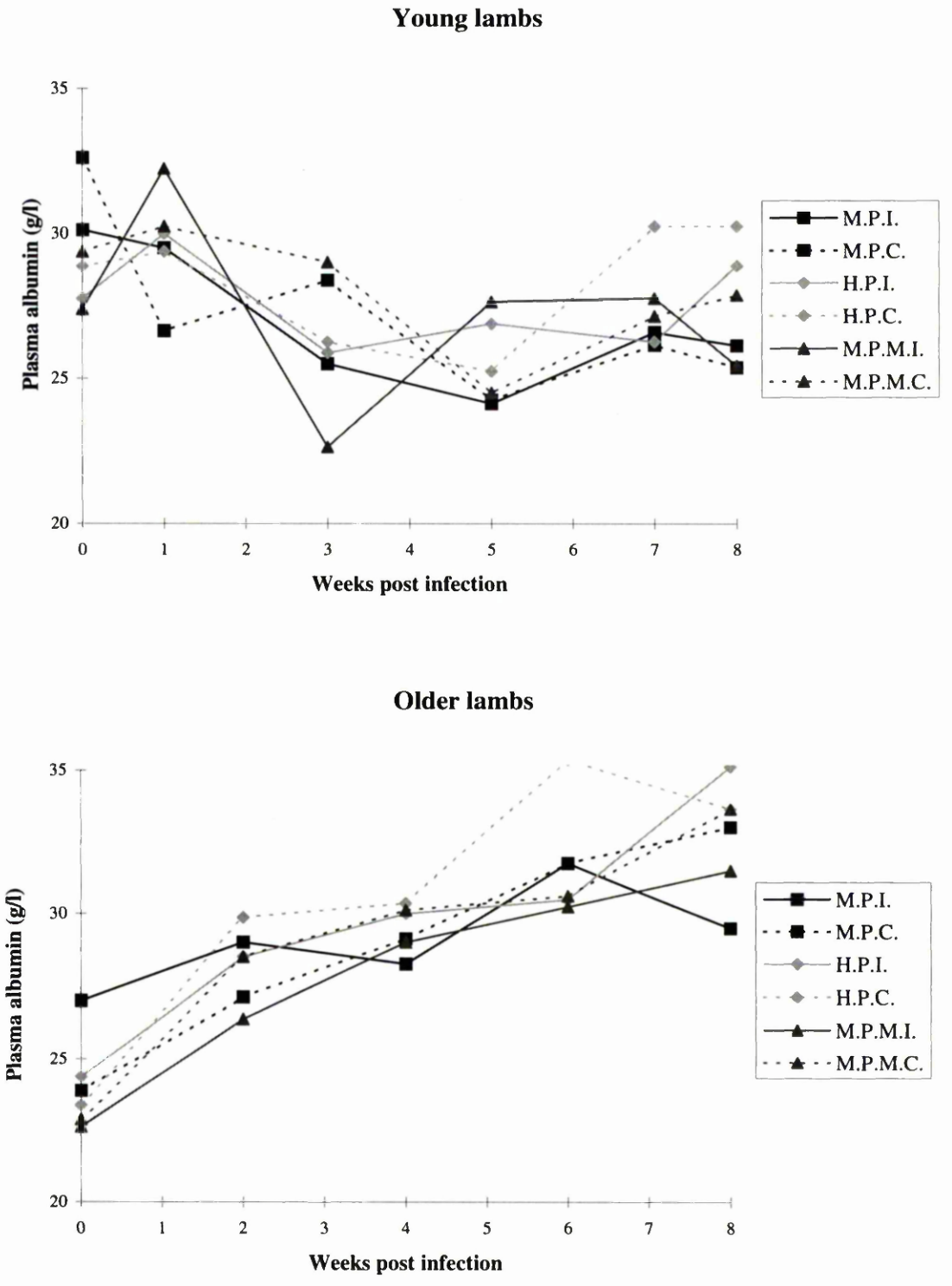


Figure 6.7 *Mean plasma albumin concentrations for the continuously challenged and uninfected lambs in experiments 1 and 2*

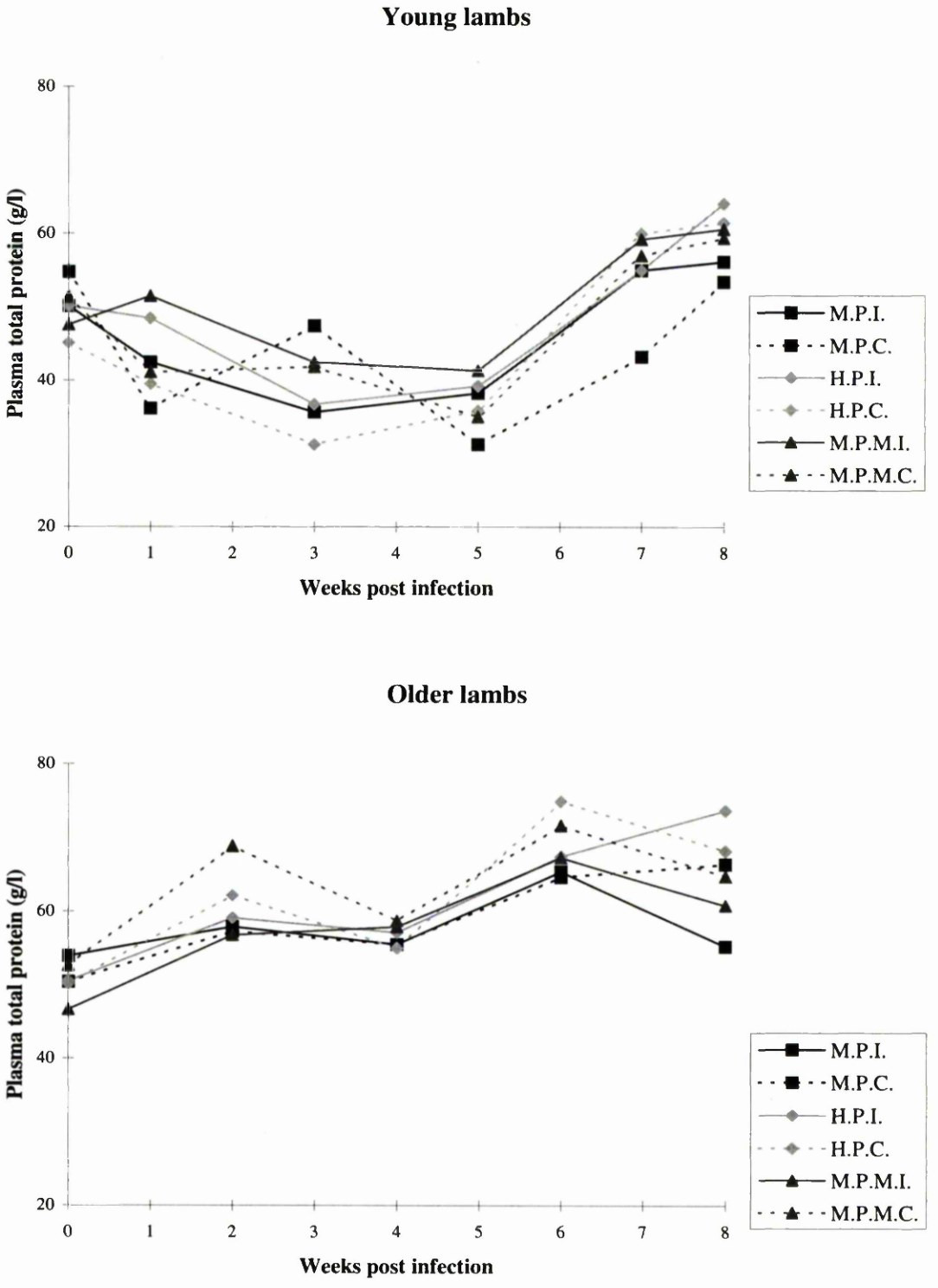


Figure 6.8 *Mean plasma total protein concentrations in the continuously infected and uninfected lambs in experiments 1 and 2*

6.3.5 Post challenge worm burdens

Figures 6.9, 6.10 and 6.11 show the individual worm burdens and stages of development of the worms recovered 10 days post challenge from the moderate protein, high protein and methionine supplemented groups respectively and Tables 6.1 and 6.2 show the individual and group average ± S.D. worm burdens from experiment 1 for the previously infected and challenge controls respectively. Tables 6.3 and 6.4 show the individual and group average ± S.D. worm burdens from experiment 2 for the previously infected and challenge controls respectively. Neither age nor diet had any significant effect upon worm establishment/persistence, except in groups 3 and 4, which were fed the high protein ration. In the previously infected animals the total worm burden was significantly lower in the younger lambs ($p<0.05$) whereas the reverse was true in the challenge controls, with the older lambs having the significantly reduced worm burden ($p<0.01$). Average establishment in the previously infected young lambs was reduced by 71.5% compared to a reduction of 58.2% in the older lambs. There were significant differences between the rate of development of the populations recovered from control and previously infected animals. There were significantly more early fifth stage worms in the challenge controls ($p<0.001$ experiments 1 and 2) whereas the previously infected animals carried significantly more early fourth stage ($p<0.001$ experiment 1 and $p<0.001$-0.05 experiment 2).

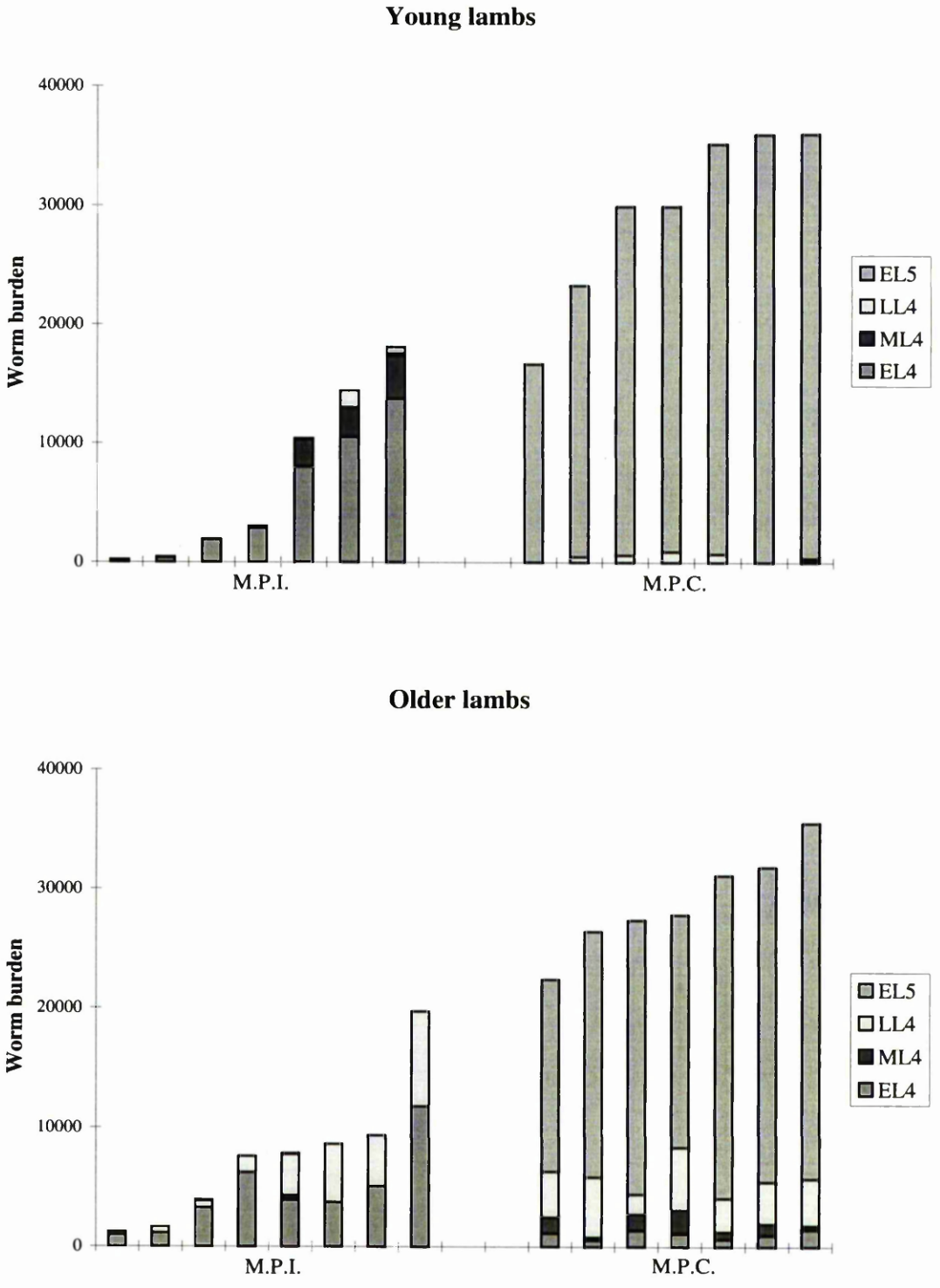


Figure 6.9 *Individual worm burdens for the lambs fed a moderate protein ration in experiments 1 and 2*

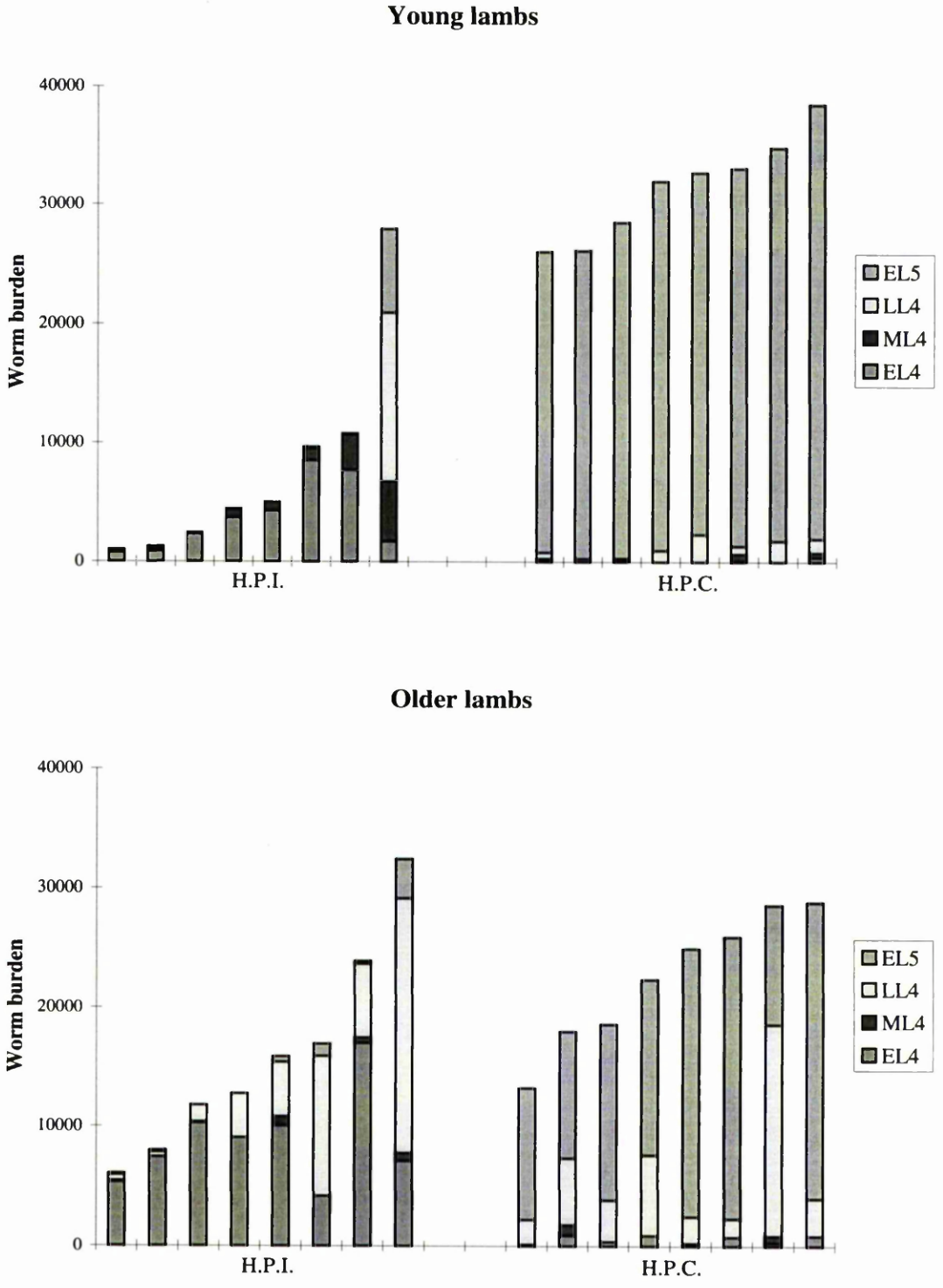


Figure 6.10 *Individual worm burdens for the lambs fed a high protein ration in experiments 1 and 2*

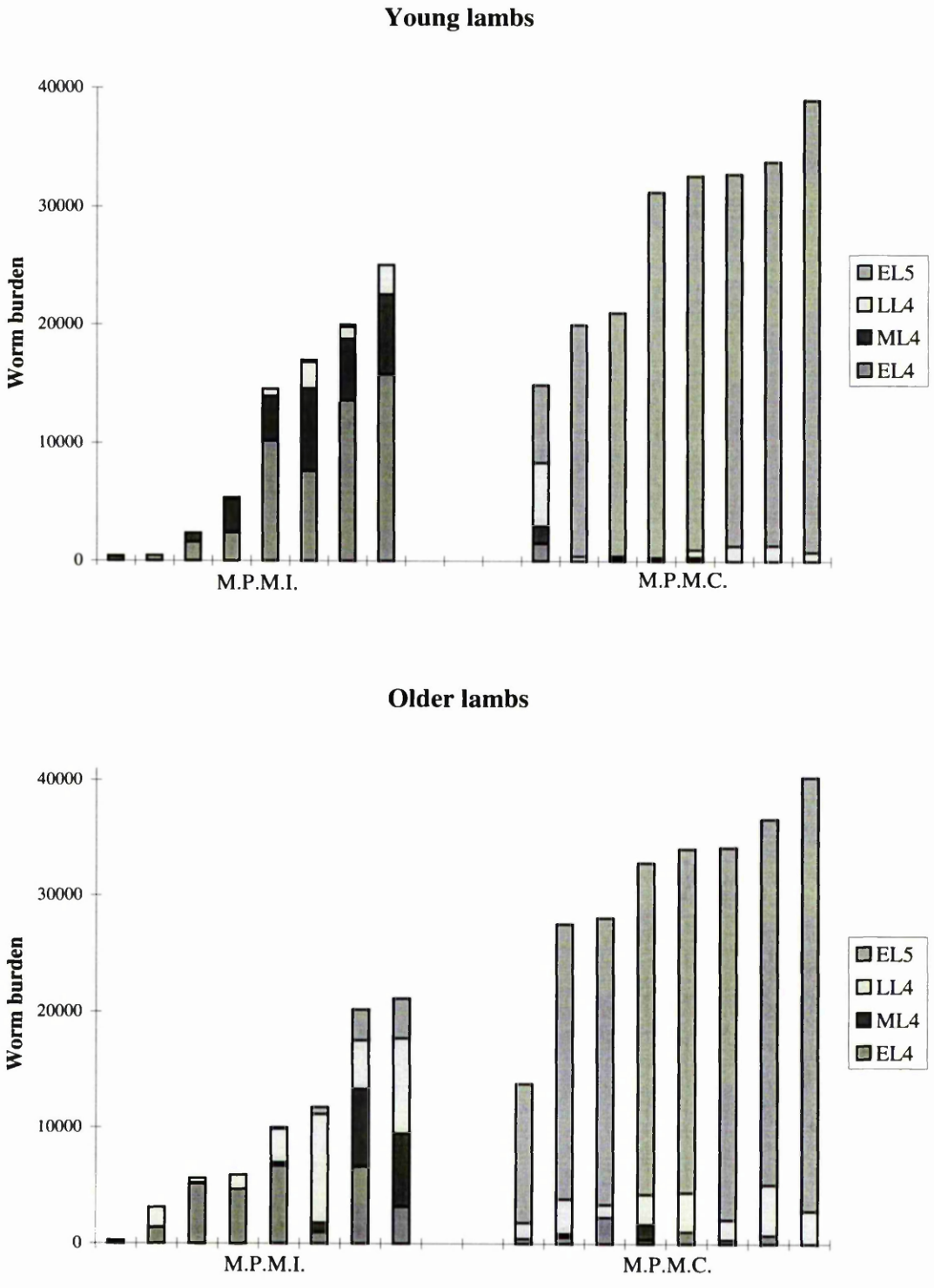


Figure 6.11 *Individual worm burdens for the lambs fed a moderate protein ration supplemented with 'protected' methionine in experiments 1 and 2*

Table 6.1 *Individual and group mean (± S.D.) worm burdens for the previously infected animals from experiment 1.*

Group	Lamb No.	EL_4	ML_4	LL_4	EL_5	Total
M.P.I.	1	0	67	0	133	200
	2	350	100	0	0	450
	3	1896	54	0	0	1950
	4	2863	187	0	0	3050
	5	8008	2288	0	104	10400
	6	10512	2448	1440	0	14400
	7	13718	3610	181	541	18050
	Mean	5335	1251	232	111	6929 ± 7287
H.P.I.	1	750	250	0	0	1000
	2	833	179	238	0	1250
	3	2300	100	0	0	2400
	4	3665	654	131	0	4450
	5	4243	707	0	0	4950
	6	8492	965	193	0	9650
	7	7668	3024	108	0	10800
	8	1674	5022	14229	6975	27900
	Mean	3703	1363	1862	872	7800 ± 8897
M.P.M.I.	1	300	0	50	50	400
	2	450	0	0	0	450
	3	1616	734	0	0	2350
	4	2430	2808	162	0	5400
	5	10185	3783	582	0	14550
	6	7650	6970	2210	170	17000
	7	13566	5187	998	199	19950
	8	15782	6763	2505	0	25050
	Mean	6497	3281	813	52	10644 ± 9673

Table 6.2 *Individual and group mean (± S.D.) worm burdens for the challenge control animals from experiment 1.*

Group	Lamb No.	EL_4	ML_4	LL_4	EL_5	Total
M.P.C.	1	0	0	0	16600	16600
	2	0	0	466	22834	23300
	3	0	0	598	29302	29900
	4	0	0	897	29003	29900
	5	0	0	703	34447	35150
	6	0	0	0	35950	35950
	7	0	360	0	35640	36000
	Mean	0	51	381	29111	29543 ± 7314
H.P.C.	1	0	260	520	25220	26000
	2	0	0	261	25839	26100
	3	0	284	0	28166	28450
	4	0	0	959	30991	31950
	5	0	0	2289	30411	32700
	6	0	661	661	31728	33050
	7	0	0	1740	33060	34800
	8	384	385	1153	36528	38450
	Mean	48	199	948	30243	31438 ± 4341
M.P.M.C.	1	1490	1490	5364	6556	14900
	2	0	0	399	19551	19950
	3	0	209	210	20531	20950
	4	0	312	0	30888	31200
	5	0	326	651	31573	32550
	6	0	0	1308	31392	32700
	7	0	0	1350	32400	33750
	8	0	0	779	38171	38950
	Mean	186	292	1258	26383	28119 ± 8384

Table 6.3 *Individual and group mean (± S.D.) worm burdens for the previously infected animals from experiment 2.*

Group	Lamb No.	EL_4	ML_4	LL_4	EL_5	Total
M.P.I.	1	1000	0	250	0	1250
	2	1155	0	495	0	1650
	3	3256	0	589	55	3900
	4	6191	0	1359	0	7550
	5	3900	390	3432	78	7800
	6	3698	0	4902	0	8600
	7	5049	0	4301	0	9350
	8	11820	0	7880	0	19700
	Mean	4509	49	2901	17	7475 ± 5853
H.P.I.	1	5368	122	488	122	6100
	2	7440	0	400	160	8000
	3	10266	118	1416	0	11800
	4	9052	0	3698	0	12750
	5	10017	795	4611	477	15900
	6	4237	0	11696	1017	16950
	7	17005	479	6227	239	23950
	8	7128	648	21384	3240	32400
	Mean	8814	270	6240	657	15981 ± 8646
M.P.M.I.	1	250	0	0	0	250
	2	1327	50	1723	0	3100
	3	5141	113	396	0	5650
	4	4701	0	1249	0	5950
	5	6700	300	2900	100	10000
	6	940	823	9400	587	11750
	7	6666	6666	4242	2626	20200
	8	3172	6345	8249	3384	21150
	Mean	3612	1787	3520	837	9756 ± 7643

Table 6.4 *Individual and group mean (± S.D.) worm burdens for the challenge control animals from experiment 2.*

Group	Lamb No.	EL_4	ML_4	LL_4	EL_5	Total
M.P.C.	1	1120	1344	3808	16128	22400
	2	528	264	5016	20592	26400
	3	1367	1368	1641	22974	27350
	4	1112	1946	5282	19460	27800
	5	623	623	2804	27101	31150
	6	956	955	3503	26435	31850
	7	1422	355	3910	29862	35550
	Mean	1018	979	3709	23222	28929 ± 4284
H.P.C.	1	132	0	2118	10988	13239
	2	899	899	5574	10608	17980
	3	372	0	3534	14694	18600
	4	896	0	6720	14784	22400
	5	0	250	2252	22523	25025
	6	780	0	1560	23660	26000
	7	286	572	17740	10015	28613
	8	866	0	3174	24811	28850
	Mean	529	215	5334	16510	22588 ± 5576
M.P.M.C.	1	414	0	1380	12006	13800
	2	551	276	3031	23693	27550
	3	2248	0	1124	24728	28100
	4	329	1314	2628	28580	32850
	5	1022	0	3405	29624	34050
	6	342	0	1708	32101	34150
	7	733	0	4398	31519	36650
	8	0	0	2821	37479	40300
	Mean	705	199	2562	27466	30931 ± 8082

6.3.6 Mucosal mast cell counts

Figure 6.12 shows the mean mucosal mast cell counts. Dietary supplementation had no effect upon mucosal mast cell counts which, as expected, were significantly increased ($p<0.001$-0.05) in the previously infected lambs compared to the challenge control lambs. The previously infected younger lamb mucosal mast cell counts were significantly higher than those of the older lambs ($p<0.01$-0.05).

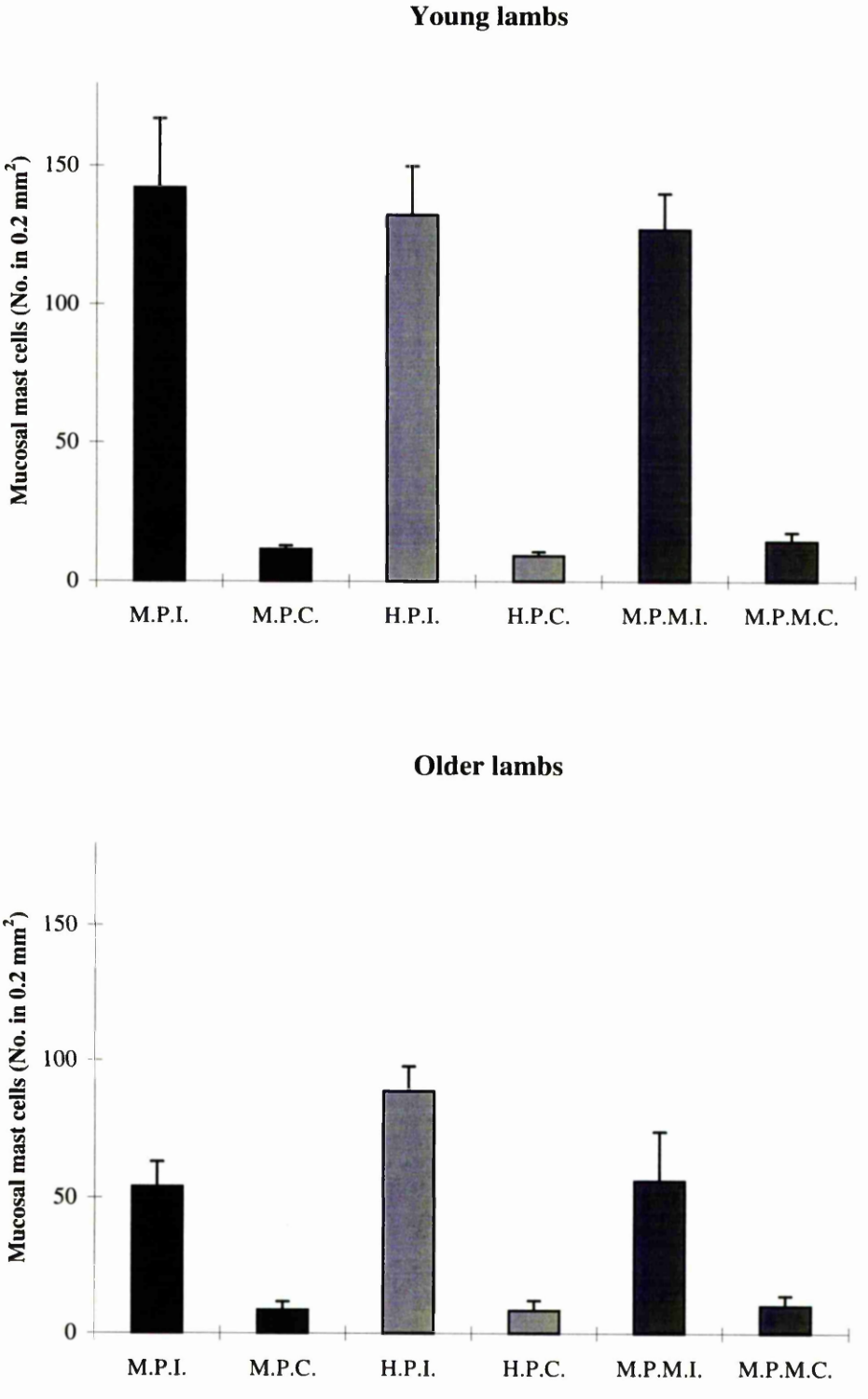


Figure 6.12 *Mucosal mast cell counts for the continuously infected and uninfected lambs in experiments 1 and 2*

6.4 Discussion

The results from these two studies provide no evidence of any enhancement of host immunity attributable to an increased availability of non-rumen degradable protein or methionine. Since the size and structure of the post-challenge worm burdens was similar in both studies it is apparent that there was no age effect upon the acquisition and expression of immunity, despite the young lambs having significantly higher mucosal mast cell counts. In a previous *T. circumcincta* study conducted at Moredun (Coop *et al.,* 1995) using the same challenge regime, there was a significant reduction in the post-challenge worm burdens and a significant increase in mast cell protease concentrations in 4 ½ month old lambs given a direct infusion of casein into the abomasum compared to lambs on a conventional diet (167 g kg^{-1} DM). The reasons for the different findings from the current and previous *Teladorsagia* studies are not clear, however these differences are not simply due to differences in total protein intake. In the former study, the average total crude protein intake was 341 gms CP per day (42.8gms as an infusion and the remainder in the diet) compared to mean total intakes of 288 gms (young lambs HP group) and 375 gms CP (older lambs HP group). It is likely that the direct infusion of casein resulted in higher levels of metabolisable protein availability. A high protein diet has been previously shown to increase host resistance against *Haemonchus contortus* (Abbott, Parkins & Holmes, 1986) and the intestinal parasite *T. colubriformis* (Kambara *et al.*, 1993; van Houtert *et al.*, 1995b). A study by Kambara *et al.* (1993) demonstrated some increased resistance in young lambs (2-6 months of age) but not in older lambs (8-12 months of age). Van Houtert *et al.* (1995b) using fishmeal as a rumen by-pass supplement at two levels (150g and 100 g day^{-1}) showed no effect upon mean egg count or worm burden over the first 70 days of the study. However, during the latter part of the study the rate of worm expulsion increased with increasing levels of fish meal supplementation. This study provides evidence, supported by other workers (e.g. Coop & Holmes, 1996; van Houtert & Sykes, 1996) that the rate of establishment of the initial worm burden is not affected by enhanced protein supplementation. It is the rate of expulsion of the worm population and effects on subsequent incoming larvae, causing either instant expulsion, inhibition,

stunting the growth of the adults and/or lowering the fecundity of the female worms which seems to be most influenced by the nutritional status of the host.

Although in the current study supplementation did not affect the acquisition or expression of immunity, protein supplementation did appear to have some effect on the pathophysiology of infection, an important component of resilience. There was a clear trend for both the high protein and, to a lesser extent, the methionine supplemented groups to have a greater daily growth rate than the moderate protein groups, although this was not statistically significant. A similar effect was seen in a study conducted at Moredun using *T. colubriformis* infection in young lambs, where there was a significant enhancement in the growth rate of the high protein groups and in lambs supplemented with methionine (Coop, Richardson, Jackson, Bartley & Jackson, 1998). Wool growth, another indicator of resilience, was also significantly improved in the high protein and methionine supplemented groups in that study. In the current study, plasma pepsinogen concentrations were lower in the high protein groups and there was increased anorexia in the moderate protein young lamb group but not in the two supplemented groups. Similar findings have been reported from a previous *Haemonchus* study (Abbott *et al.*, 1986) where lambs on the poorest plane of nutrition were more prone to becoming anorexic.

The results from these studies confirm the important role that parasite specific characteristics play in influencing the potential of a 'nutrition mediated' approach to control of gastrointestinal nematodes species such as *Teladorsagia*, whose principal effect is a reduction in voluntary food intake of growing lambs, rather than an effect upon efficiency of nutrient utilisation/absorption. This is particularly the case where the key aim of supplementation is to enhance the rate of acquisition of immunity and thus reduce pasture contamination. For species such as *Teladorsagia* however, the 'nutrition mediated' approach may still play an important role in maintaining acquired immunity at times of climatic stress and during the peri--parturient relaxation in immunity, as has been recently demonstrated by Donaldson *et al.*, (1997, 1998). It was shown that the periparturient rise in faecal egg count, usually associated with parturition and lactation, can be reduced by increasing the level of metabolisable protein in the diet of the mature ewe. This in turn has implications for the young grazing lamb, as the major source of infection for the

lamb are the eggs excreted onto the pasture by the periparturient ewe (Dunsmore, 1965; Familton, 1991).

Chapter 7

The use of computer generated mathematical models in the understanding and prediction of parasite epidemiology

7.1 Introduction

The host-parasite relationship is complex and relies on many different factors, such as the hosts' immune status, age, plane of nutrition and genetically determined resistance, rate of larval intake, weather and anthelmintic strategy. Similarly, the relationships within populations of both host and parasite can be complex, as diverse genetic populations exist within these groups. Generic (Smith, 1989, 1990; Leathwick, Barlow &Vlassoff, 1992; Leathwick, Vlassoff & Barlow, 1995) and specific (Barnes & Dobson, 1990 (*T. colubriformis);* Dobson, personal communication, (*Ostertagia)*; Gettinby, Bairden, Armour & Benitez-Usher, 1989 (*Ostertagia)*; Paton, Thomas & Waller, 1984 (*Ostertagia)*; Beecham, Wright, Gettinby, Coop & Jackson, 1995 (*Ostertagia*)) mathematical models have been developed to model important elements of the host/parasite relationship including infra and suprapopulation dynamics, aspects of host immunity and disease and the selection of anthelmintic resistance (Barnes and Dobson, 1990; Smith, 1990). The current models are relatively strong as far as the suprapopulation dynamics are concerned, being able to model challenge from pasture attributable to seasonal effects of climate upon the development and translation of the free living stages reasonably accurately. However, these models all have limitations, particularly with regard to their descriptive/modelling capacity for the acquisition and expression of immunity and in predicting subclinical disease. Most of the current models are relatively unsophisticated in predicting the density dependant effects upon productivity using mortality as a marker of the impact of infection.

Despite their acknowledged weaknesses, computer models have been used to predict the development of anthelmintic resistance and as a tool to devise strategies to delay its onset. They have also been used to provide predictions about the impact of alternative control strategies such as genetically resistant hosts, vaccines and nematophagous fungi influence, and how they influence both the host and parasite population dynamics (Barnes *et al.*,1995). These strategies can be modelled over long periods of time, enabling effective sustainable solutions to be investigated.

To date, none of the models has sufficient immunoregulatory capacity to enable them to be used to in the direct investigation of the impact of protein supplementation. However, specific models with user friendly front ends' such as

the *T.colubriformis* and *Ostertagia* models developed by CSIRO scientists, enable different stocking and pasture management strategies to be evaluated in combination with anthelmintic control strategies. It is possible within the *T.colubriformis* and *T.circumcincta* WormWorld™ computer models developed by Barnes and Dobson to 'administer' a bolus of known efficacy to different classes of animal. In this way it is possible to simulate approximately the effects of reducing contamination though supplementation, albeit very crudely and only at a constant rate of efficacy.

Although the supplementation studies described in Chapter 6 used a lamb model to investigate the impact of specific amino acid supplementation on resistance and resilience against *T.circumcincta,* supplementation of grazing lambs poses practical difficulties. Protein or specific amino acid supplementation is most likely to be used in the field in the UK in order to minimise the impact of the ewe peri-parturient rise (PPR). Studies in New Zealand (Donaldson, 1997) have shown that supplementation with non-rumen degradable protein (NRDP) can ameliorate the effects of the PPR, particularly in twin bearing animals. The aim of this experiment was to use UK weather and farm management data generated in a study at Moredun Research Institute (Barrett, PhD Thesis, Glasgow University 1997) in the WormWorld™ *T.circumcincta* computer model to investigate the impact of ewe NRDP supplementation on the dynamics of challenge from pasture.

7.2 Materials and methods

7.2.1 Meteorological data

Meterological data were kindly supplied by the Rothamsted Institute, situated in Hertfordshire, UK. Daily values for the minimum and maximum temperature, rainfall and evaporation were sent to Drs. Robert Dobson and Elizabeth Barnes at CSIRO in Armidale, Australia. The data were parameterised for *T. circumcincta* and this meteorological file was then used in the WormWorld™ model.

7.2.2 Management Strategies

Two management strategies were compared. The first management strategy, a non-suppressive regime (Strategy 1), was used in a three year epidemiological study on benzimidazole resistance (Barrett, 1997) at one of the Institute farms, Firth Mains.

The second employed a grazing management strategy designed to limit infection, a 'dose and move' strategy (Strategy 2).

In both simulations, a total of 120 ewes (flock 1) lambed on day 73 of the simulation (mid-March), each ewe having twin lambs. The ewes were turned out with their lambs onto Paddock 1, on day 105 (mid-April). The ewes were given a 'flushing' drench on day 282 (early October) prior to going to the tup. They were housed on day 335 (early December) and drenched again on day 336.

In the non-suppressive regime, the lambs were weaned on day 230 (mid-August), and the ewes were moved onto Paddock 2. The lambs were drenched twice, once on day 140 (mid-May) and on day 230 (mid-August) and were sold on day 268 (late September).

In the 'dose and move' regime, on day 170 (mid June) the lambs were drenched with anthelmintic and moved to Paddock 2, a clean pasture, while the ewes remained on Paddock 1. Flock 2, consisting of half of the lambs were sold 'early' as fat lambs on day 210 (end of July), and received two anthelmintic drenches on days 135 (mid May) and 170 (mid June). Flock 3, the other half of the lamb flock, were sold 'late' as store lambs on day 330 (mid November) and received three anthelmintic drenches on days 135 (mid May), 170 (mid June) and 205 (mid July).

All simulations were run over a ten year period, using the same 2 paddocks, allowing the effect of the carry over between grazing seasons to be investigated.

In order to simulate a reduction in ewe egg count attributable to supplementation an anthelmintic bolus was 'given' to the ewes on day 79 (March 20th), effective for 60 days, i.e. effective until day 139 (May 19th). Ten year simulations were run at 5 different efficacies, i.e. 0, 25, 50, 75 and 100% effectiveness. Data on ewe and lamb faecal egg output and worm burdens were obtained from the simulations as were pasture larval contamination data.

7.3 Results

The effect of the strategy and bolus treatment upon faecal egg output is shown in Figures 7.1-10 and summarised in Table 7.1, the mean worm burdens are shown in Figures 7.11-20 and summarised in Table 7.2, and the herbage contamination for paddocks 1 and 2 are shown in Figures 7.21-30 and summarised in Table 7.3. The model provided no mortalities in either of the flocks of lambs in any of the simulations.

Table 7.1. *The effect of strategy and bolus treatment upon mean egg count over a ten year simulation*

Bolus efficacy (%)	Mean egg count (epg)			
	Flock 1 (ewes)		Flock 2/3 (lambs)	
	Strategy 1	Strategy 2	Strategy 1	Strategy 2
100	1	4	1	18
75	3	8	3	19
50	4	14	4	21
25	6	16	4	12
0	8	22	8	12

Table 7.2. *The effect of strategy and bolus treatment upon mean worm burden over a ten year simulation*

Bolus efficacy (%)	Mean worm burden			
	Flock 1 (ewes)		Flock 2/3 (lambs)	
	Strategy 1	Strategy 2	Strategy 1	Strategy 2
100	5	30	20	279
75	24	69	56	302
50	37	141	75	348
25	50	220	110	356
0	80	220	185	356

Table 7.3. *The effect of strategy and bolus treatment upon mean larval pasture counts over a ten year simulation*

Bolus efficacy (%)	Mean pasture larval count (L3/kg DM)			
	Paddock 1		Paddock 2	
	Strategy 1	Strategy 2	Strategy 1	Strategy 2
100	40	54	14	600
75	141	147	58	633
50	194	219	76	670
25	263	250	92	698
0	389	325	120	689

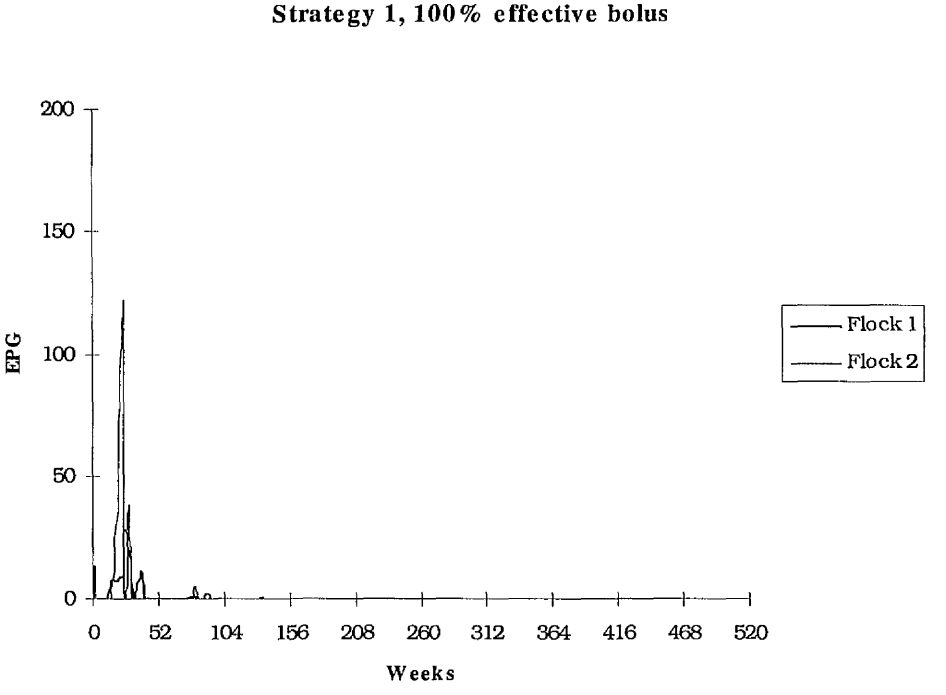


Figure 7.1. *Mean egg counts for Flocks 1 and 2 modelled using Strategy 1 and 100% effective bolus*

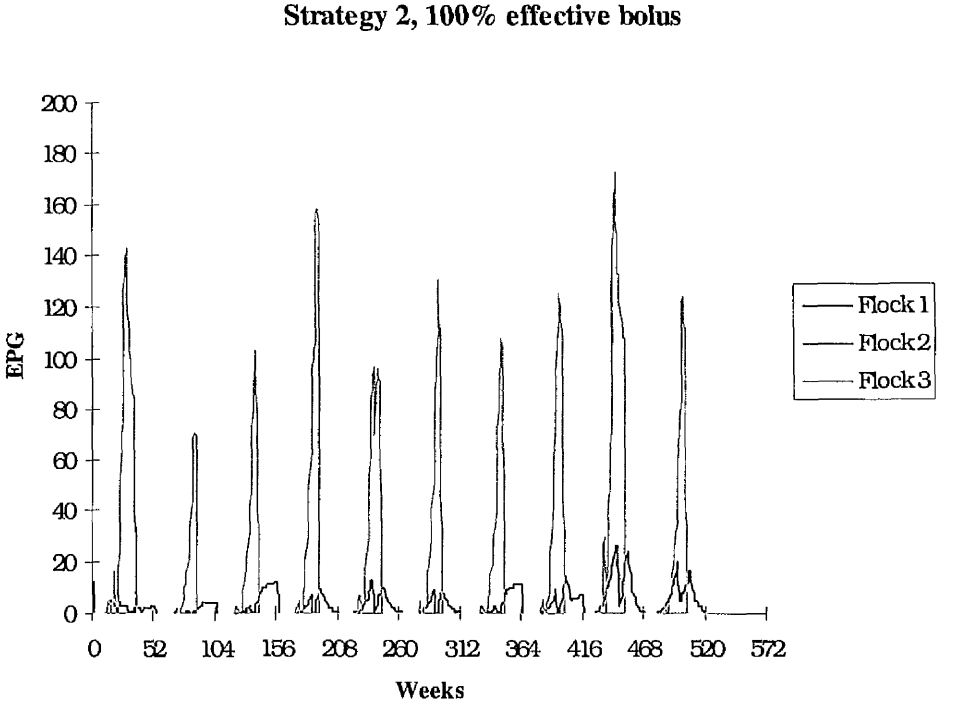


Figure 7.2 *Mean egg counts for Flocks 1, 2 and 3 modelled using Strategy 2 and 100% effective bolus*

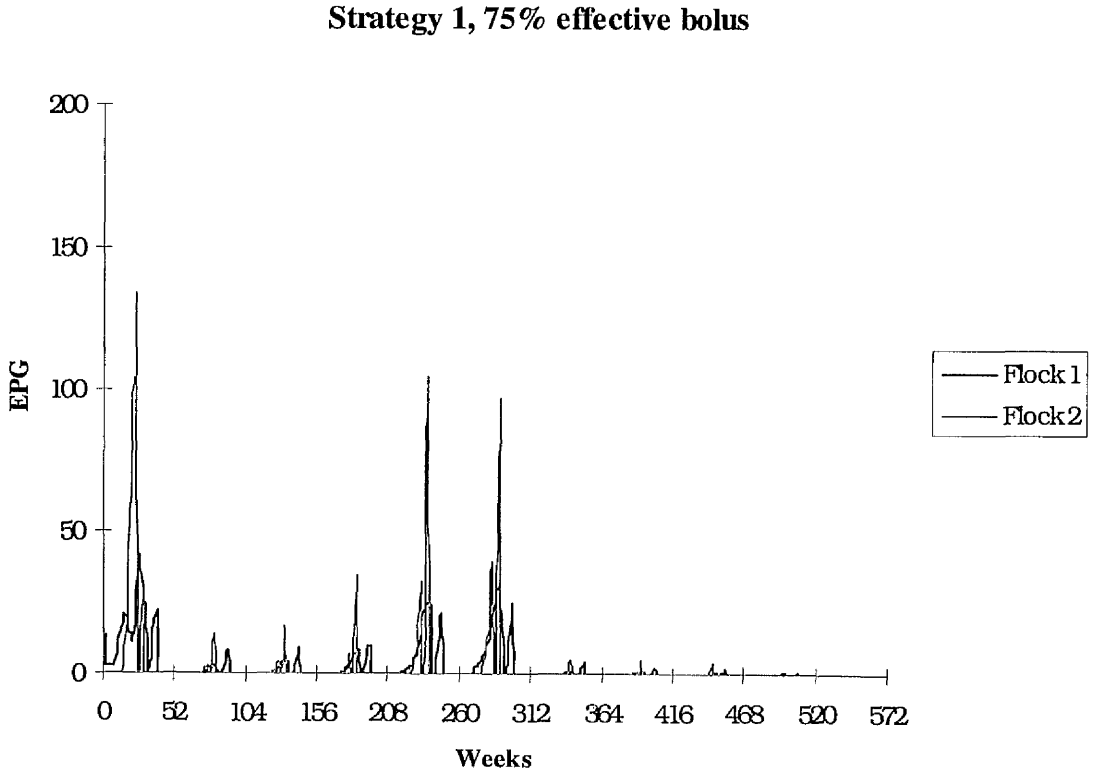


Figure 7.3 *Mean egg counts for Flocks 1 and 2 modelled using Strategy 1 and 75% effective bolus*

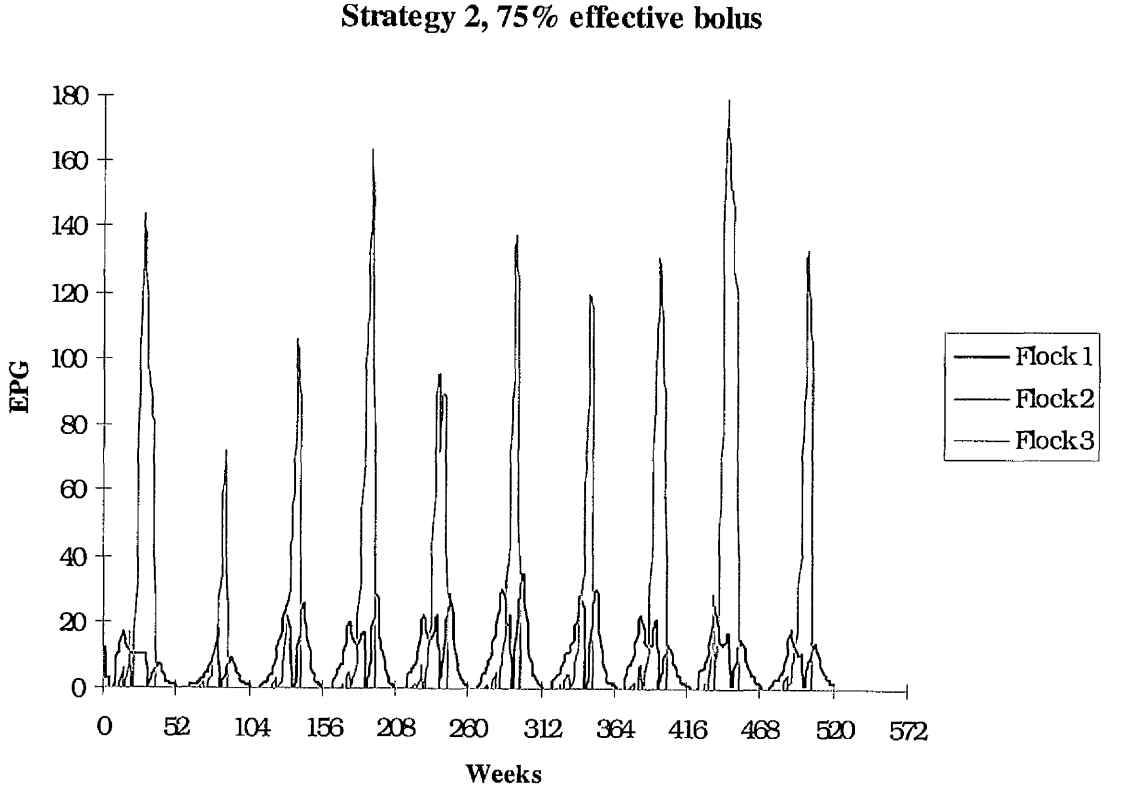


Figure 7.4 *Mean egg counts for Flocks 1, 2 and 3 modelled using Strategy 2 and 75% effective bolus*

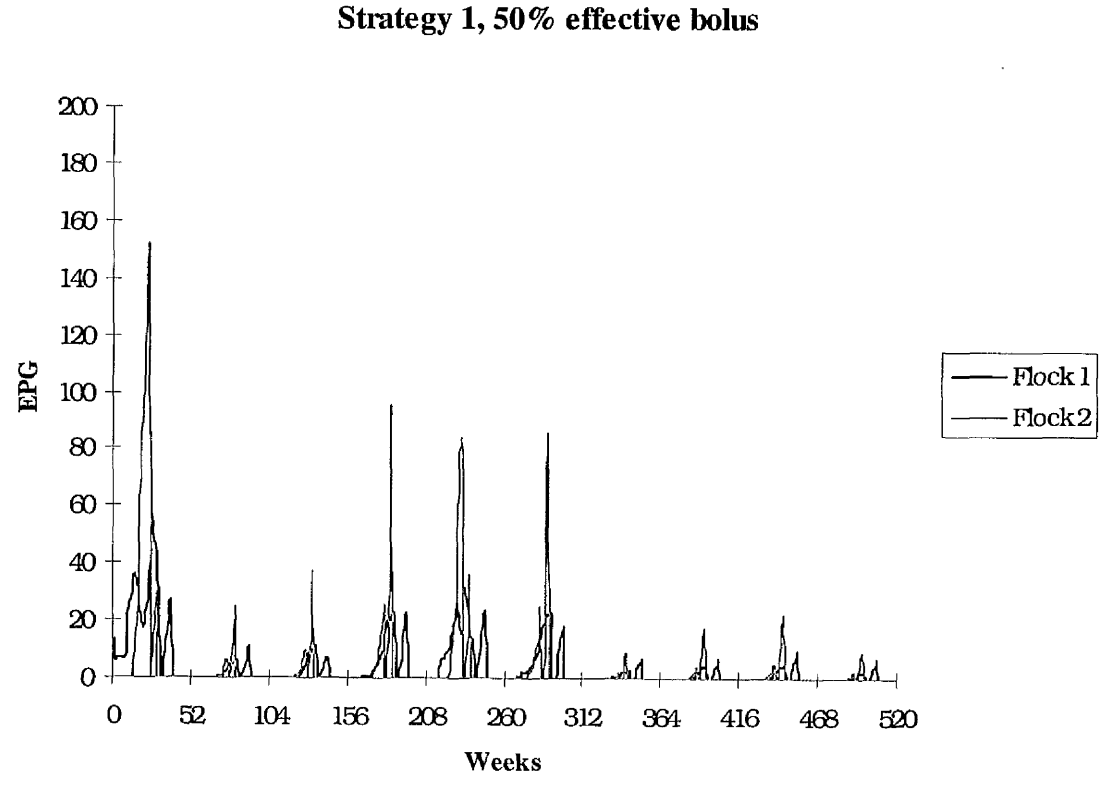


Figure 7.5 *Mean egg counts for Flocks 1 and 2 modelled using Strategy 1 and 50% effective bolus*

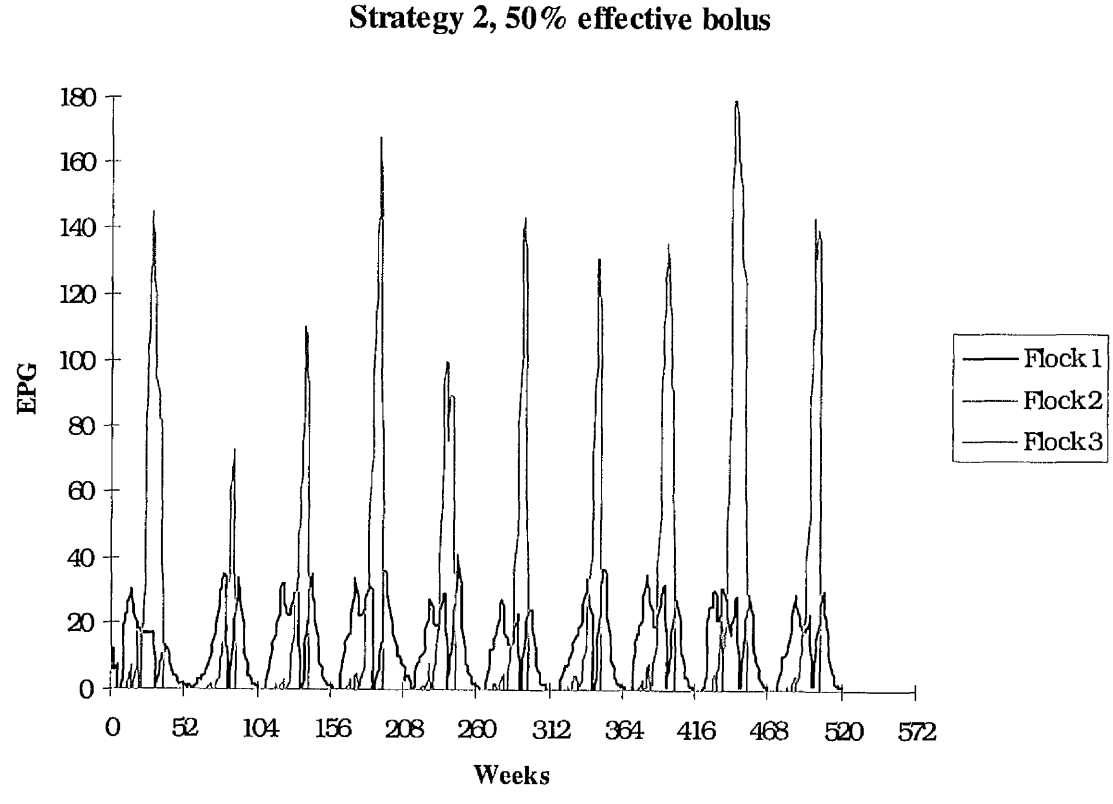


Figure 7.6 *Mean egg counts for Flocks 1, 2 and 3 modelled using Strategy 2 and 50% effective bolus*

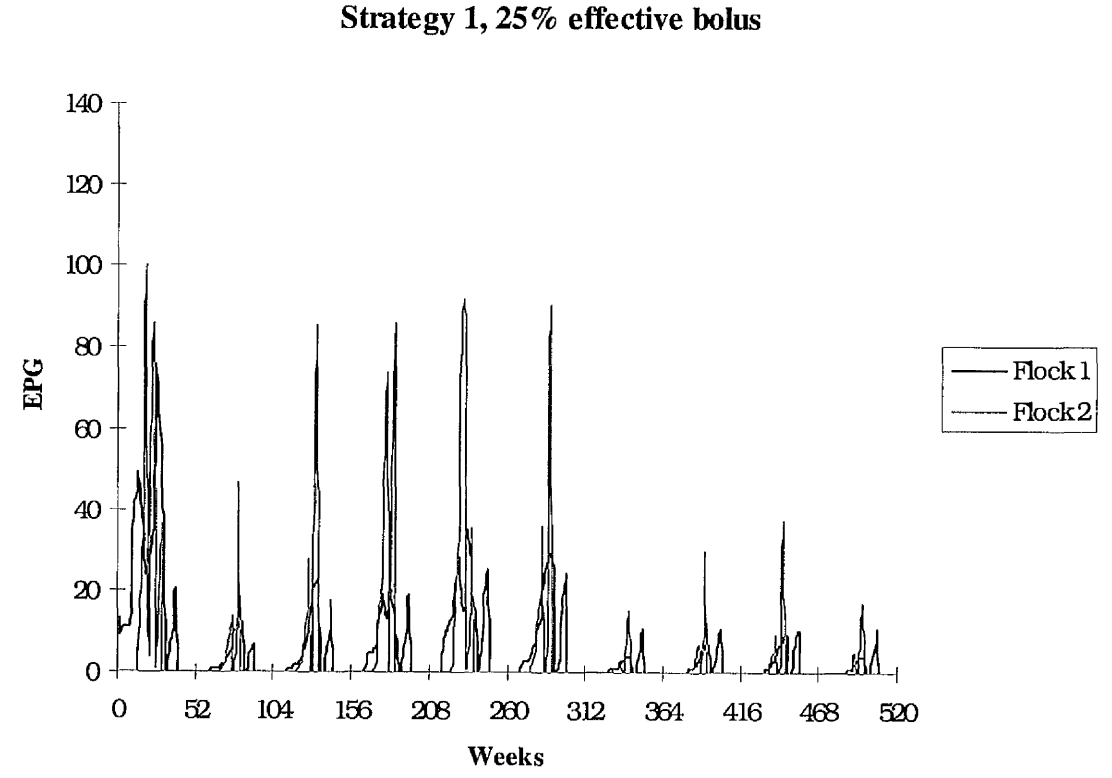


Figure 7.7 *Mean egg counts for Flocks 1 and 2 modelled using Strategy 1 and 25% effective bolus*

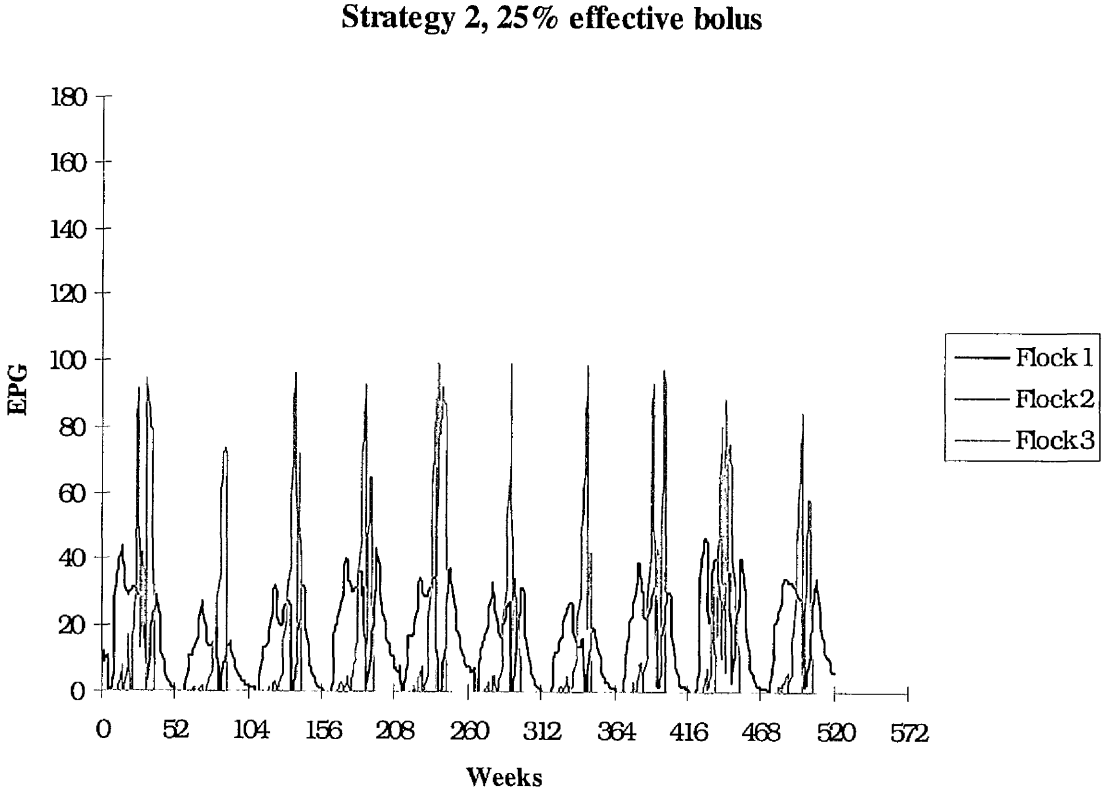


Figure 7.8 *Mean egg counts for Flocks 1, 2 and 3 modelled using Strategy 2 and 25% effective bolus*

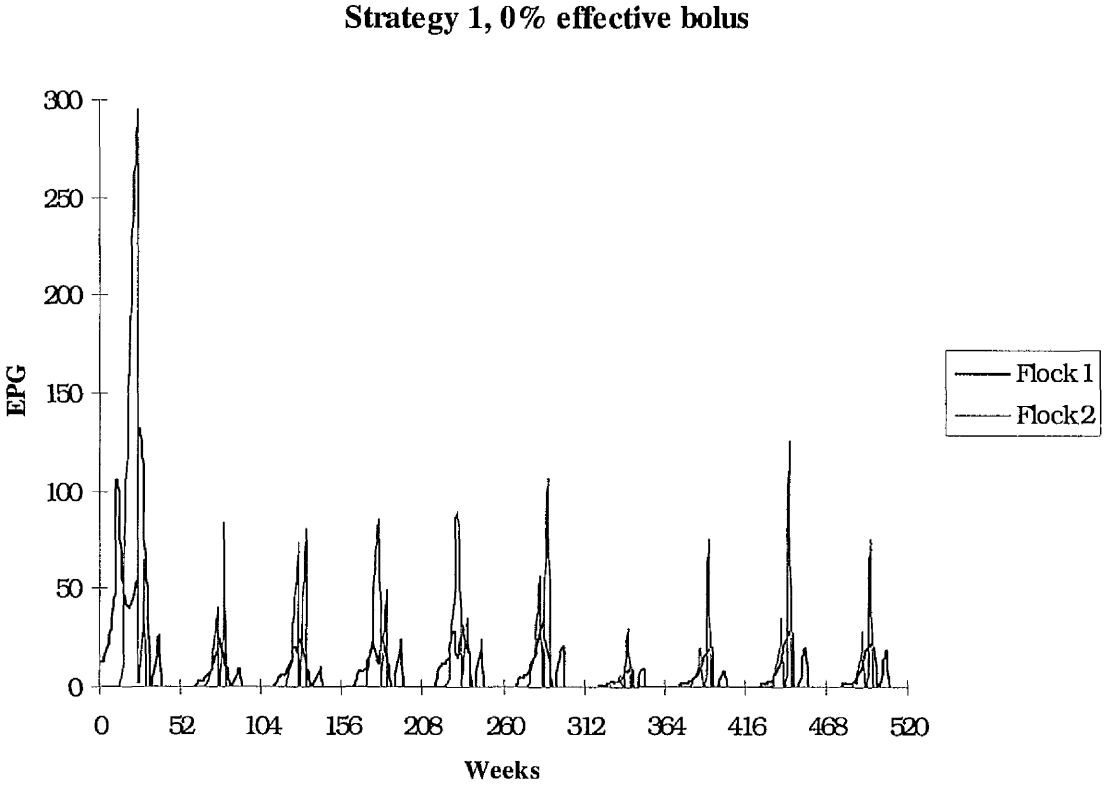


Figure 7.9 *Mean egg counts for Flocks 1 and 2 modelled using Strategy 1 and 0% effective bolus*

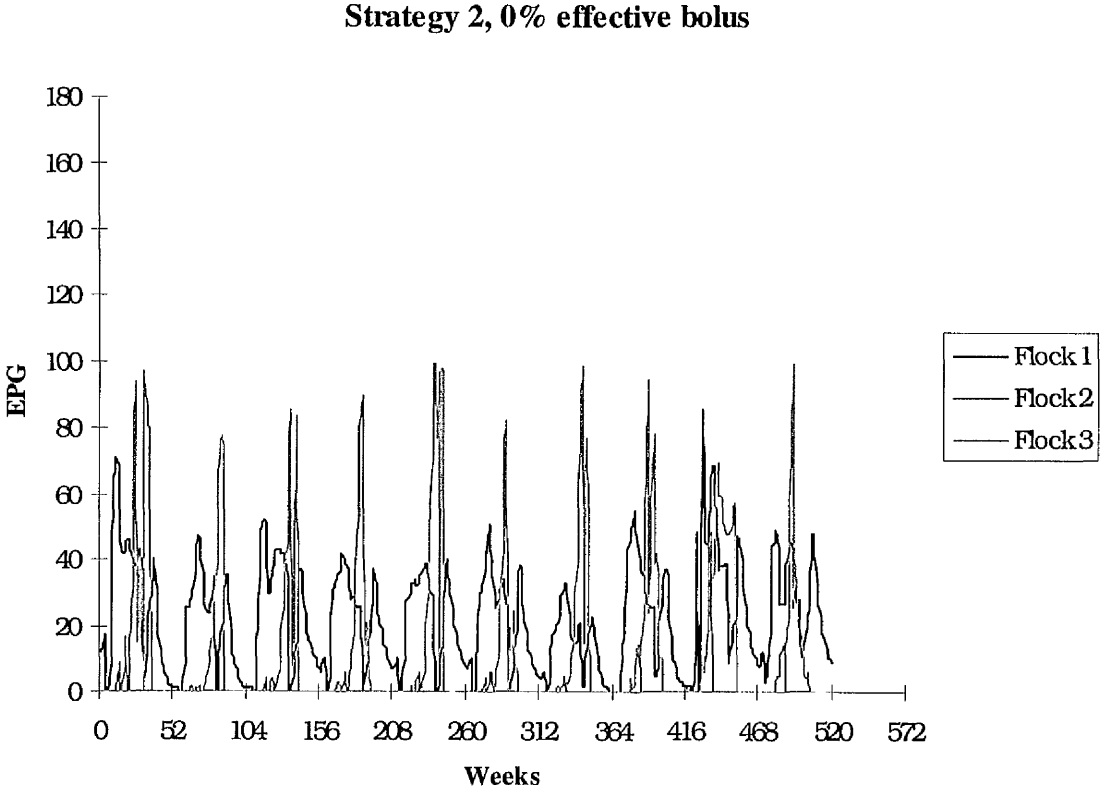


Figure 7.10 *Mean egg counts for Flocks 1, 2 and 3 modelled using Strategy 2 and 0% effective bolus*

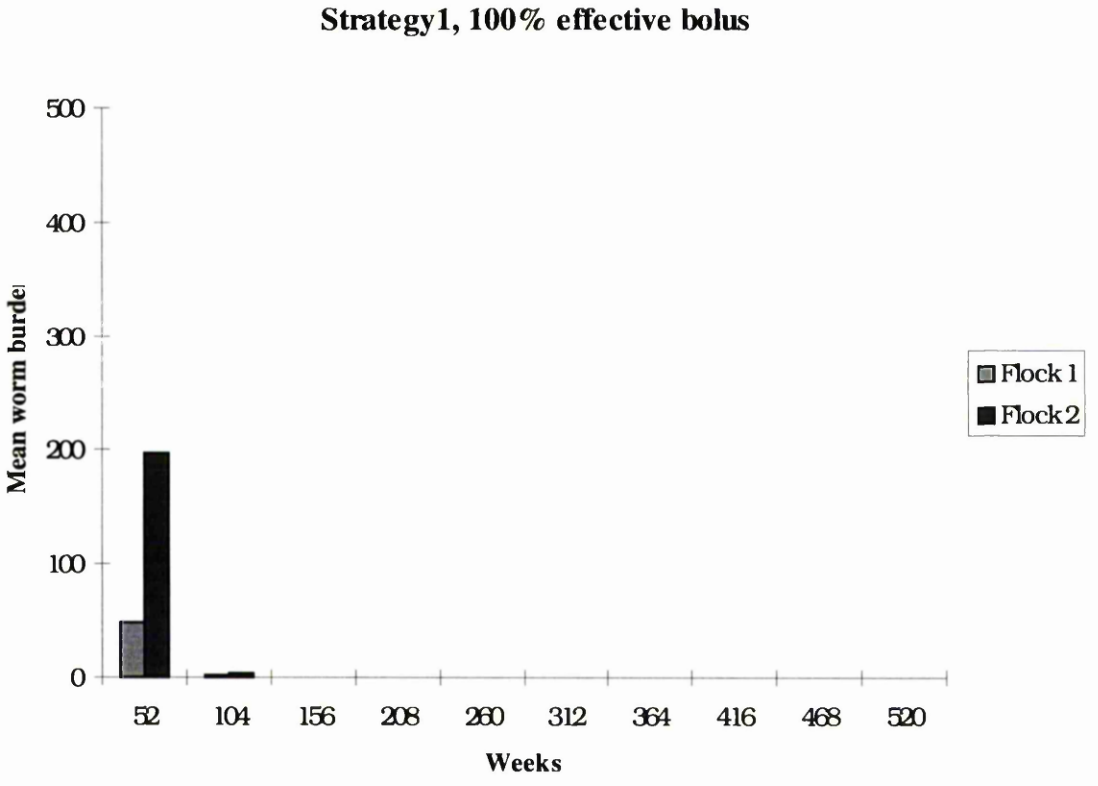


Figure 7.11 *Mean worm burdens for Flocks 1 and 2 modelled using Strategy 1 and 100% effective bolus*

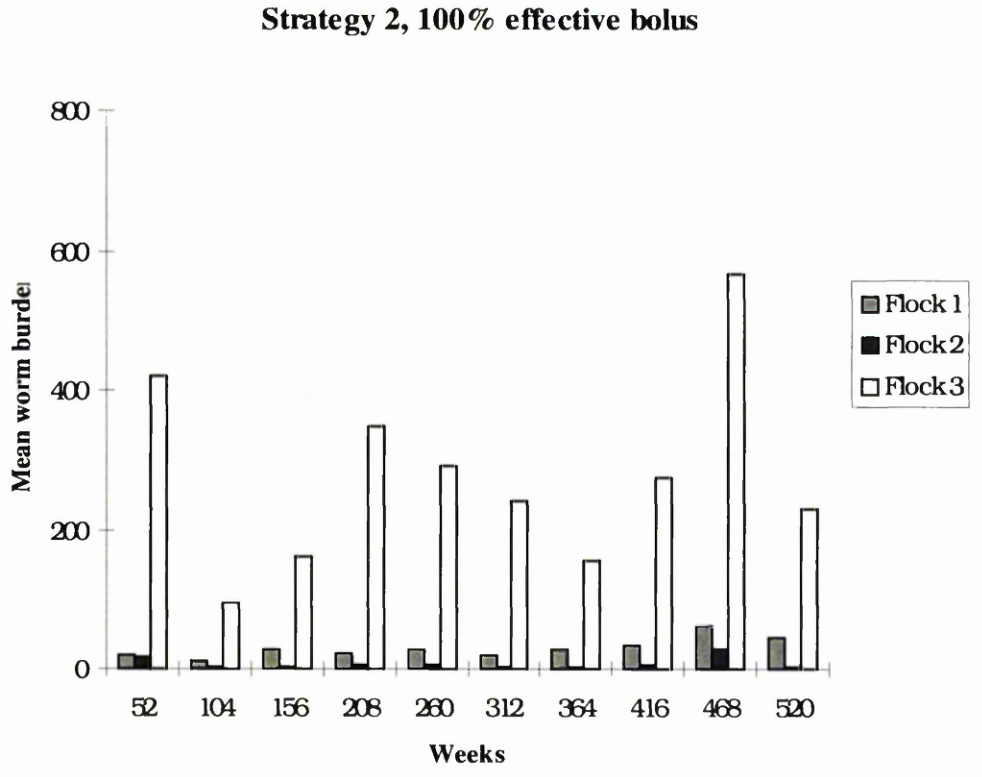


Figure 7.12 *Mean worm burdens for Flocks 1, 2 and 3 modelled using Strategy 2 and 100% effective bolus*

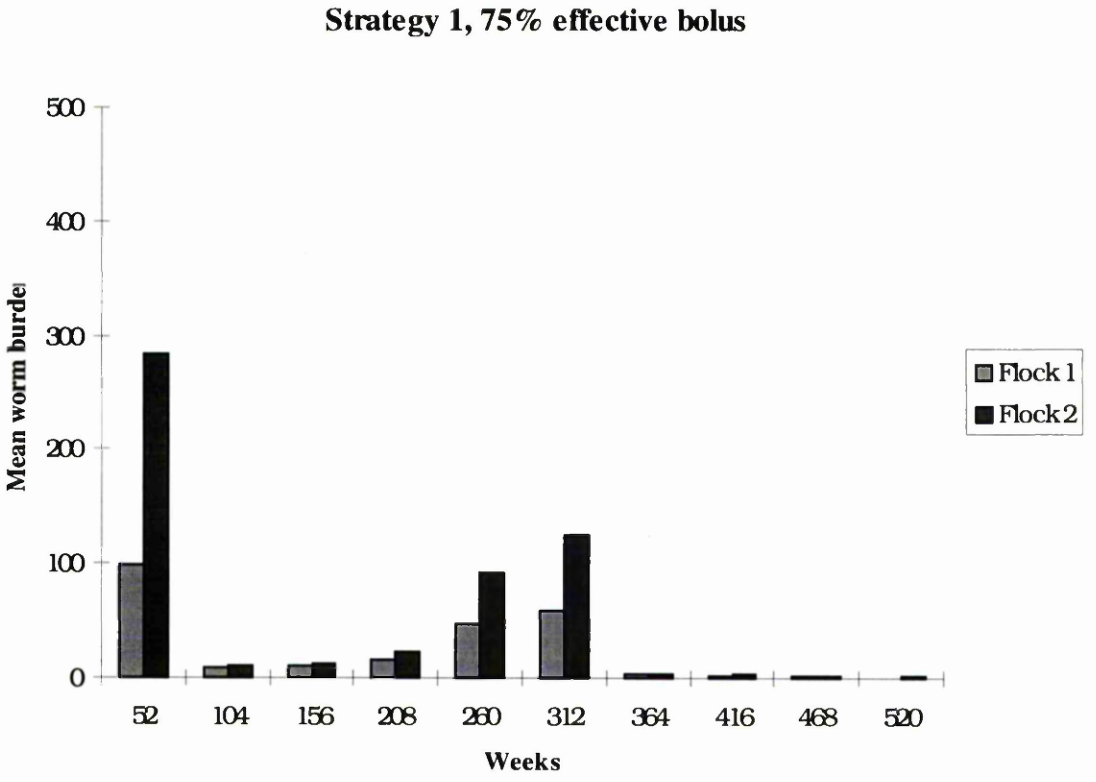


Figure 7.13 *Mean worm burdens for Flocks 1 and 2 modelled using Strategy 1 and 75% effective bolus*

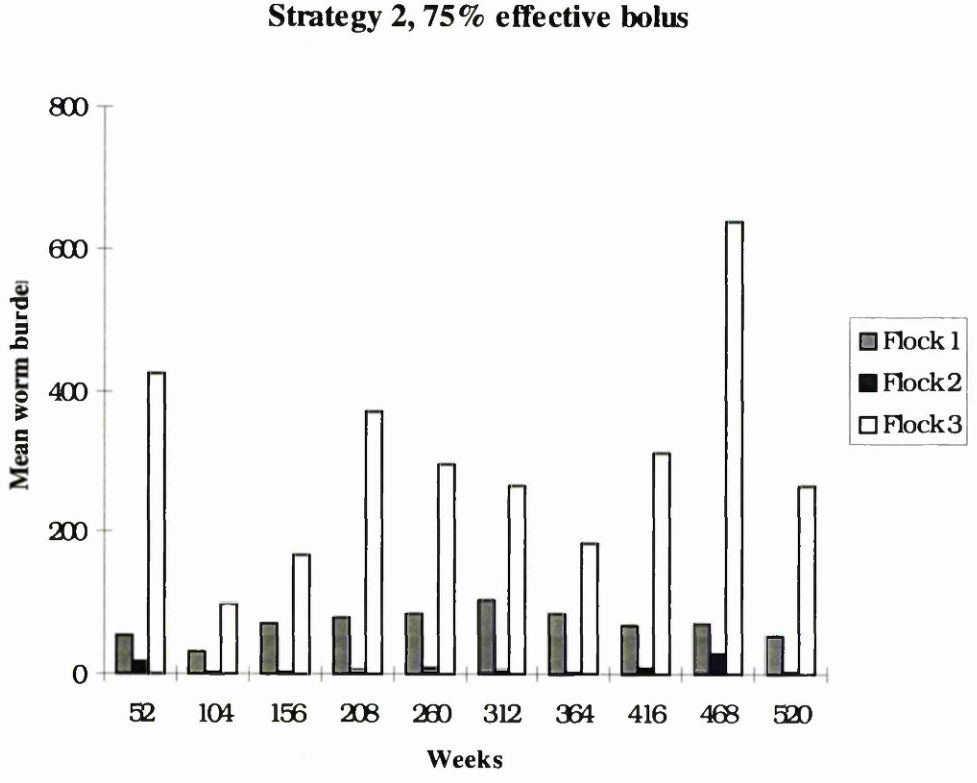


Figure 7.14 *Mean worm burdens for Flocks 1, 2 and 3 modelled using Strategy 2 and 75% effective bolus*

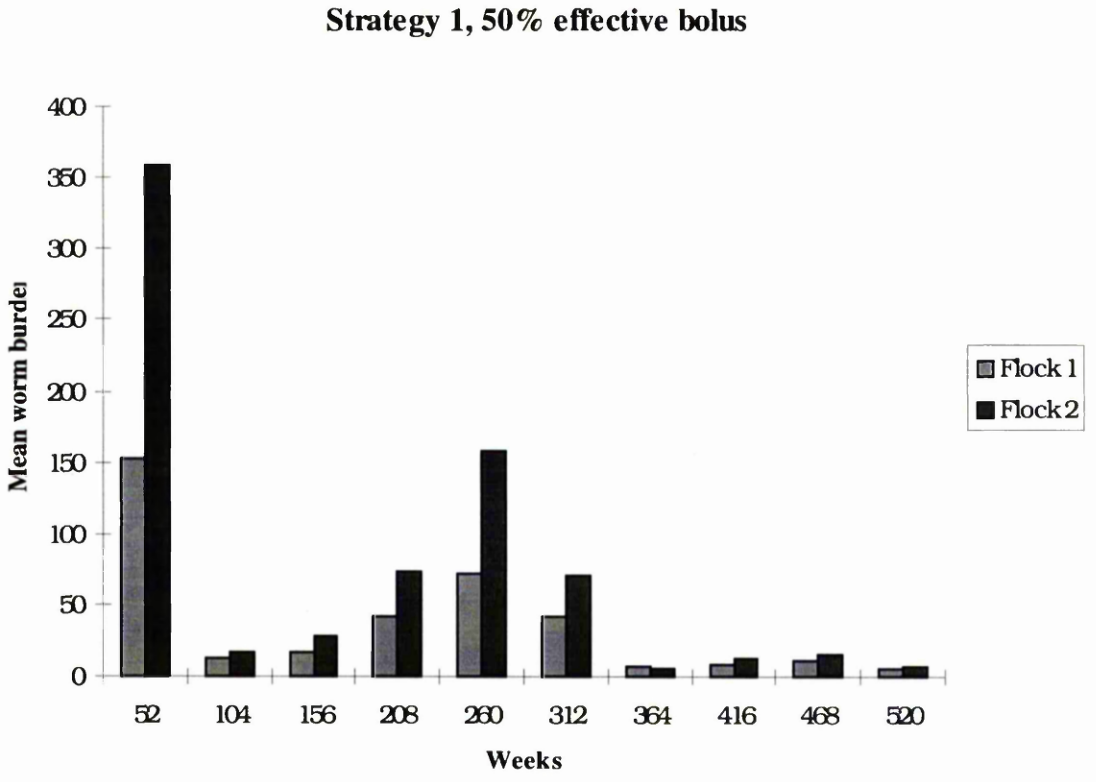


Figure 7.15 *Mean worm burdens for Flocks 1 and 2 modelled using Strategy 1 and 50% effective bolus*

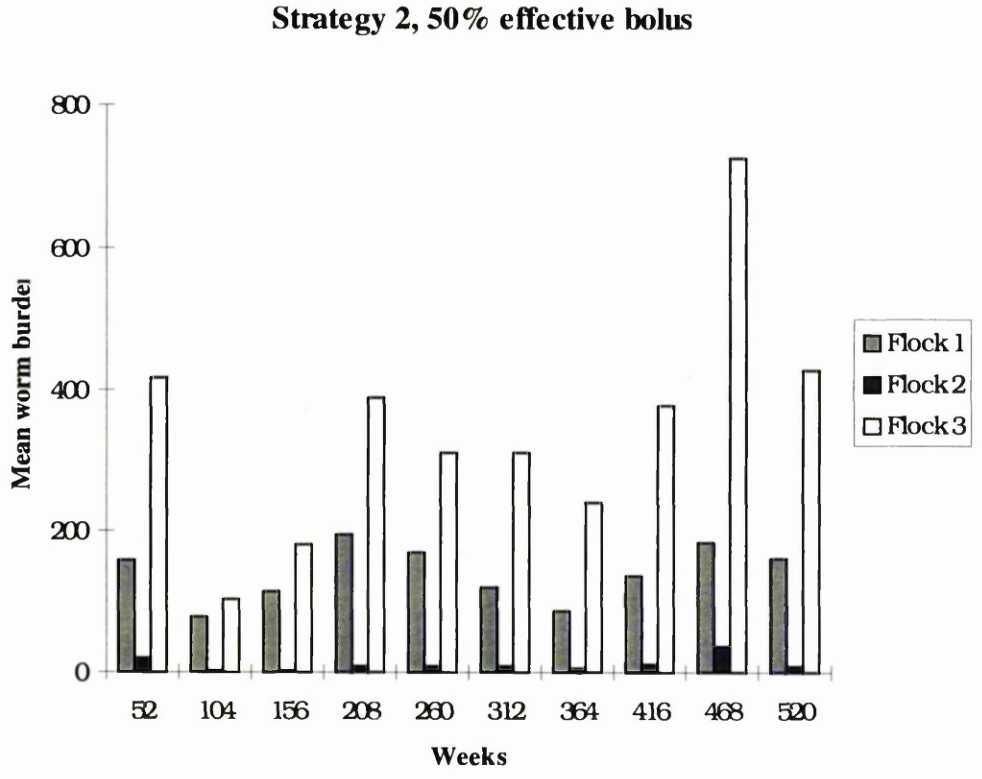


Figure 7.16 *Mean worm burdens for Flocks 1, 2 and 3 modelled using Strategy 2 and 50% effective bolus*

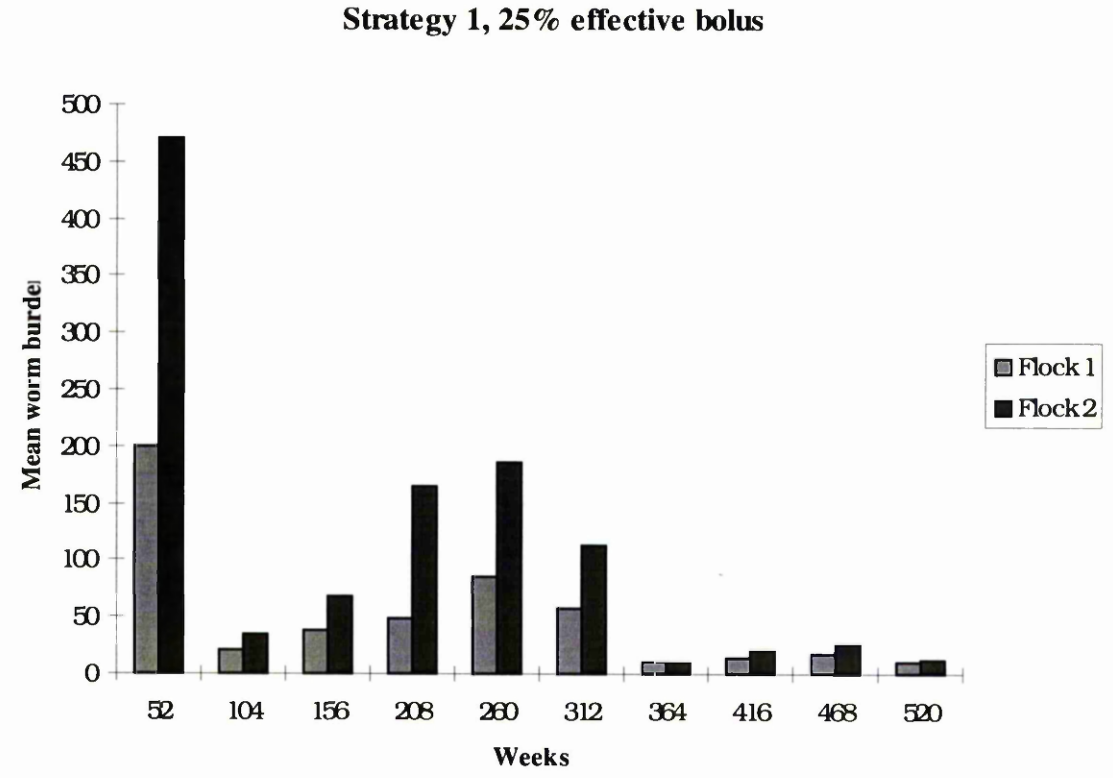


Figure 7.17 *Mean worm burdens for Flocks 1 and 2 modelled using Strategy 1 and 25% effective bolus*

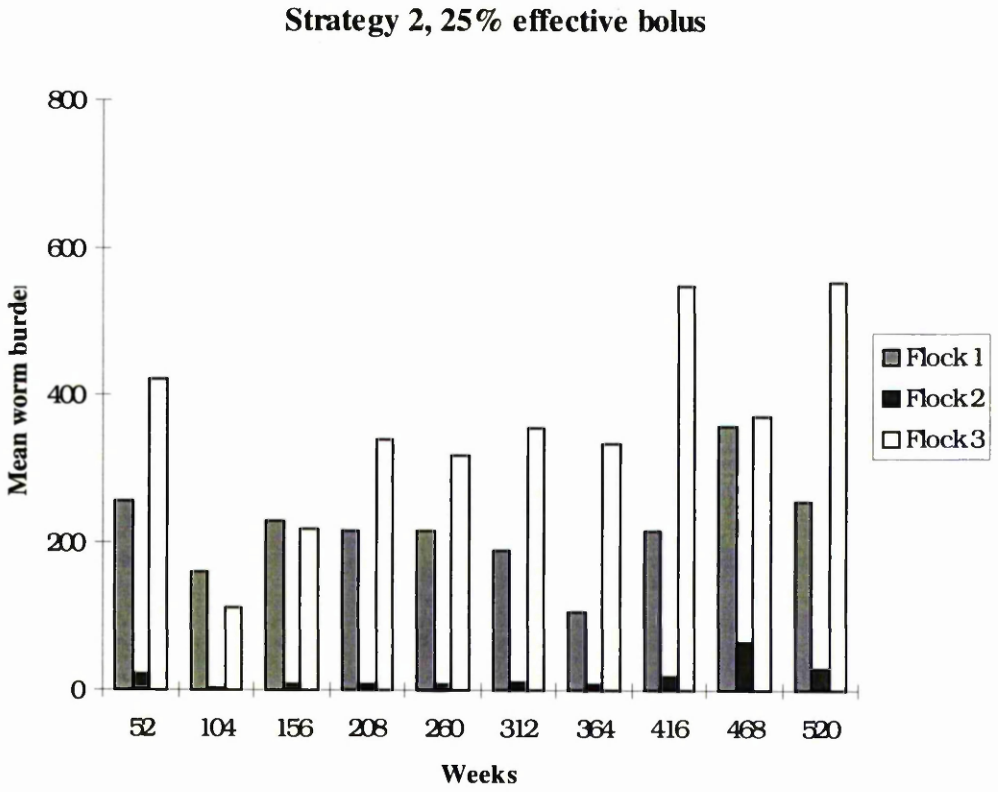


Figure 7.18 *Mean worm burdens for Flocks 1, 2 and 3 modelled using Strategy 2 and 25% effective bolus*

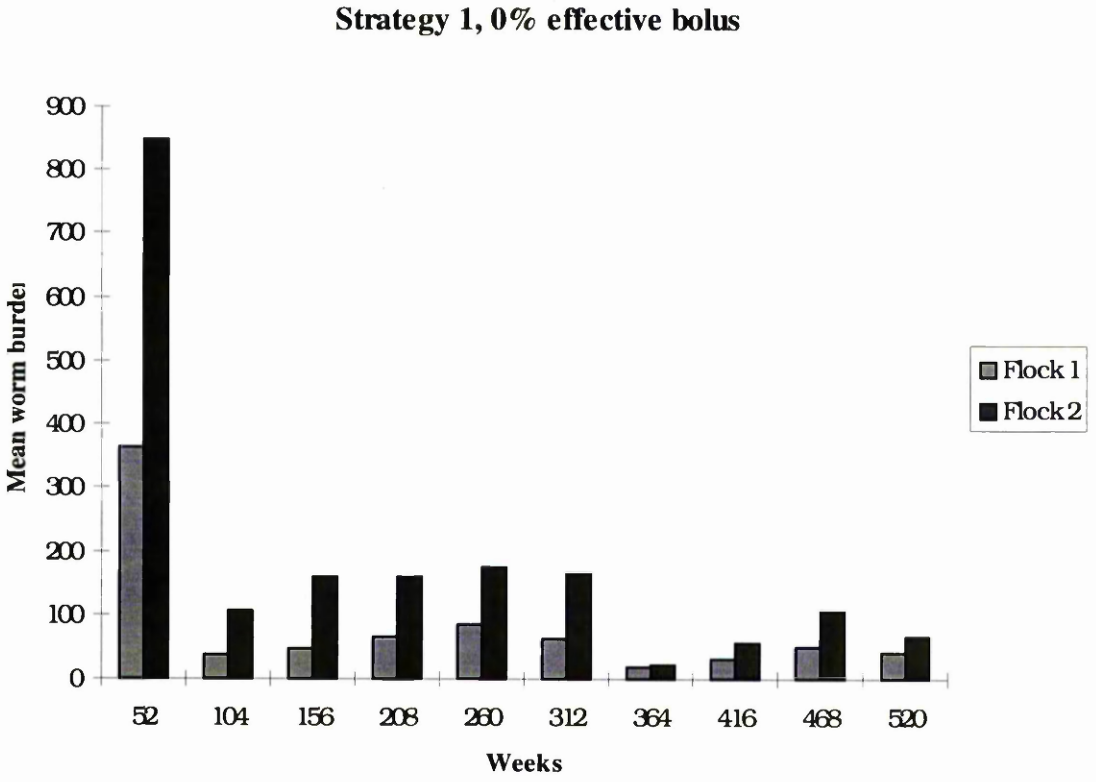


Figure 7.19 *Mean worm burdens for Flocks 1 and 2 modelled using Strategy 1 and 0% effective bolus*

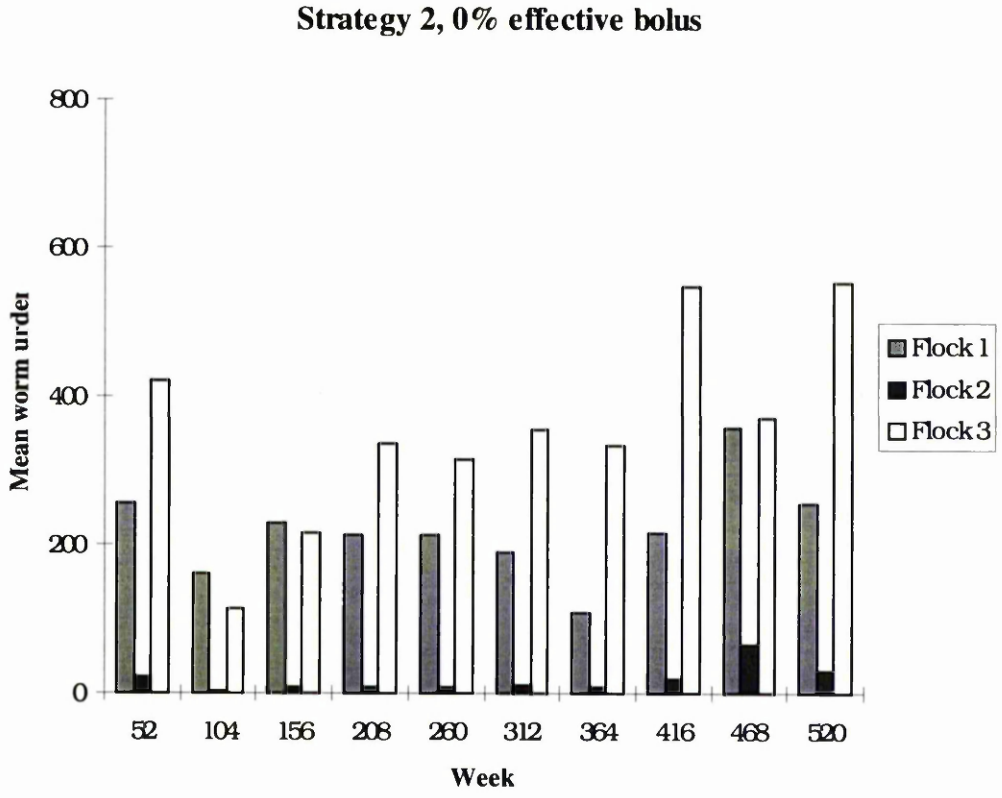


Figure 7.20 *Mean worm burdens for Flocks 1, 2 and 3 modelled using Strategy 2 and 0% effective bolus*

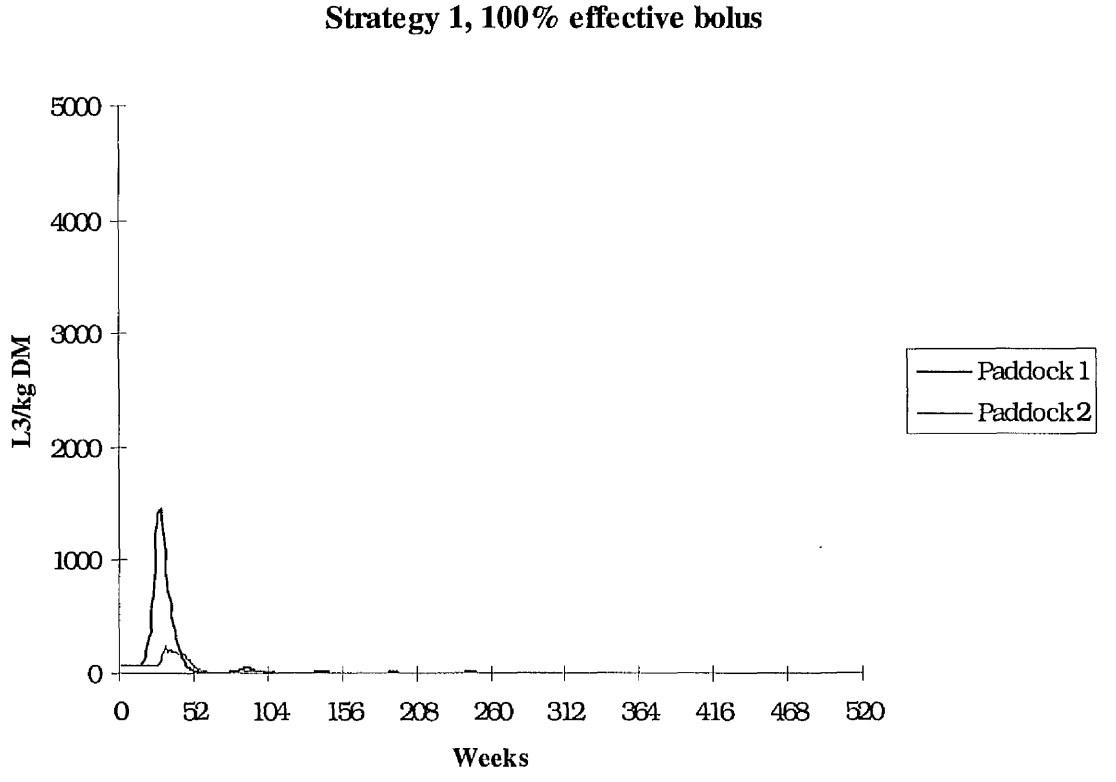


Figure 7.21 *Mean pasture larval counts for Paddocks 1 and 2 modelled using Strategy 1 and 100% effective bolus*

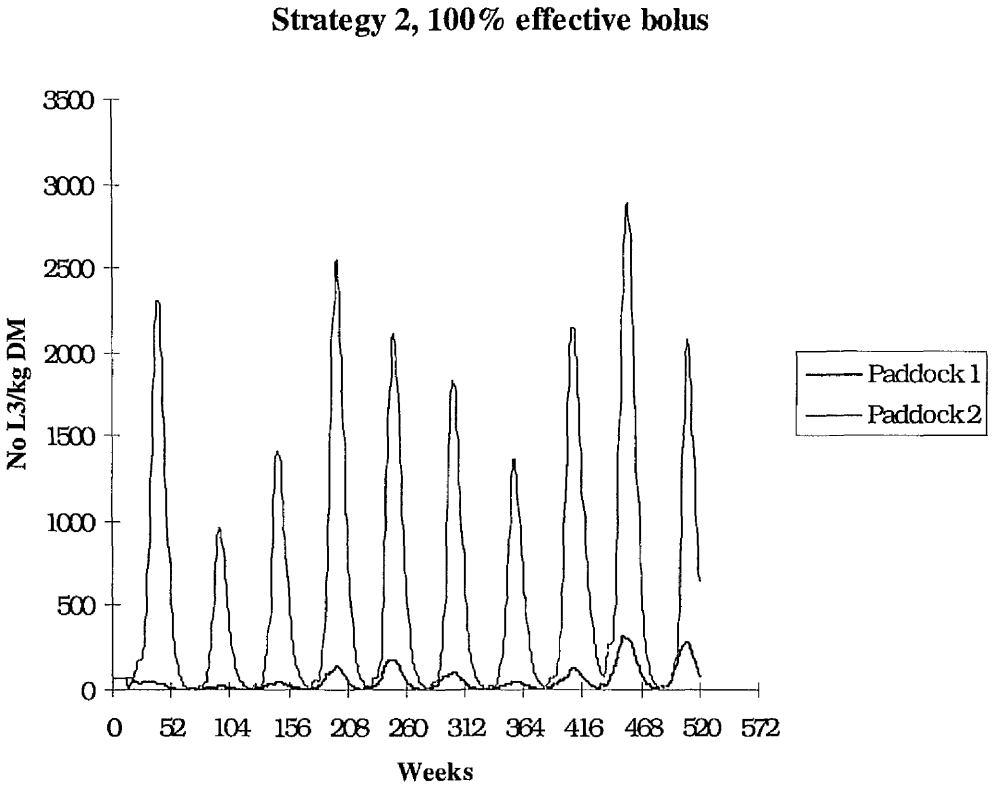


Figure 7.22 *Mean pasture larval counts for Paddocks 1 and 2 modelled using Strategy 2 and 100% effective bolus*

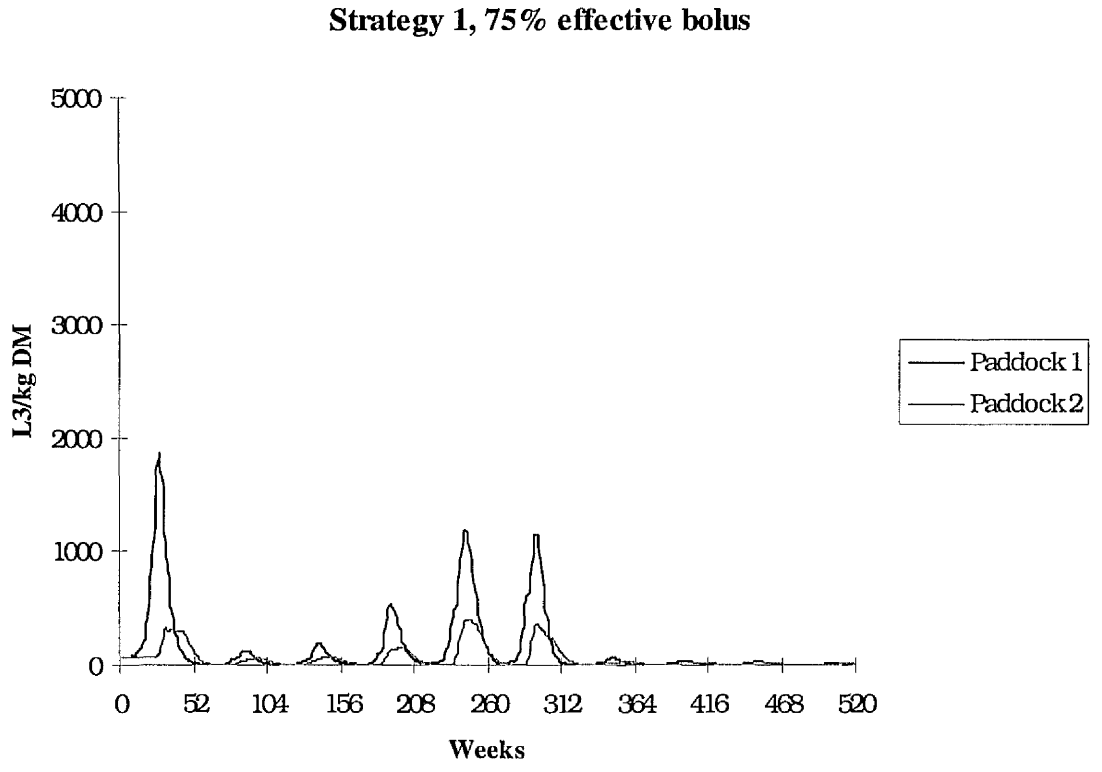


Figure 7.23 *Mean pasture larval counts for Paddocks 1 and 2 modelled using Strategy 1 and 75% effective bolus*

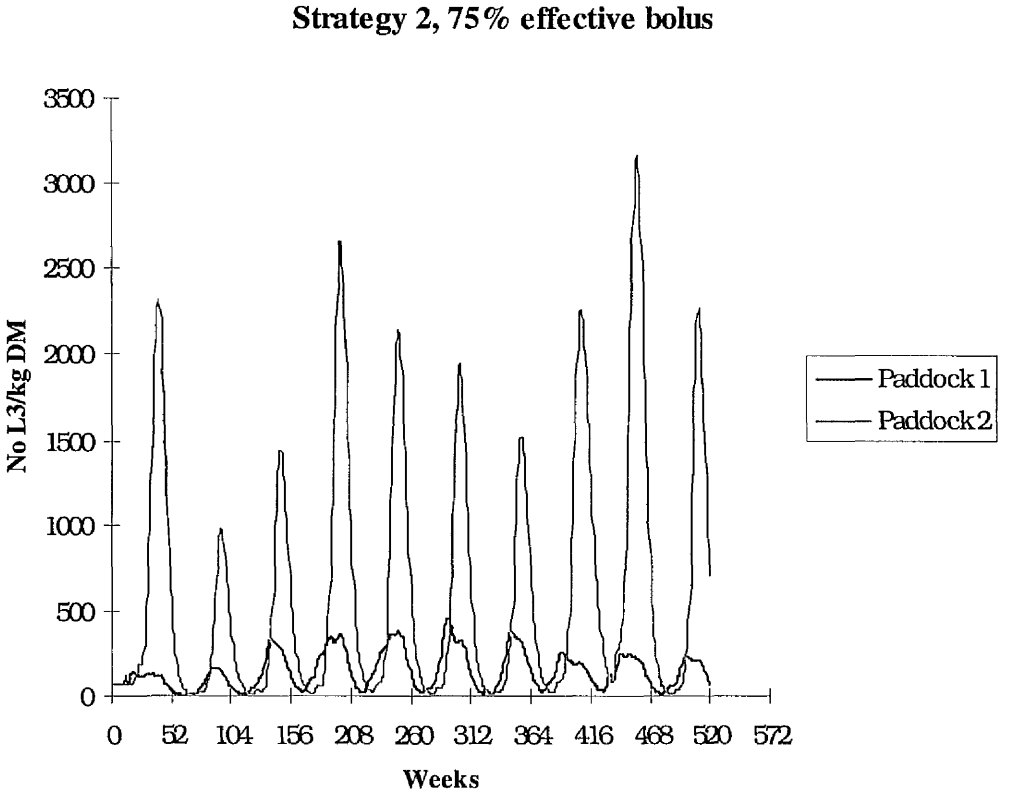


Figure 7.24 *Mean pasture larval counts for Paddocks 1 and 2 modelled using Strategy 2 and 75% effective bolus*

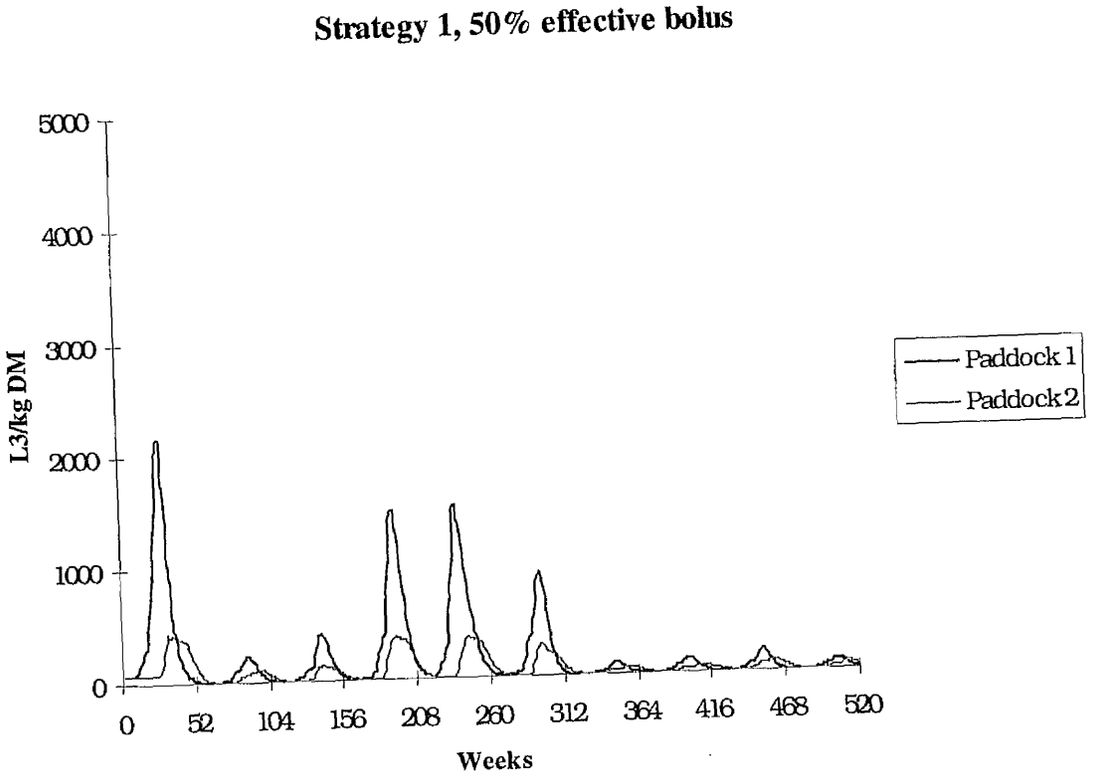


Figure 7.25 *Mean pasture larval counts for Paddocks 1 and 2 modelled using Strategy 1 and 50% effective bolus*

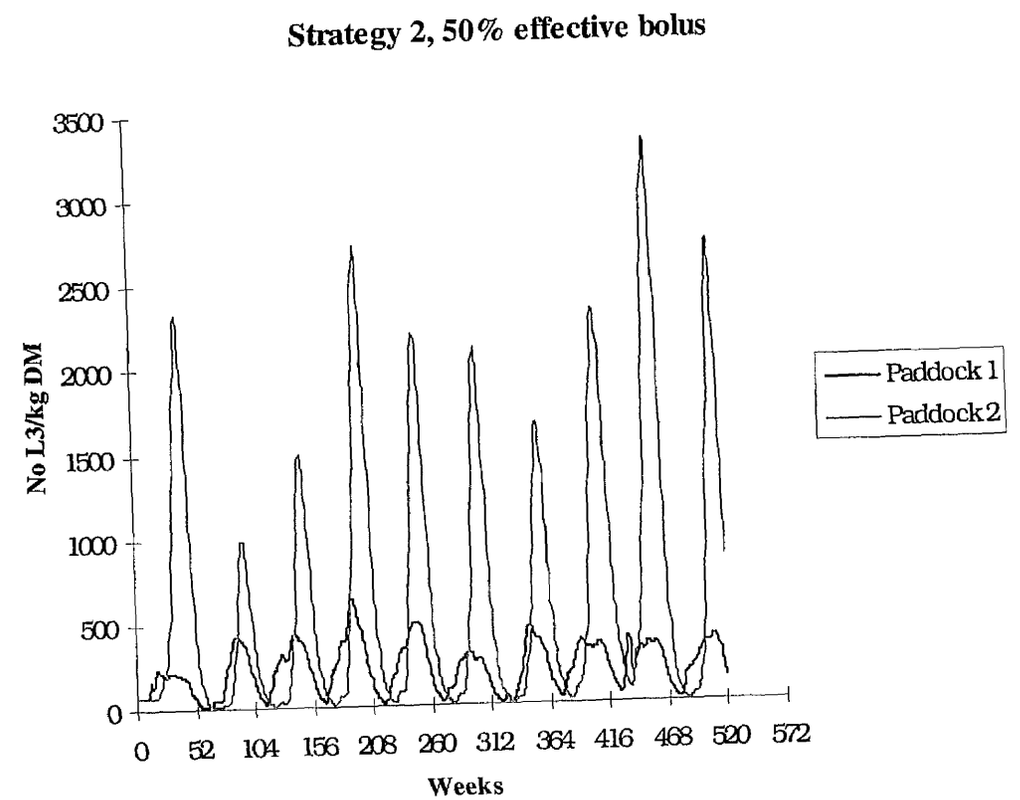


Figure 7.26 *Mean pasture larval counts for Paddocks 1 and 2 modelled using Strategy 2 and 50% effective bolus*

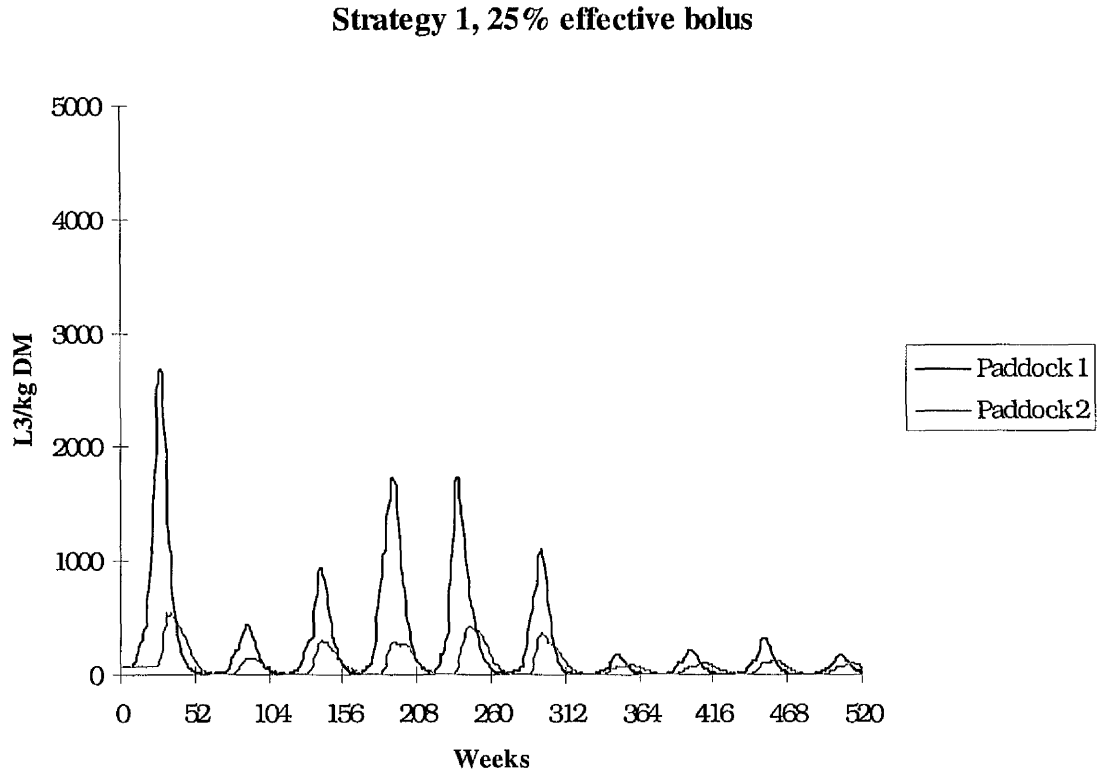


Figure 7.27 *Mean pasture larval counts for Paddocks 1 and 2 modelled using Strategy 1 and 25% effective bolus*

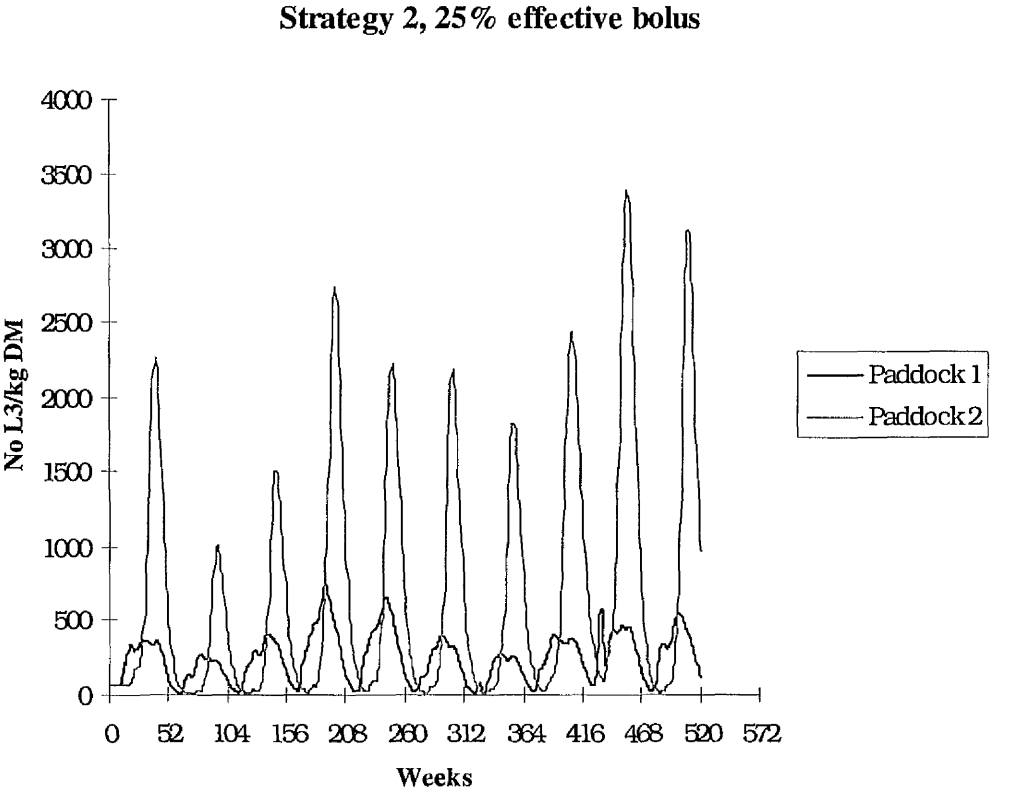


Figure 7.28 *Mean pasture larval counts for Paddocks 1 and 2 modelled using Strategy 2 and 25% effective bolus*

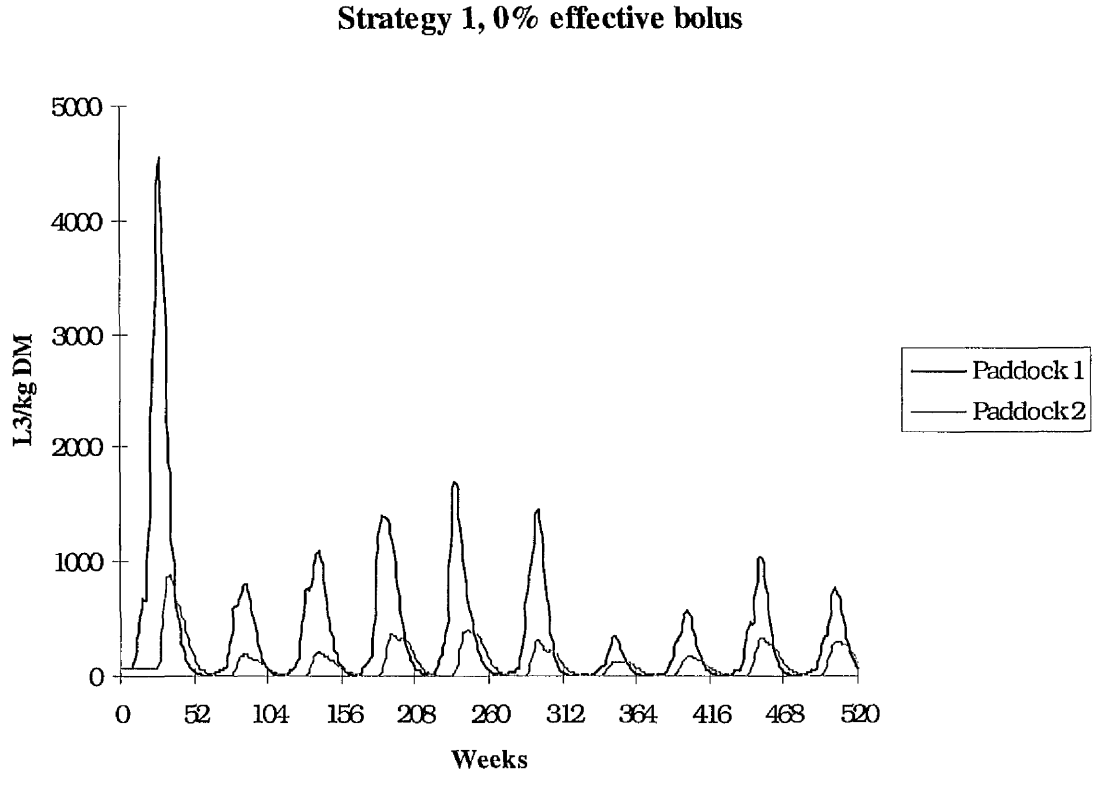


Figure 7.29 *Mean pasture larval counts for Paddocks 1 and 2 modelled using Strategy 1 and 0% effective bolus*

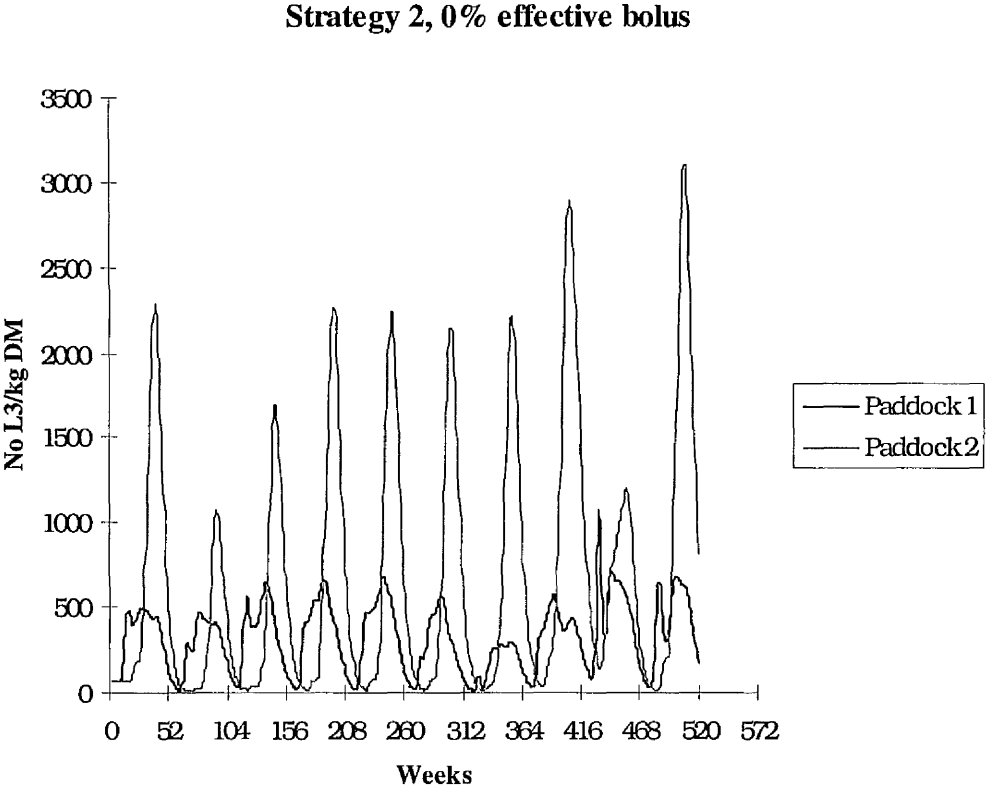


Figure 7.30 *Mean pasture larval counts for Paddocks 1 and 2 modelled using Strategy 2 and 0% effective bolus*

7.4 Discussion

The model was parameterised using a relatively modest stocking rate of 10 ewes and twin lambs to the hectare with routine anthelmintic treatments that were intended to minimise the impact of gastrointestinal nematodes. The numbers of ewe and lamb treatments administered are similar to those recorded in a recent survey in Scotland which showed that ewes were given on average between 2 and 3 treatments per annum and lambs were given just over three treatments (Jackson, personal communication). Whilst the patterns of infection seen in the model were similar to those seen in the study at Firth Mains (Barrett, 1997), the parasitological data generated within the model were several orders of magnitude lower than might have been expected. These very apparent differences highlight one of the weaknesses of the model: its inability to deal realistically with low pasture infectivity and low worm burdens in terms of faecal egg output and the acquisition of immunity. Faecal egg counts of *Ostertagia* spp in both cattle (Michel, 1970) and sheep (Jackson & Christie, 1979) have been shown to be stereotypic in that, above some level of challenge, they appear to be unrelated to larval challenge. This aspect of the host parasite relationship may be poorly described by the fixed per capita fecundity descriptors used in models. Predictors for rate of acquisition of immunity used in models are invariably based upon data generated in studies using moderate-high levels of larval challenge which invoke immunity relatively rapidly. There is evidence from field studies that immunity against *Ostertagia (Teladorsagia)* is acquired by lambs towards the end of the first grazing season (Thomas & Waller, 1979; Barrett, 1997). However, there is also evidence from field studies using minimally contaminated pastures that immunity against *Teladorsagia* is acquired more slowly under these circumstances. Studies in Scotland using clean grazing have demonstrated a marked lack of immunity towards *Teladorsagia* at the end of the grazing season in lambs reared on clean pasture but a marked immunity in lambs reared on pastures carrying overwintered contamination (Jackson, 1989). The low worm burdens generated by the model most probably result from the 'apparent' acquisition of immunity which reduced establishment and enhanced adult rejection. Given that all models are parameterised using data generated in moderate-high challenge studies, it is perhaps not surprising that they may have obvious limitations in dealing realistically with low challenge situations. Despite these obvious

shortcomings, it is possible, by relating the data to the 100% effective bolus, to use the findings from the model to gain some insight into the impact of reducing ewe egg output. Table 7.4 shows the ratios for average annual egg count, average worm burden and average annual pasture larval count using the data from the 100% effective bolus group as the comparator.

Table 7.4 *Ratios of worm burden, FEC and pasture larval count in ewes given 100%, 75%, 50%, 25% and 0% effective boli together with their lamb ratios using the 100% bolus data as the comparator (strategy 1)*

Bolus efficacy	EWB Ratio	Lamb WB Ratio	Ewe FEC Ratio	Lamb FEC Ratio	Pasture larval count
100%	1	1	1	1	1
75%	4.8	2.8	3	3	3.5
50%	7.4	3.8	4	4	4.9
25%	10	5.5	6	4	6.6
0%	16	9.3	8	8	9.7

Table 7. 5 *Ratios of worm burden, FEC and pasture larval count in ewes given 100%, 75%, 50%, 25% and 0% effective boli together with their lamb ratios using the 100% bolus data as the comparator (strategy 2)*

Bolus efficacy	EWB Ratio	'Fat' Lamb WB Ratio	Store Lamb WB Ratio	Ewe FEC Ratio	'Fat' Lamb FEC Ratio	Store lamb FEC Ratio	Pasture larval count
100%	1	1	1	1	1	1	1
75%	2.3	1.1	1.1	8.8	1	1.1	1.1
50%	4.7	1.3	1.2	3.5	1.3	1.1	1.1
25%	7.3	2.3	1.3	4	1.7	0.7	1.2
0%	7.7	2.5	1.3	5.5	2.3	0.7	1.2

The data in Table 7.5 show, as would be expected, a more than 7 fold difference in worm burden and a more then 5 fold difference in egg count between ewes treated with an wholly effective bolus and those left untreated. However, the data also suggest that the benefits accruing to the lamb as a result of reduced dam periparturient pasture contamination are less marked and are mostly evident in the short term. The worm burdens and egg counts of fat lambs from untreated dams were 2.5 and 2.3 times higher than those from lambs whose dams were given the 100% effective bolus. In store lambs maintained on pasture for an additional 120 days these differences were smaller with regard to worm count and in the case of faecal egg counts were negligible. Within the low challenge model system studied here there was also little benefit of reducing ewe egg output as far as average pasture larval populations were concerned.

Taken together, the data generated within the model suggest that, under the management strategy used in this study, the peak larval populations seen in the late summer/autumn months are largely derived from contamination laid down by lambs. This finding is entirely in agreement with those reported by Paton *et al.* (1984) whose model showed that eggs deposited during the summer months were responsible for the peak pasture larval populations.

Although under some circumstances the ewe may not make a significant contribution to the peak larval populations which are often associated with disease in lambs, the periparturient ewe usually acts as an important initial source of infection for lambs (Armour, 1980). Empirical studies have also shown that using anthelmintics to reduce ewe egg output during the periparturient period can produce production benefits (Pomroy, 1998).

The WormWorld™ *T.circumcincta* model is arguably the most user friendly computer model and, unlike the model developed by Beecham *et al.* (1995), using data generated within this thesis, can readily accept new environmental, managemental and drug resistance parameters. In that respect the model might be expected to form the basis for a decision support system, however, as the results in this chapter show, the model lacks sophistication in two inter-related critical areas. Firstly, it clearly requires some threshold dependant adjustment to the immunological function within the model as shown by the models' difficulty in coping with low *T.circumcincta* challenge simulations. Secondly, and more

importantly, the only measure of disease within the model is mortality. Although *Teladorsagiosis* is sometimes a cause of death, it is most commonly associated with sub-optimal performance in growing animals (Coop *et al.,* 1985a). Developing the disease function of mathematical models is a challenge because subclinical disease is not only challenge density dependant but is influenced by the expression of host immunity, the acquisition of which depends upon the degree and duration of previous exposure. Given that increasingly sophisticated multigeneric computer models can be developed, then they have clear roles as epidemiological tools to investigate the long term impact of different control strategies and as on farm decision support systems.

Chapter 8

General Discussion

8.1 Discussion

The continuing emergence of anthelmintic resistance, environmental concerns over anthelmintic residues and consumer driven interest in animal welfare issues all make the need for sustainable control measures increasingly important. Any control programme needs to be based on a sound understanding of the epidemiology of the parasite and the complex host-parasite relationship. To this end greater knowledge of the ecology of the free living stages and what influences changes in population dynamics of the infective larvae on the pasture are of utmost importance. The data generated within this thesis have provided important information on both the suprapopulation and infrapopulation dynamics of *T. circumcincta*, which is one of the most important parasites in small ruminants.

The experiments designed and performed in the growth chambers were to investigate the influence of changes in the environment on the migratory behaviour of infective larvae and to provide data for use in investigating the relationship between feeding behaviour and infective processes (Beecham *et al.*, 1995). Two swards of different ages were used, a mature sward prepared from cultivated turf and a young sward grown from seed. There was a huge difference in the migration rates between these swards. This may be due to the tillering of the mature sward trapping moisture and so creating a different microclimate, or the larvae themselves may become trapped in these nodules.

The optimum temperature for migration was around 20°C for the mature sward and around 15°C for the sward grown from seed. This was similar to results from Callinan & Westcott (1986), also using *T. circumcincta*, who found optimum migration at around 15°C. Differences in migration rates between nematode species have been described by several authors (Rogers, 1940; Krecek *et al.*, 1991, 1992, 1995) although Callinan & Westcott (1986) found no difference in migration rates between species. As different parasite species have different global distribution areas, it would seem to be perfectly feasible that different larval species react more favourably to certain environmental conditions. As many experiments are performed under field conditions, using naturally occurring populations, this effect is often not fully parameterised. Clearly this area warrants further investigation.

The percentage of all the larvae recovered from the sward planted from seed at 10°C and 20°C show a clear trend for there to be a greater proportion of larvae on

the top of the sward as time progresses. This process seemed to occur quicker at the higher temperature, which may be explained by the increased motility seen at these increased temperatures (Urquhart *et al.*, 1991). This trend is not clear at 15°C, where no discernible pattern could be demonstrated.

The use of young swards resulted in greatly reduced migration rates when compared to the mature sward obtained from commercial turf. Rose & Small (1985) also found very low migration rates, on average, only 0.2% of the larvae seeded were recovered from the swards. Some authors do not place infective larvae directly onto the soil but use contaminated faeces instead, making it harder to make comparisons, as the hatching and development rate can be variable. This difference may be due to the fact that the mature sward had tillered so making the environment very similar to that of the field. Also the mature sward had a well developed mat at the base of the grass whereas the young swards had not developed a mat at all. This will affect the micro-environment and may also mean that the larvae were pipetted onto the mat as opposed to the soil, and so be further up the sward at the start of the experiment. The size of this error cannot be quantified, unfortunately, and so this is merely an assumption.

The ability to measure the environmental conditions on this small scale, made possible by the use of the microthermistors, is extremely useful, as measurements taken at standard points, i.e. 1 metre above ground temperatures, can often be misleading and bear little relation on the micro-environment present between the swards of herbage. The moisture readings taken from the leaf were particularly relevant.

This micro-environment is affected by many different factors such as sward species (Moss & Vlassof, 1993) and sward density (Silangwa & Todd, 1964), as well as those factors that affect the entire environment such as relative humidity, temperature and wind speed (Rogers, 1940). The density of the young swards should have been of similar magnitude in both experiments as the same number of seeds were planted per square cm. There was no information on the mature sward density but it appeared to be of a similar density, judging by eye. Unfortunately, no measurements were taken of herbage weight so no direct comparison could be made. Similarly no direct comparison could be made between the numbers of larvae recovered per gram dry weight of herbage. In the second and third experiments the

pots were sprayed with water every hour and so the weight of the herbage sample would have been affected by the amount of water present on the sward.

The wetting of the sward would have ensured a high humidity throughout the chamber, as did the trays of water placed in the chamber. Even so, the humidity level did not get over approximately 85%, although the readings from the micro-thermistors showed that the leaf surface did not dry out but remained moist throughout the experiment. This would have ensured that the moisture film on the sward surface would have remained constant and so allowed the infective larvae to move freely in any direction. It would be assumed that any movement would not be random but would be a trophic response. This theory is supported by the work of Rogers (1940) who used glass surfaces for the larvae to migrate upon. Further experiments on the precise nature of this response would seem to be in order.

The evidence in the literature is conflicting about whether or not there is a differentiation between nematode species in their migratory responses to external stimuli. Differences in migration rates between nematode species have been described by several authors (Rogers, 1940; Krecek *et al.*, 1991, 1992, 1995) although Callinan & Westcott (1986) found no difference in migration rates between species. Clearly this area warrants further investigation. It is well documented that different environmental conditions affect development and hatching rates of the different larval species (Urquhart *et al.*, 1991), so it would not be entirely unexpected if a difference was also seen in migration rates, especially involving species with different geographical distributions. There is some evidence to suggest that different isolates of the same species could have differing optimum development conditions. Echevarria and colleagues (1993) using a susceptible, laboratory selected ivermectin resistant and multiply resistant field strain of *H. contortus* in field studies found that there was a difference in the optimum development temperature. There was also a difference in the percentage hatch rate, with the multiply resistant strain showing a significantly lower hatch rate.

The use of growth chambers to investigate the migration of larvae under different environmental conditions is useful in that all of the conditions can be carefully controlled and monitored. However, it does have its drawbacks in that the light is derived from diffuse bulbs and fluorescent tubes, making it extremely artificial. The wavelength of light emitted may be totally different from that of the

sun and this in turn could have dramatic effects on the migrating larvae. There has not been a great deal of work done on the effects of light intensity, as its fairly difficult to extract its effect from other environmental parameters. Rees (1950) found the greatest migration of *H. contortus* at low light intensities, at around dawn and dusk. This work agrees with the findings of Rogers (1940) who found the greatest migration in the laboratory at low intensities, using *T. colubriformis*. There may be an effect from the intensity and wavelength of the light or the effect may be on the moisture film in which the larvae move.

The information provided by the fistulated lambs can be used to provide data on the actual numbers of larvae that are being ingested and which part of the sward the sheep is grazing, providing it can be shown that the lambs do not exhibit any behavioural changes as a result of the fistulation. When grazing behaviour has been studied in the field, fistulated and unfistulated animals have shown no difference in grazing behaviour (Bryan & Kerr, 1988). There is evidence to suggest that parasitised and non-parasitised animals behave differently when grazing pastures contaminated by faeces (Cooper, 1997; Hutchings *et al.*, 1998). Avoidance behaviour is at its greatest when fresh faeces are present, although this is the time when the risk of infection is at its lowest.

Although differences were recorded in the bite depths between the lambs, there was no difference in larval intake. It has been demonstrated that migration rates vary on different sward structures (Niezen *et al.*, 1998c), which may offer the opportunity to manipulate the grazing environment to favour low levels of migration. This is the one area where the host dependent processes cannot be manipulated through other means, e.g. by improving the host's nutritional status, or by genetic selection.

The accuracy of the bite depth recording method using the sward stick is questionable, and in order to get a reliable indication of bite depth a more sophisticated method would be required. There are systems available that use a laser but these are obviously costly and require specialist knowledge to operate.

The framework suggested by Coop & Kyriazakis (1999) provides a basis for understanding the priorities of a parasitised animal. There are two stages of an animals' life to consider, the young, growing animal and the reproductive animal undergoing pregnancy or lactation. In the young growing animal there are two

phases to consider, the acquisition of immunity in the very young animal and the expression of immunity in the growing animal. In the reproductive animal there is the periparturient relaxation in immunity, which is one of the greatest sources of contamination for the young grazing lamb (Brunsdon, 1966; Armour, 1980; Familton, 1991).

The greatest period of susceptibility to *T. circumcincta* in the periparturient ewe was investigated, as this knowledge would be useful when implementing a grazing management strategy. The greatest period of susceptibility in the ewe appeared to be between 7 and 28 days *post partum*. This was in contrast to a study done previously by Jackson (1982) using the intestinal parasite *T. vitrinus*, where the most susceptible period was shown to be at around 28 days *post partum*. The contamination of the pasture by the periparturient ewe is the major source of infection for the lamb, so clearly any measures that can reduce this rise in faecal egg count are highly significant. The work done by Donaldson and colleagues (1997, 1998) in New Zealand demonstrating an almost total lack of periparturient rise by using protein supplementation is extremely significant. The management systems are obviously very different when comparing the British situation, as ewes are not kept outdoors for the entire year, nevertheless this demonstration could have far reaching benefits.

Benefits from adding protein to the diet of young lambs has also been recently shown to have long lasting benefits, even if the protein is only provided for a short length of time (Datta *et al.*, 1999). The effects of nine weeks of offering a high protein ration while the lambs were artificially infected with *H. contortus* lasted for the length of the 69 week sampling period, with the supplemented lambs having a higher liveweight gain, increased wool growth, increased antibody responses and lower faecal egg count.

Manipulation of the hosts' plane of nutrition is another possible method of reducing the loss of production associated with gastrointestinal nematode parasitism. The work presented in this thesis suggests that supplementation with a specific amino acid, methionine, offers no benefits on either resilience or resistance to the abomasal parasite *T. circumcincta*, although benefits on resilience were demonstrated on lambs infected with the intestinal parasite *T. colubriformis* (Coop *et al.*, 1998). Supplementation with urea or molasses blocks, a much cheaper and more

readily available substance have also shown a positive effect on production traits when used in the tropics and other poor areas and when used with both sheep and goats (Knox & Steel, 1996). This could be used as part of an integrated approach.

T. circumcincta is a very well adapted parasite and covers a large geographical area. The free living stages are extremely well adapted to the temperate environment, being able to withstand mild dessication, to survive over-winter on the pasture or to survive within the host via hypobiosis. It has a relatively short generation time, so allowing the parasite to adapt fairly rapidly to any selection pressures, for instance that of chemotherapy. It is this development of anthelmintic resistance that has led to different control methods being explored. Methods such as the use of nematophagous fungi, breeding for enhanced genetic resistance and the development of vaccines are being explored in increasing numbers. Progress is being made, for instance, a reduction of between 30-50% in egg count has been demonstrated by vaccination using the O12 antigen (W. D. Smith, personal communication). As a relatively small proportion of the host population harbours a large proportion of the parasite population, targeting this most susceptible section of the host population is another area being explored. This has been demonstrated to be of benefit in dairy goats, where the highest producing does are also the least resilient or resistant to parasite infection (Chartier & Hoste, 1997).

Computer generated mathematical models are an extremely useful and versatile tool in that they can be used to look at the long term consequences of changes in management procedures and at other factors, such as the development of anthelmintic resistance and effects of vaccination. It has been predicted, for instance, that protecting 80% of the herd is sufficient to cause enough of a fall in faecal egg count that the following grazing season the herd will be virtually free of risk (Dobson & Barnes, 1998). The models offer the opportunity to run a twenty year field study in minutes, making this a very cost effective method of, for instance, investigating the development of anthelmintic resistance, ways of slowing this development and novel control methods.

The simulations run using the anthelmintic bolus over a ten year period show the importance of allowing young animals the opportunity to gain sufficient parasite exposure to allow the acquisition of an effective immune response to develop. While this is important, a balance is required as the acquisition of a pathogenic worm

burden is obviously undesirable. The grazing management system that was modelled demonstrated that the greatest effect on the young lambs parasitic status came from their own faecal egg output, once their initial infection was acquired from the deposition of eggs by the periparturient ewes.

The most likely candidate for the greatest success in biological control is the nematophagous fungi *D. flagrans*. This can be given as a feed supplement or as part of a feed block and has been shown to be very effective in reducing pasture contamination not just with sheep but also with cattle, goats, pigs and horses (Larsen, 1999). It is effective against all of the most common trichostrongylid parasites and even against those with slightly different lifecycles i.e. *Nematodirus* spp. and *Dictyocaulus viviparus*. This method of control also has the added bonus of being environmentally friendly and acceptable in an organic farming system. The fungi is currently being trialed in many areas especially in the tropics in small ruminants, the most common small holdings animal. The loss of such an animal is proportionally more important.

The advent of vaccines seems to be a likely prospect either from a conventional antigen or from a 'hidden' antigen. The conventional antigen approach has the advantage of being stimulated when infective larvae are ingested, the 'hidden' antigen is one which is not normally seen by the hosts' immune system, e.g. the antigen present on the inside of the parasites' gut, which in blood feeders such as *H. contortus* will be recognised by antibodies taken in while feeding. The mass production of such a vaccine may pose problems, for instance, expression in a eukaryotic system will be needed in order to ensure the correct folding of the protein. Further research in this area is clearly required.

Consumer driven interest in organic production methods has led to a rapid expansion in this area in recent years. Organic production carries a premium with organic lamb costing on average 40% more than conventionally reared animals. With the reduced stocking rate associated with this method of production, the profit margins can be maintained due to the increased revenue from the meat. Welfare issues surrounding the use of anthelmintics are still the subject of discussion but the use of effective alternative control strategies in this system would obviously be the ideal solution.

The elimination of all parasitic nematodes is an unrealistic goal and almost certainly not attainable. A balance between the need to stimulate and maintain an effective immune response and avoid clinical disease is the ideal situation. The young lamb is the most vulnerable and most important in terms of generating revenue and so requires the most attention. The scope for further investigation in many of these areas is great.

It seems unlikely that supplementation with a single amino acid will produce any significant benefits when the main parasite is *T. circumcincta*. However, the use of urea and other cheaper supplements may of great benefit in areas where the plane of nutrition is poor. Evidence suggests that nutrition cannot be used to increase the resilience or resistance of those animals already genetically more refractory and on a fairly good plane of nutrition (Wallace *et al.*, 1996). However, those animals that are genetically more susceptible are shown to have benefits from the nutritional supplementation (Wallace *et al.*, 1995).

There is little doubt that 'traditional' sheep production systems are likely to change in future as a consequence of the political changes affecting subsidies and the public desire to minimise the use of chemicals in food animals (Thamsborg, Roepstorff & Larsen, 1999). Since our present understanding suggests that *Teladorsagia* is arguably one of the most adaptive GI parasites, it seems certain that *Teladorsagia* will remain as one of the key parasites in these emerging systems in temperate areas. If, as has been suggested, we are now entering the post anthelmintic era (Thamsborg *et al.*, 1999), then researchers may need to be equally adaptive in order to develop and implement successful control regimes.

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Appendix

Helminthological iodine

250 g potassium iodide (Fisons)

50 g resublimed iodine (BDH chemicals Ltd.)

Dissolve in 500 mls tap water.

Phosphate-buffered Saline (PBS) pH 7.4

200 g NaCl (Fisons)

28.75 g Na_2HPO_4

5 g KH_2PO_4

5 g KCl

Dissolve in 2.5 litres in distilled water. This is a ×10 concentrate of PBS and an appropriate dilution was made before use.

1% Bovine serum albumin (BSA)

0.25 g BSA dissolved in 25 ml distilled water.

Glycine buffer (0.1M, pH 2)

7.5 g glycine

5.85 g sodium chloride

Dissolve in 800 ml distilled water, stirring vigorously with a magnetic stirrer. Adjust the pH to 2 with hydrochloric acid (HCl). Make up to 1 litre using distilled water.

1N Sodium hydroxide (NaOH)

40 g NaOH pellets dissolved in 1 litre of distilled water.

10% Trichloroacetic acid (TCA)

100 g TCA crystals dissolved in 1 litre of distilled water.

Folins working reagent

5 ml Folins and Ciocalteau's reagent mixed with 10 ml distilled water.

Stock tyrosine solution

0.0362 g tyrosine dissolved in 100 ml of 0.1N HCl. (Note: shelf life 1 month at 4°C).

Working standard is a 1 in 10 dilution of stock with distilled water.